Determining heterotic orientation of South African maize inbred lines towards USA temperate and CIMMYT- tropical testers and genetic analyses under contrasting environments

By

SHANDU, Siphiwokuhle Funani
BSc. MSc. (UKZN)

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Breeding

UNIVERSITY OF
KWAZULU-NATAL

School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

South Africa

June 2018

ABSTRACT

Drought and low soil fertility are major abiotic stresses limiting maize productivity in South Africa. Developing drought and low nitrogen-tolerant varieties is part of a long-term solution to improved maize productivity under climate change. The employment of well-defined heterotic groups has been the prime cause of success of most hybrid breeding programs in the private sector. The public maize program in South Africa utilises seven heterotic groups. These require many different testers, and the resultant many cross combinations require a lot of resources for extensive field testing. Reducing the number of heterotic groups is essential for improving breeding efficiency. The objectives of this study were to classify the South Africa maize inbred lines into fewer heterotic groups based on their orientation towards temperate and tropical testers, and to identify superior genotypes under stress and non-stress environments. A sample of 42 lines drawn from the seven heterotic groups was genotyped with 56110 SNP DNA markers. The lines were also crossed to two inbred line testers representing the heterotic groups A and B for tropical CIMMYT and temperate USA Corn Belt. The resultant hybrids were evaluated in an (0, 1) α-lattice design under stress and non-stress conditions during the 2014/15 and 2015/16 summer seasons. Data were collected on grain yield and secondary traits. Using the specific combining ability and SNP-marker data, the seven heterotic groups could be reorganised into two major clusters. This information would be useful in designing superior hybrids. Correlation between genetic distance with grain yield and specific combining ability was negligible, making it prudent to perform multi-location trials to identify superior genotypes. The lines FO215W, I-42, I-16 and K64 displayed good general combining ability for grain yield. The most superior hybrids were FO215W x CML444 and I-42 x CML444, which combined high productivity with stability. However, performance of hybrids generally differed under stress and non-stress conditions. Overall, results showed success in simplifying the heterotic grouping of the public maize germplasm in South Africa and the possibility of improving heterosis and obtaining high yields under low input and water limited environments by exploiting temperate × tropical hybrid combinations.

DECLARATION

I, **Siphiwokuhle Funani Shandu**, certify that this is my original work and it contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by any other person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of KwaZulu-Natal and where applicable, any partner institution responsible for the joint-award of this degree. I also declare that;

1. This thesis has not been submitted for any degree or examination at any other university.
2. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    a. Their words have been re-written but the general information attributed to them has been referenced.
    b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
3. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Signed:

……………………………………………………………Date………..…………..

Siphiwokuhle Funani Shandu

As the candidate’s supervisors, we agree to the submission of this thesis

…………………………………………………….........Date……22 **June 2018**..………………………………..

Professor John Derera (Supervisor)

…………………………………………………….........Date……22 **June 2018**………………………………..

Dr Edmore Gasura (Co-Supervisor)

…………………………………………………………….........Date…22 **June 2018**……………………………

Dr Kingstone Mashingaidze (Co-Supervisor)

ACKNOWLEDGEMENTS

***Nyaz' LweZulu,** thank you Lord for guidance and strength through it all; never would have made it without you!*

Many people helped in the assembly of data and all the information for this thesis. Special thanks are due to the Agricultural Research Council (ARC) for giving me the remarkable opportunity to develop myself, and the National Research Foundation (NRF) for financial support. Many thanks to Prof John Derera for his substantial contribution to the quality and accuracy of this work, and for being the primary source of knowledge and inspiration. I am grateful to Dr Kingstone Mashingaidze for being a great source of wisdom and courage, and for consistently instilling hard work and patience in me. Many thanks to Dr Godfree Chigeza for mentorship and his limitless knowledge and untiring efforts and support during the start of the project. Without the help of Dr Edmore Gasura during data analysis, and thesis write-up, I would not make it to the end; the sleepless nights he spent on my work are invaluable.

Thanks to the maize breeding team under the Agricultural Research Council – Grain Crops (ARC-GC), for hard work and countless hours spent on my trials, without them, this work would not be possible. I greatly thank my colleagues and sisters, Jabulile Mkhatshwa, Lucky Molefe and Mirriam Sithole for everything they assisted with. I thank the ARC-Biotech Platform and the ARC-GC biotech team (Lebogang Madubanya, Thulo Sejake, Thuli Mthombeni and other team members) for providing the molecular service. Excellent inputs and support were provided by Eric Ndou, Egas Nhamucho, Dr Dries Fourie, Dr Nemera Shargie and Dr Cousin Musvosvi during the study. Thanks to Dr Sylvester Oikeh for his assistance and encouragements. Dr Alfred Odindo has always been a catalyst for my continued learning and development; I greatly acknowledge his ability to see potential in me since I was an undergraduate student.

Invaluable inputs and assistance were provided by the fellow students; Londiwe Mabuza, Penny Makhumbila, Lesedi Sere, William Makgoga, Barnaba Makavu, Lisedi Hlema, Goodluck Ringo, Awhadzi Mashombo, Lucia Ndlala, Ntombi Maduna, McDonald Nundwe, Claudia Tesha, Sale Masudu, Chimwemwe Josia and Zawadi Mwaikonyole. I cannot thank my family enough for their understanding and support in my education endeavors. I was much encouraged by many friends who believed in me throughout; Dr Hangwani Muedi, Dr Murenga Mwimali, Dr Regina Tende, Dr Alina Mofokeng, Andile Nene, Zinhle Shabalala, Kwenzakele Mthombeni, Nelile Ngubo, Zinhle Ntombela, Nqobizwe Mahlangu,Thembakazi Mpofana, Ayanda Ngema, Ondwela Mudau, Humbelani Thenga, Sfisokuhle Ndlanzi, Xolani Ntombela, Zanele Tembe, Philile Zondi, Sbusiso Ngobese, Thando Xulu, Sanele Duma, Nomcebo Mchunu, Yvone Hadzi and Pinky Mojapelo.

DEDICATION

This work is dedicated to:

1) My God Almighty Shembe Nyaz' LweZulu, who constantly prove to me that because he is the one who strengthens me each day, nothing is impossible through him.

2) The memories of my mother Idah Shandu and father Mbabazeni Shandu who unfortunately did not live to see my work, but who never doubted me and who taught me that the essence to success is simply hard work.

3) My incredible sisters (Nontokozo, Sbekezelo, Ntando, Gugu and Phakamile), brothers (Mxolisi and Phakamani), nieces (Skhulile and Thando) and nephews (Sluleko, Nhlakanipho and Bandile) who constantly supported me and who were so loving and enduring during the period of the study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

AD	Anthesis date
AMMI	Additive main effect and multiplicative interaction
ASI	Athesis-silking interval
ASV	AMMI stability value
CIMMYT	International Maize and Wheat Improvement Center
CML	CIMMYT maize line
DAFF	Department of Agriculture, Forestry and Fisheries
DF	Degrees of freedom
ECV	Environmental coefficient of variation
EH	Ear height
EPO	Ear position
EPP	Ears per plant
F_1	Filial 1
FAW	Fall Armyworm
GD	Genetic distance
G × E	Genotype x environment interaction
GCA	General combining ability
GCAf	General combining ability due to female
GCAm	General combining ability due to male
GCV	Genotypic coefficient of variation
GGE	Genotype plus genotype by environment interaction
GY	Grain yield
HG	Heterotic group
MD	Managed drought
MOI	Grain moisture
MS	Mean square
Mse	Error mean square
MSV	Maize streak virus
N	Nitrogen
OPV	Open - pollinated varieties
PCA	Principal component analysis
PCV	Phenotypic coefficient of variation
PH	Plant height
QPM	Quality protein maize
RD	Random drought
SCA	Specific combining ability
SD	Silking date
SEN	Senescence
SNP	Single nucleotide polymorphism
SP	Shelling percentage
SS	Sums of squares
t ha^{-1}	tonnes per hectare

CHAPTER 1

General Introduction

1.1 Economic importance and production levels of maize in South Africa

Maize (*Zea mays* L.) is the most dominantly produced cereal crop in Africa (Figure 1.1) and most parts of the world. In South Africa (SA), the maize crop is a major staple food commodity that serves as an important source of carbohydrates for both humans and animals (BFAP, 2015), and constitutes the largest crop size of different crops, followed by wheat (*Triticum aestivum*), soya bean (*Glycine max*) and sunflower (*Helianthus annuus*) (The Maize Trust, 2016). Maize can be processed into a variety of food and industrial products. In general, normal maize contains approximately 72% starch, 10% protein, and 4% fat, supplying an energy density of 365 Kcal/100g (Ranum *et al*., 2014). Currently, the total production in SA is comprised of 52% and 48% white and yellow maize, respectively (DAFF, 2016). White maize is used as a staple food for human consumption and yellow maize is mainly for animal feed (DAFF, 2016). The total maize cultivated constitutes about 85% genetically modified (GM) maize (The Maize Trust, 2016). About half of the maize produced is used as food, 40% serves as a major feed grain for animals and 10% is utilised in industrial uses (DAFF, 2016); due to food security concerns, no maize is used in biofuel production to produce maize-based ethanol (BFAP, 2015).

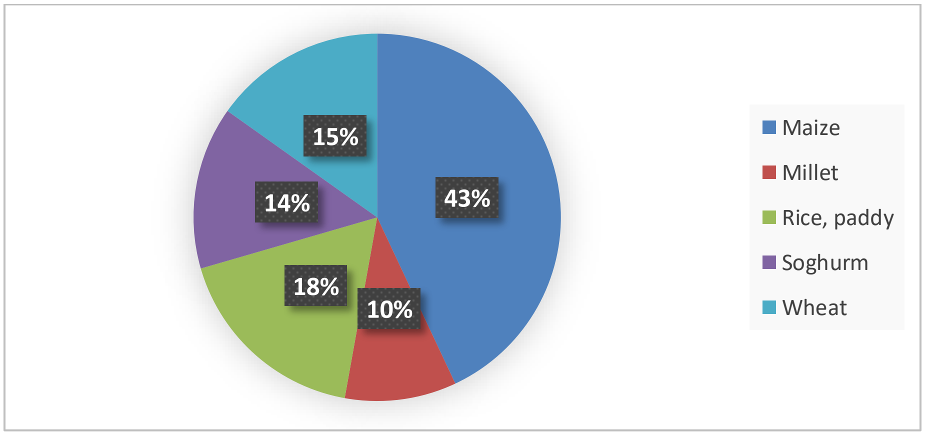

Figure 1.1: Major cereal crops produced in Africa (FAOSTAT, 2015)

South Africa is leading the continent with technology adoption (The Maize Trust, 2016), as a result it remains among the top 10 world maize producers, and the main maize producer in the African continent (Agri SA, 2016). Approximately 10 - 12 million tonnes of maize are produced in SA annually on an average of 2.5 - 2.75 million hectares of land, leaving an average surplus of 1.8 million tonnes for the export market (Grain SA, 2015). Commercial maize producers contribute about 98% of the SA maize crop while 2% is produced by the small scale-farmers (Grain SA, 2015). The majority of maize production is concentrated in the North West, Free State, Mpumalanga and KwaZulu-Natal provinces (DAFF, 2016).

The production regions (maize belt) are divided into four major regions according to climatological characteristics. These are (i) the warm Western Region which includes the western parts of the Free State and the North West province, (ii) the temperate eastern region in Gauteng and the central parts of the Free State, (iii) the cold eastern region which is the Mpumalanga Highveld and eastern Free State and (iv) the KwaZulu-Natal Region comprised of the western/upland and the central/midland parts of the KwaZulu-Natal province (The Maize Trust, 2016). White maize is mainly produced in the western parts of the maize belt, while yellow maize is planted in the eastern parts. The planting season normally starts during late spring to early summer between October to December, with optimal planting times in November and December in most regions (DAFF, 2016). The rainfall pattern and other weather conditions generally determine the planting time and the length of the growing season. Planting can therefore start as early as October and extend to January. Depending on the planting period, harvests can begin from April or late May to end of August (DAFF, 2016).

1.2 **Maize production constraints**

South Africa is currently the main maize producer on the African continent; however, sustainable and sufficient maize production is still constrained by several biotic and abiotic factors. Biotic constraints such as preponderance of diseases, insects and pests are responsible for unstable yields (ARC, 2014). The most destructive pests are particularly the African stem borer (*Busseola fusca*), spotted stem borer (*Chilo partellus*), weevils in storage by smallholder farmers and the new invasive fall armyworm (*Spodoptera frugiperda)* (ARC, 2016*).* Various options including the use of chemicals are available to control pests, however the new invasive fall armyworm is difficult to control with pesticides. Predominant pathogenic diseases include, grey leaf spot (*Cercospora zeae-maydis*) caused by the fungus *Cercospora zeaemaydis*, the Maize streak virus (MSV) disease caused by a leafhopper transmitted virus; although the occurrence of MSV is sporadic, its outbreak can lead to serious implications on maize yields and quality (The Maize Trust, 2016). Turcicum leaf blight (*Exserohilum turcicum*) and common rust (*Puccinia sorghi*) are also among the major fungal diseases.

Several abiotic stresses also play a role in the limitation of maize production is SA, and these are mostly climate and soil related stresses. Among them, there is a prevalence of acidic soils and poorly drained soils is some areas; the problem of soil erosion especially in marginalised areas such as the Northern Cape, North West and in some parts of the Eastern Cape has also posed a challenge in maize production (ARC, 2014). The country is also characterized by above-average temperatures (ARC, 2014) and unevenly distributed rainfall (Table 1.1).

Table 1.1: Annual rainfall (mm) distribution and climatic classification in South Africa

Rainfall (mm)	**Classification**	**Land surface (%)**
<200	Desert	22.8
201-400	Arid	24.6
401-600	Semi-arid	24.6
601-800	Sub-humid	18.5
801-1000	Humid	6.7
>1000	Super-humid	2.8

Source: ARC, 2014

The *El Nino* induced drought stress and low soil fertility particularly nitrogen (N) rank high among major abiotic stresses threatening maize production (ARC, 2014), and consequently food security in SA and other parts of eastern and Southern Africa. South Africa is vulnerable to low N stress and drought stress because of the small proportion of fertile land and the importance of dryland production, respectively. An estimated 83% of SA maize is under dryland production and only 3% of the total land is deemed fertile (Agri SA, 2016); this increases the susceptibility of many maize growing regions to the effect of drought and low soil fertility. Nitrogen (N) forms the core element in several biochemical processes in plants and in the constitution of compounds including amino acids, proteins, enzymes and chlorophyll (Below, 1997). For every maize tonne to be produced, approximately 16 kg N are required in the grain (Banziger *et al.*, 2000). Occurrence of soil nitrogen stress during maize growth and development thus hinders the final crop yield. Low soil fertility is associated with poor cultural practices and the little use of agricultural inputs, due to high costs, especially for smallholder farmers (Tesfa *et al*., 2012). Soil nitrogen supply is expected to vary among years and locations mainly due to climate change and variation in soil characteristics over time (Lory and Scharf, 2003). This therefore accelerates the need for developing cultivars that are efficient is the use of available soil nitrogen.

Drought is a serious constraint in SA crop production particularly in the maize growing regions. Drought effects are exacerbated by factors including low natural soil fertility, inefficient crop management practices and biotic stresses (Santos *et al.*, 1996). The significance of drought in SA maize production was observed mainly in the 2015/16 season when the majority of the country including the main maize growing areas were characterized by severe to extreme drought (Figure 1.2), coupled by heat waves (ARC, 2016).

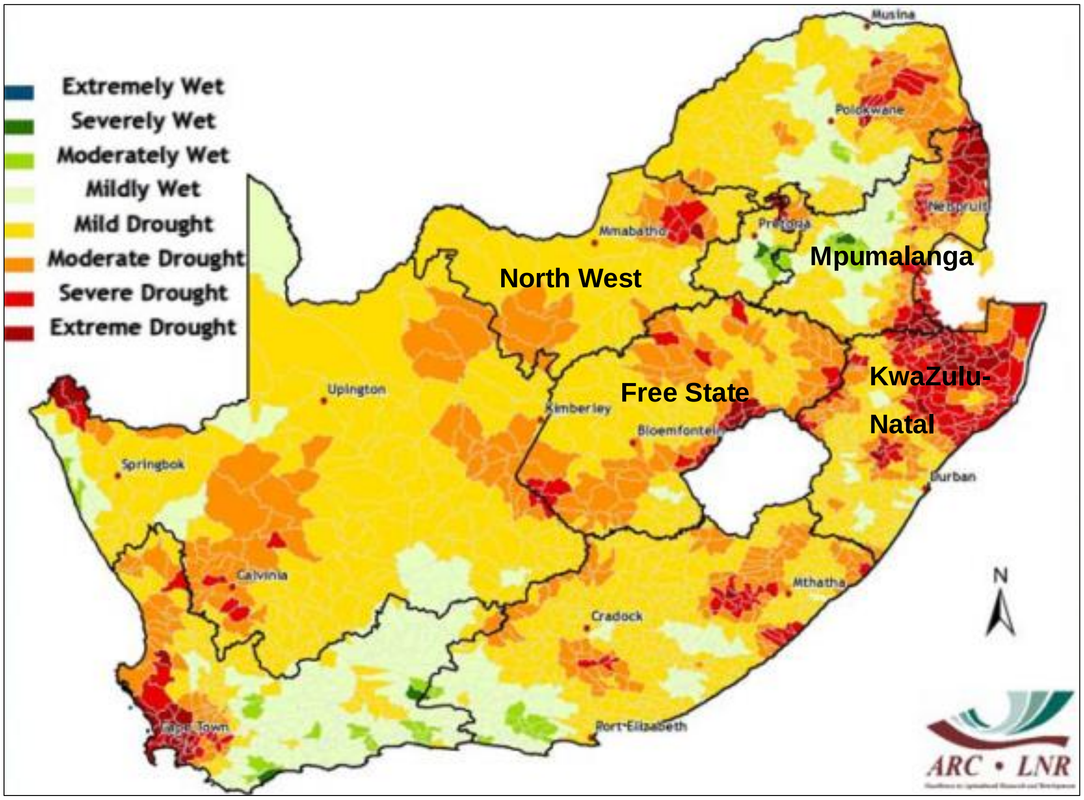

Figure 1.2: Drought occurrence in SA maize growing regions during the 2015/16 summer season

The impact of drought was preceded by a decrease in production area and delayed maize planting season; this significantly lowered the total annual maize harvest from the annual average of 10 -

12 million tonnes to about 7.78 million tonnes (Grain SA, 2015), which was reported as the lowest production since 2007. As a result, maize producer prices were pushed higher by approximately 50% (Figure 1.3). Drought also caused SA to import large quantities of about 943 000 tonnes of yellow maize for the first time in 2015 (Grain SA, 2015).

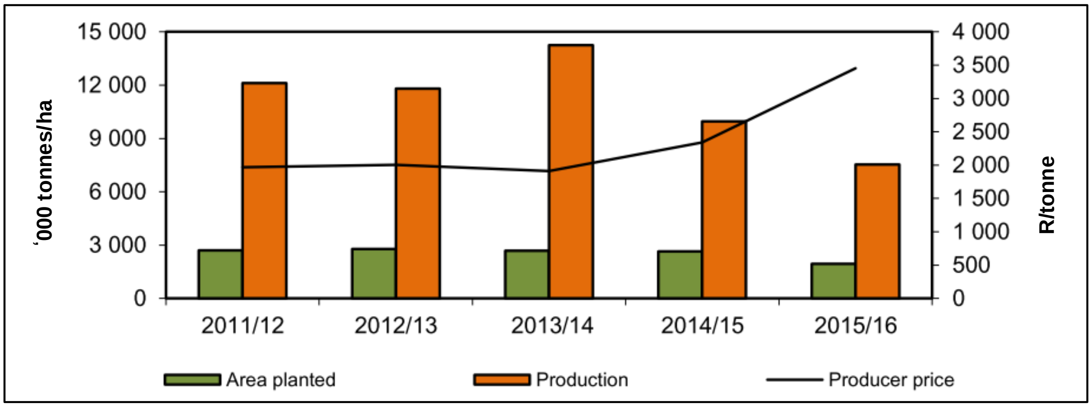

Figure 1.3: Maize total area planted, yields and producer prices from 2011/12 to 2015/16 season (DAFF, 2016)

1.3 Problem statement – drought, low nitrogen and too many heterotic groups

South Africa (SA) is among the major potential contributors to the world total maize production. Therefore, any production constraint hindering maize production in the country affects not only SA but the rest of the continent, particularly the neighboring countries in Southern Africa that rely on SA maize exports. The *El Nino* induced drought stress and low soil fertility, particularly nitrogen (N) are the primary threats to maize production in SA. An estimated 13% of the area planted to maize is under irrigation and 87% is dryland. The proportion of dryland production worsen the effect of drought. Smallholder farmers' crops are more vulnerable to moisture stress because they rely mainly on in-season rainfall for production. Nitrogen is an essential and expensive input of grain maize production. The majority of farmers need to increase the fertility of their soils to achieve high yields; however, this option is not possible for some farmers, particularly poor-resource smallholder farmers in marginal areas. Maize yields obtained by smallholder farmers are therefore generally low because they use extremely low inputs due to lack of financial resources required to optimise yields. Furthermore, in most farmers' fields, drought and low N stress generally occur simultaneously during the same growing season. The combined effect of these abiotic stresses has created a huge yield gap between smallholder and large-scale farmers, and it has impacted negatively on food security as well as food prices.

The major objectives of the Agricultural research council (ARC) breeding program are (i) high grain yield, (ii) drought tolerance, (iii) low nitrogen tolerance, (iv) resistance to major diseases (Northern corn leaf blight, Grey leaf spot, Ear rots, etc.) and (v) good agronomic traits (resistance to root and shoot lodging, maturity, grain colour – pure white, good plant and ear height, etc.). The program targets single-cross and three-way hybrids. The ARC has made substantial progress in developing drought and low N tolerant cultivars working in partnership with the International Maize and Wheat Improvement Center (CIMMYT). The germplasm used included parental lines originating mostly from breeding programs at both ARC and CIMMYT. The employment of well-defined heterotic groups has been the prime cause of success of most breeding programs, particularly in the private sector. The public maize program in SA, which is based at ARC works with seven heterotic groups which have been designated as the F, I, K, L, M, P and R. These seven heterotic groups have been utilised extensively in different combinations to develop commercial maize hybrids. The employment of too many heterotic groups is associated with high costs, delayed outcomes and reduced breeding efficiency. Many heterotic groups, from different populations also complicate the breeding process and decision making during selection of parental lines, and this has been criticised in breeding programs.

1.4 Rationale of the study

Both drought and low soil fertility have caused significant losses in maize crop yields thereby imposing wide fluctuations in the industry. The current trends are expected to increase due to global climate changes, causing more variations in soil fertility and water availability within farmers' fields. The cumulative and long-term effects will severely prevent SA from achieving high yields, with further reductions in the cropped area and increased vulnerability of most South African households to food insecurity. Identifying ways of mitigating drought and low soil N risks, in order to stabilise maize yields are fundamental to realising food security and improving livelihoods in the country. Developing and deploying varieties with improved performance under both drought and low N stress will therefore improve SA maize crop yield and will minimise production risks; these cultivars will also bridge the yield gap between small and large-scale producers.

For an efficient breeding program, it is crucial to simply the heterotic grouping of public maize germplasm in SA. The tropical maize program at CIMMYT uses a well-arranged germplasm which classifies inbred lines into heterotic groups A, B and/or AB. Most of the germplasm used by the national research programs in sub-Saharan Africa mostly come from CIMMYT, and most programs have adopted the CIMMYT heterotic group pool. The SA maize germplasm is built on a collection of products that were derived from temperate and tropical germplasm. Depending on the predominance of either temperate or tropical material in the genetic background of the inbred lines, some lines may be more aligned towards tropical CIMMYT than USA temperate Corn Belt testers, in the same vein, some might be more inclined towards the temperate lines and others could possibly show lack of association with either temperate or tropical material. Therefore, including both tropical and temperate lines will properly discriminate between inbred lines. Further validation using molecular marker genotyping based on thousands of single nucleotide polymorphism markers is necessary to justify pedigree data.

Furthermore, knowledge of the combining ability of inbred lines is important for devising appropriate breeding strategies and identifying superior parental lines for use in hybrid development. Based on combining ability effects, inbred lines to use as potential inbred testers for discriminating genotypes and defining heterotic groups may be identified. Information on the combining ability of lines is also useful for studying the mode of gene action controlling yield and major secondary traits under stress and non-stress conditions and thus will assist in predicting breeding progress. Maize is a widely grown crop in SA; as a result, developed hybrids may vary in their performance from region to region due to genotype by environment interaction (G × E). Therefore, in addition to high grain yield and stress tolerance, understanding the magnitude of G × E is crucial for identifying hybrids with a broad and narrower adaptation, especially when stress environments are the primary target. Quantifying the level of heterosis in new hybrids is another essential aspect for measuring breeding progress and therefore to attain the specific South African maize breeding objectives.

1.5 Research objective

The main objective of the study was to simplify the heterotic grouping of public maize germplasm in SA on the basis of their orientation towards tropical CIMMYT and USA temperate Corn Belt testers, and to assess the combining ability of the SA lines with tropical CIMMYT and temperate

USA Corn Belt lines, and hybrid performance (G x E and heterosis) under multiple stress conditions.

1.6 Specific objectives

The specific objectives of the study were to:

(i) Classify SA maize inbred lines into heterotic groups on the basis of their orientation towards tropical CIMMYT and temperate USA Corn Belt testers.
(ii) Determine combining ability and gene action involved in controlling grain yield under stress and non-stress environments.
(iii) Determine the genotype by environment interaction and stability of maize single-cross hybrids under stress and non-stress environments.
(iv) Determine standard heterosis in maize grain yield under stress and non-stress environments.

1.7 Research questions

The following research questions were answered in the study:

(i) Is there a possibility of narrowing the heterotic grouping of public maize germplasm of South Africa based on the orientation of inbred lines towards tropical CIMMYT and USA temperate Corn Belt testers?
(ii) Given that SA falls within subtropical and near warm temperate production environment, how well do the SA maize inbred lines combine with tropical and temperate material under stress and non-stress environments, and what is the nature of gene action controlling grain yield?
(iii) Are there some higher yielding and stable hybrids that include both tropical and temperate germplasm, under non-stress and stress environments of SA?
(iv) Are there desirable levels of standard heterosis in maize grain yield, for specific hybrid combinations under stress and non-stress environments?

1.8 Research hypotheses

The following research hypotheses were tested:

(i) The heterotic groups of public maize germplasm in SA can be narrowed on the basis of their orientation towards tropical CIMMYT and USA temperate Corn Belt testers.

(ii) There is desirable combining ability of the SA material with both tropical and temperate lines under stress and non-stress environments, with different types of gene action conditioning grain yield under different growing conditions.

(iii) There are high yielding and stable hybrids derived from both the tropical and temperate materials across stress and non-stress environments.

(iv) There are desirable levels of standard heterosis in maize grain yield for specific hybrid combinations under stress and non-stress environments.

1.9 Thesis outline

This thesis is comprised of four research chapters based on specific objectives; each chapter is presented as a stand-alone research chapter that will be submitted independently as a manuscript for publication. Some of the information on introduction and materials and methods is therefore a repetition. The general introduction, literature review and the overview chapters are included.

Chapter 1: General introduction

This section outlines the setting of the study; it provides background information and rationale of the study. Research aims, objectives, research questions and hypotheses are presented in this section.

Chapter 2: Literature review

This chapter creates a frame of reference for the study. The chapter provides description and critical evaluation of the major concepts including, heterosis, genotype by environment interaction, heritability and combining ability in relation to breeding for drought and low N tolerance. Progress made in breeding for drought and low N tolerance and the mode of gene action underlying the inheritance of traits under stress and non-stress environments is reviewed. Methods employed in constituting heterotic groups in maize are also outlined in this section. The chapter thus serves as reference guideline for the study.

Chapter 3: Heterotic orientation of South African public maize (*Zea mays* L.) inbred lines towards tropical CIMMYT and temperate USA- Corn Belt testers

This chapter presents the classification of SA public maize inbred lines from seven heterotic groups using tropical CIMMYT and temperate USA Corn Belt testers, along with the single nucleotide polymorphism-markers genotyping. Results on major findings on the heterotic orientation of lines are discussed and summarised.

Chapters 4: Combining ability and gene action controlling major traits in maize (*Zea mays* L.) under stress and non-stress environments

This chapter is based on quantifying genetic variability, combining ability of inbred lines and understanding the mode of gene action controlling the inheritance of major traits under stress and non-stress environments. The yield potential of experimental hybrids is shown, and the results and discussions sections are detailed.

Chapter 5: Genotype by environment interaction and yield stability of newly developed maize (*Zea mays* L.) hybrids across stress and non-stress environments

Chapter 5 focuses on determining the genotype by environment interaction among the F_1 experimental hybrids evaluated, and identification of cultivars for broad and narrow adaptation. Ideal test environments, higher yielding, and stable cultivars across stress and non-stress environments are identified and recommended.

Chapter 6: Heterosis in maize (*Zea mays* L.) grain yield under non-stress and stress environments

This section assesses the level of heterosis in grain yield under stress and non-stress environments. Results of the section are discussed in detail and the conclusions on major findings are made.

Chapter 7: General discussion and research overview

This chapter provides a general review of the research findings by highlighting the major objectives, implications of the findings and recommendations for future research in heterotic orientation, and breeding for improved hybrid performance under stress and non-stress environments in SA.

.

References

Agri SA. 2016. “A raindrop in the drought”. Report to the Multi-Stakeholder Task Team on the drought, Agri SA’s status report on the current drought crisis. Agri South Africa, Pretoria, South Africa. http://www.nstf.org.za/wp-content/uploads/2016/06/Agri-SA-Drought-Report_CS4.pdf (Accessed 15/07/2016).

ARC. 2014. An economic value of the national cultivar Trials in South Africa. Agricultural Research Council, Pretoria, South Africa. http://www.arc.agric.za/Economic%20Impact%20Assessment%20Studies/The%20value%20of%20National%20Cultivar%20Trials%20in%20South%20Africa.pdf (Accessed 23/03/2017).

ARC. 2016. The new invasive fall armyworm (FAW) in South Africa. Agricultural Research Council, Potchefstroom, South Africa. http://www.arc.agric.za/arc-ppri/Documents/Armyworm/Fall%20Armyworm%20English%20FS4%20%282017%29.pdf (Accessed 18/09/2017).

Banziger, M., G.O. Edmeades, D. Beck, and M. Bellon. 2000. Breeding for drought and Nitrogen stress tolerance in maize: From theory to practice. CIMMYT, Mexico, D.F.

Below, F.E. 1997. Growth and productivity of maize under Nitrogen stress. pp 235-240. In: Edmeades, G.O., M. Banziger, H.R. Mickelson, and C.B. Pena-Valdivia (eds.). Developing Drought- and Low N-Tolerant Maize. Proceedings of a Symposium, March 25-29,1996. CIMMYT, El Batan, Mexico D.F.

BFAP. 2015. Adding value in the South African maize value chain. The Bureau for Food and Agricultural Policy, Pretoria, South Africa. http://www.bfap.co.za/documents/research%20reports/ADDING%20VALUE%20IN%20THE%20SOUTH%20AFRICAN_%20MAIZE%20VALUE%20CHAIN%2013%20April%202017.pdf (Accessed 08/11/2016).

DAFF. 2016. Trends in the Agricultural Sector 2016. Department of Agriculture, Forestry and Fisheries, Directorate Plant Production. Pretoria, South Africa. http://www.nda.agric.za/doaDev/sideMenu/Marketing/Annual%20Publications/Commodity%20Profiles/field%20crops/Maize%20Market%20value%20Chain%20Profile%202016.pdf (Accessed 11/09/2016).

FAOSTAT. 2015. Statistical databases and data sets of the Food and Agriculture Organization of the United Nations. FAO. http:// faostat.fao.org/default.aspx (accessed 25/05/2016).

Grain SA. 2015. Supply and demand scenarios for South Africa's maize market: Looking into the 2015/2016 and 2016/2017 marketing years. Grain South Africa, Pretoria, South Africa. http://www.grainsa.co.za/supply-and-demand-scenarios-for-south-africa-s-maize-market:-looking-into-the-2015/2016-and-2016/2017-marketing-years (Accessed 04/05/2017).

Lory, J.A., and P.C. Scharf. 2003. Yield goal versus delta yield for predicting fertilizer Nitrogen need in corn. *Agronomy Journal* 95: 994–999.

Ranum, P., J.P. Pena-Rosas, and M.N. Garcia-Casal. 2014. Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences* 1312: 105–112.

Santos, M., X., M.A. Lopes, A.M. Coelho, P.E.O. Guimaraes, S.N. Parentoni, E.E.G, Gama, and G.E. Franca. 1996. Drought and Low N Status Limiting Maize Production in Brazil. pp 20-23. In: Edmeades, G.O., M. Banziger, H.R. Mickelson, and C.B. Pena-Valdivia (eds.). Drought-and Low N Tolerant Maize: Proceedings of a Symposium. March 25-29. CIMMYT, El Batan, Mexico, D.F.

Tesfa, B., A. Tolera, M. Tewodros, H. Gebresilasie, D. Temesgen, W. Tenaw, M. Waga, and H. Hussen. 2012. Status and future direction of maize research and production in Ethiopia. pp 24-33. In: Worku, M., S. Twumasi-Afriyie, L. Wolde, B. Tadesse, G. Demisie, G. Bogale, D. Wegary and B.M. Prasanna (eds.). Meeting the challenges of global climate change and food security through innovative maize research. Proceedings of the 3rd National Maize Workshop of Ethiopia.18-20 April 2011. Addis Ababa, Ethiopia.

The Maize Trust. 2016. Prospectus on the South African maize industry. The Maize Trust, Pretoria, South Africa. http://sacota.co.za (Accessed 11/18/2016).

CHAPTER 2

Literature Review

2.1 Introduction

In this literature chapter, progress in developing drought and low nitrogen (low N) tolerant cultivars is reviewed. The impact of drought and low N stress on maize production, mechanism of drought and low N tolerance, principles underlying breeding for drought and low N tolerance in maize are also reviewed. The phenomenon of heterosis, heritability and combining ability are discussed in relation to breeding for stress tolerance. The section further highlights the mode of gene action involved in the inheritance of grain yield under stress and non-stress environments, and the significance of genotype by environment interaction in breeding for stress tolerance. Major approaches to constitute heterotic groups in maize and identification of suitable testers for use under stress and non-stress environments are also reviewed. This chapter therefore creates a frame of reference for the entire study.

2.2 The concept of drought

Drought can be defined as any duration without rainfall, which is long enough to reduce plant growth (Njoroge *et al.*, 1997). The effect occurs when available soil water fails to meet the plant’s transpiration demand for a reasonable period during growth. The concepts can be partitioned into meteorological, agricultural and hydrological drought (Blum, 2011). Meteorological drought occurs when precipitation falls below average over a large area for a long period. Agricultural drought is brought about when there is insufficient moisture for maximum or potential growth of crops, this type of drought can be expressed on a very wide range of plant growth reductions up to complete crop failure, but small reductions when mild (Blum, 2011). Hydrological drought occurs when the level of precipitation or available water reserves is significant and severe enough to reduce crop yields below average. Thus, the rainfall, soil water storage capacity, potential evapotranspiration, crop phenology and crop development stages must all be considered when assessing the impact of drought on production (Njoroge *et al.*, 1997).

2.3 Mechanism of drought and low nitrogen tolerance in maize

Resistance to drought is achieved through drought escape or drought tolerance depending on the type of crop (Levitt's, 1980) as cited by Baker (1989). Drought escape is achieved through early maturation. Early maturing crops or cultivars complete their cycle quickly to avoid the onset of severe water stress. Anthesis date is used to distinguish drought escaping genotypes from drought-tolerant ones (Banziger *et al.*, 2000). Edmeades *et al.* (1997) defined drought tolerance as the ability to produce relatively high grain yield potential despite showing symptoms of water deficiency. Yield potential is defined as the maximum production when the cultivar is grown in a favorable environment where it is completely adapted, with no limiting factors such as mineral nutrients, water, weeds and pests (Evans and Fisher, 1999). Drought tolerance is achieved through postponement of dehydration by maintaining water uptake and minimising water loss and through desiccation tolerance by osmotic adjustment, increased root depth and density. Plants also respond to stress through expression of stress-responsive genes. Phytohormones are also used in the stress response; for example, the stress tolerant genes are activated by the stress hormone abscisic acid (ABA) produced in the root tip (Xiong *et al*., 2006). When plants are subjected to abiotic stress, the biosynthesis of ABA is activated. When ABA is synthesised, the ion channel is regulated, ABA is then transferred to the shoots to subsequently reduce stomatal opening and therefore plant transpiration rate is lowered (Baker, 1989).

2.3.1 Early maturity as a drought escape mechanism

Most maize breeding programs are aimed to partially overcome the effects of drought by developing early maturing maize cultivars that escape drought. Earliness, for example, allows the crop to avoid terminal drought by completing its life cycle within a given season length, and may also allow the crop to avoid coincidence between flowering and a mid-season dry spell (Edmeades *et al.*, 1997). However, early maturing cultivars have associated with yield penalties especially when rainfall is above average (Banziger *et al*., 2000). Shorter growing seasons generally reduce leaf area development, which in turn limits the amount of captured radiation and subsequently accumulation of photosynthetic products required for full grain filling; as a result, earliness is linked with undesirable characters including small cob size and lower yields (Banziger *et al.*, 2000). Moreover, early maturing cultivars have been observed to be very sensitive to early-season moisture stress, which consequently leads to lower yields.

Weber *et al*. (2012) evaluated the late and early maturing maize cultivars under drought stress and observed that the early maturing cultivars had higher yields than the late maturing group under drought stress. In the study by Bello *et al.* (2012), it was observed that late/intermediate maturing cultivars were higher in plant and ear heights and higher yielding, compared to the early maturing group. It has been explained that the higher yielding late maturing cultivars have an extended time for accumulating and utilising photosynthetic products during grain filling, which enable for a long duration in metabolic transformation into grain yield and stover (Bello *et al.*, 2012). Therefore, earliness generally limits yield potential of cultivars. However, since early maturing cultivars carry an effective drought avoidance strategy by enabling plants to complete flowering prior to the onset of drought, most farmers generally prefer early maturing cultivars that combine drought tolerance and high yield potential (Cairns *et al.*, 2013; Derera, 2005). When breeding for drought stress, earliness should not be considered solely as a primary attribute, the emphasis should also be focused on high yield potential under stress conditions.

2.4 **The role of nitrogen and maize response to low nitrogen stress**

Nitrogen (N) is the most essential element in crop species, various biochemical processes that occur in plants require N. Several organic compounds including amino acids, proteins, enzymes and chlorophyll are constituted from N (Below, 1997). Approximately 50% of all leaf N is directly involved in photosynthesis either as enzymes or as chlorophyll (Banziger *et al.*, 2000). According to Banziger *et al.* (2000), about 16 kg N are required in the grain for every maize tonne to be produced. Therefore, N limitation during maize growth and development has a large effect on the final yield. Nitrogen stress that occurs before flowering affects leaf development by reducing the leaf surface area and sink size, as a result, the rate of photosynthesis is reduced (Banziger *et al*., 2000). Nitrogen stress also accelerates early leaf senescence, this therefore decreases the size of sink by reducing the supply of assimilates to the ear, which enhances kernel and ear abortion (Banziger *et al.*, 2000). Nitrogen stress thus interferes with final grain yield mainly by altering the number of kernels; cultivars that are efficient in using available soil N are therefore desired.

2.5 **Maize response to drought stress**

Drought can affect maize at all growth stages; however, sensitivity to drought stress differs with stages. The maize crop is more sensitive to drought during flowering particularly when drought stress occurs between 7-10 days prior flowering (Banziger *et al.*, 2000). Figure 2.1 provides a summary of estimate of water use in inches per day from vegetative (V) to reproductive (R) growth stages and the effect of moisture stress at different growth stages. The susceptibility of maize at flowering is due to the separation of male and female inflorescence by approximately 1 m (Edmeades *et al.*, 1993). The male inflorescence and male gametophytes appear to be less affected under drought stress than the female (Edmeades *et al.*, 1993).

Silking is an extreme style elongation process dependent on high water potential, therefore under drought conditions, silk emergence delays as a result of a reduced proportion of assimilates partitioned to the ear than to the tassel (Edmeades *et al.,* 1993). Schussler and Westgate (1995) as cited by Banziger *et al*. (2000) stated that silk growth and the number of kernels is directly dependent on the flow of photosynthetic products during the first three weeks prior flowering, therefore, the reduced leaf area and the rate of photosynthesis due to drought during pre-flowering period delays silk growth. Silking is also more susceptible because in maize, female florets develop at the same time and are borne on a single ear on a single stem (Banziger *et al.*, 2000). The Anthesis-to-silking interval (ASI) thus becomes large and significant, leading to reduced pollen availability; high synchrony of silking; anthesis is therefore critical for yield under drought stress.

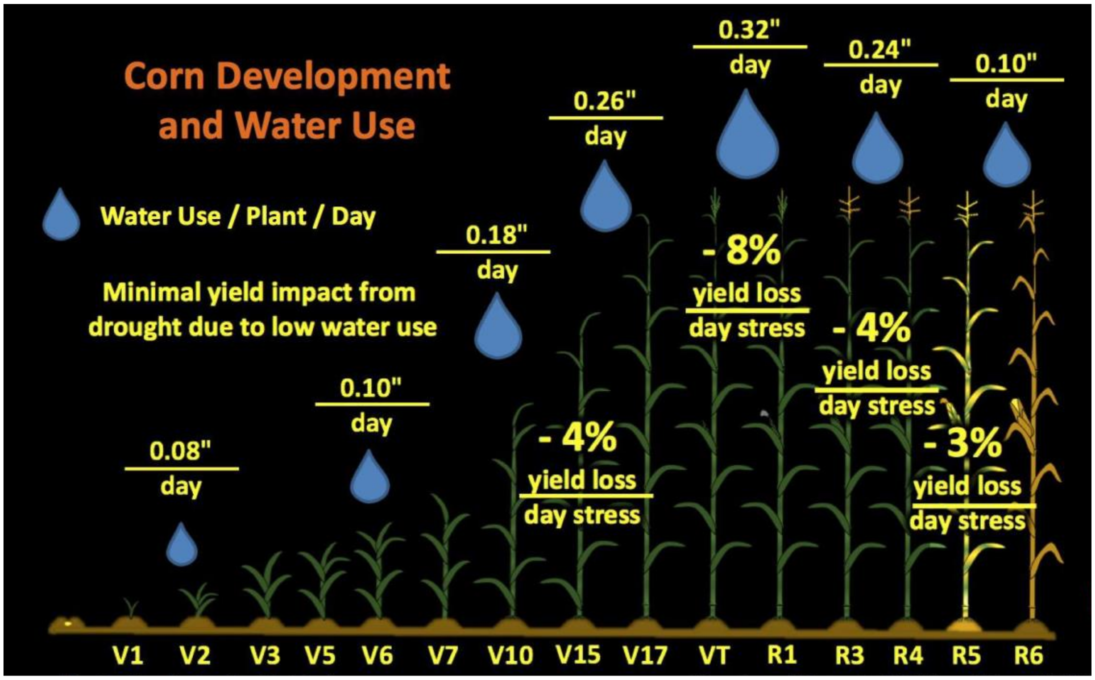

Figure 2.1: Effects of drought on maize at different growth stages (adapted from Shaw 1988; Rhoads and Bennett, 1990)

In addition, the exposure of both silks and pollen to drought leads to reduced number of fertilisation occurrences because of dehydrated silks which are not supportive for pollen tube development, as a result proper pollination is hampered resulting in reduced productivity (Khalil *et al*., 2013). The effect of drought on newly fertilised ovaries leads to embryo (kernel) abortion shortly after fertilisation (Edmeades *et al*., 1992). This occurs as a result of the inhibition of the photosynthesis rate by drought stress, and hence subsequent reduction in assimilates accumulation. Kernel abortion therefore occurs due to insufficient provision of photosynthetic assimilates mainly sucrose to the developing kernels (Aslam *et al.*, 2015). This may therefore lead to reduced kernel size, number of kernels per ear or complete barrenness (Edmeades *et al.*, 1992). The duration of drought stress determines the rate and success of grain filling which is the key component of seed yield. Overall, maize plants under drought stress generally show symptoms of stunted growth, wilting, rolling of the lower leaves, early leaf senescence, top-firing, tassel blast, silk delay, poor seed set, barrenness and eventually low grain yield (Banziger *et al*., 2000).

2.6 **Grain yield reduction due to drought and low nitrogen stress**

Drought and low N are the major constraints to maize production in sub-Saharan Africa. Massive losses due to drought and low N stress are outlined in the literature. Banziger *et al.* (2000) reported that yield reductions of 15-20% can be observed under moderate drought stress conditions and 25-35% under low N stress conditions. Betran *et al.* (2003) reported high reductions of up to 65% in maize hybrids under low N, 13% and 50% under intermediate and severe drought stress, respectively; a different site resulted in 5% and 48% yield reductions under mild and severe drought stress. Yield losses due to drought reported by Ziyomo and Bernardo (2012) were 48%. Ndlela (2012) observed 61% yield losses under drought stress and 15% reductions under low N stress. Some studies reported greater than 50% grain yield losses due to drought (Ahamed *et al.*, 2015; Cairns *et al.*, 2013) and low N (Meseka *et al.*, 2013), with mean grain yield of 1.70 t ha^{-1} under drought (Cairns *et al.*, 2013) and 1.5 -3.5 t ha^{-1} under low N (Banziger *et al.*, 2004). Ertiro *et al.* (2017a) observed 50% and 69% yield reductions in maize hybrids due to drought and low N, respectively. The reported variations in maize grain yield reduction under low N and drought stress suggest that yield losses due to drought and low N stress are influenced by the hybrids evaluated, test environments used, duration and intensity of stress.

2.7 **Phenotyping for drought and low nitrogen stress**

Drought experiments are conducted during rain-free periods while low N stress experiments are conducted in fields previously depleted of N for several years. The phenotyping protocol implemented by the CIMMYT maize breeding program (Banziger *et al.*, 2000) suggests that stress should be managed such that grain yield under managed low N stress is reduced by 25 - 35% of yield obtained under well-fertilised environments. Under managed drought, the stress should at least reduce yields to about 15 - 20% of yields obtained under well-watered conditions. The common strategies proposed by CIMMYT to manage N stress are (i) using fields that are naturally depleted of N such as those with sandy soil texture, however no factors should be limiting apart from the targeted N, (ii) recurrently using the same low N field and (iii) growing non-leguminous crops with a high biomass production or at high plant density such as wheat and oats in the previous season and removing all stover biomass after harvest. It is also proposed that where yields are expected to fall below 20% of well-fertilised yields, supplementary N fertiliser of no more than 20 kg N ha^{-1} may be applied, with additional fertiliser applied during crop development.

Managed drought stress trials should be carried out during the dry or rain-free season. Full irrigation is applied at regular intervals for germination and crop establishment; stress is then imposed by withholding irrigation from 2 weeks before flowering begins with no irrigation applied during flowering. However, after flowering stress, additional irrigation is applied to ensure adequate grain filling. Application of additional irrigation after flowering stress is determined by four scenarios based on the average anthesis-silking interval (ASI) of the block under managed drought stress. These are (i) if the average ASI is less than 3 days, no further irrigation is applied after flowering, (ii) if the average ASI ranges between 3 - 5 days, irrigation is applied two weeks after the end of male flowering, (iii) if the average ASI is 5 - 8 days, irrigation is applied once, one week after the end of male flowering and (iv) if the average ASI exceeds 8 days, irrigation is applied at the end of male flowering from 80 - 100% of the plots (Banziger *et al.*, 2000). In general, some cultivars tend to mature earlier than others, irrigation therefore has to be stopped early for the early maturing group than the late; maturity grouping is therefore crucial. Furthermore, when different drought experiments are undertaken in one block where all experiments will be subjected to one stress treatment, experiments should be grouped to ensure that flowering time coincides for all experiments under stress. This can be successfully achieved by for example, where there are early and late maturing groups, the early maturing trials should be planted later or otherwise different experiments by maturity should be conducted in different blocks with different stress management schedules (Banziger *et al.*, 2000).

2.8 Breeding strategy for drought and low nitrogen tolerance in maize

2.8.1 Usefulness of secondary traits in identifying drought and low nitrogen tolerance

Drought and low N tolerance are complex responses that are mostly accompanied by various interactive traits that are linked to stress tolerance. Grain yield is also a complex quantitative trait influenced by several interactive traits, thus making the process of selecting for stress tolerance genetically difficult (Dass *et al*., 1996). Use of adaptive traits which are secondary to the primary trait, grain yield is therefore emphasised when improving cultivars for stress tolerance. Compared to measuring only grain yield, secondary traits improve the precision with which drought or low N tolerant genotypes are identified (Banziger *et al.*, 2000). The genetic correlation between grain yield and most secondary traits also increases under stress conditions (Banziger *et al.*, 2000). Many secondary traits related to drought and low N tolerance have been proposed. Secondary traits examined at CIMMYT for drought tolerance can be listed in the order of their utility as ears per plant > anthesis-silking interval (ASI) > leaf senescence > tassel size > leaf rolling. Traits that are useful in identifying low N tolerance include ears per plant, leaf senescense and anthesis-silking interval (Banziger *et al*., 2000). Several studies suggest that selecting for a combination of these traits for example, a shortened anthesis-silking interval, reduced barrenness and delayed leaf senescence, in addition to yield should result in rapid yield improvements and yield stability under drought and low N conditions than selection on the basis of yield alone (Lafitte and Banziger, 1997).

A characteristic of maize mainly under drought stress is a delay in silking resulting in an increase in the ASI, incomplete to no fertilization, and subsequently decreased to no grain formation (Magorokosho *et al*., 2003). Anthesis-silking interval has been reported to be the most valuable diagnostic trait for cultivar performance under stress environments; this is due to the fact that ASI is largely independent of maturity differences among cultivars (Abdalla *et al*., 2010). Ziyomo and Bernardo (2012) also reported the strong association between grain yield and ASI under drought environments, which was also consistent with many previous findings (Banziger *et al*., 2000; Bolanos and Edmeades, 1996; Edmeades *et al*., 1993). This therefore underscores the reliability of ASI as an indicator of stress tolerance. Selecting cultivars with reduced ASI under stress environments is a routinely used breeding strategy in most programs. A strong correlation between grain yield and other secondary traits including plant height, leaf chlorophyll content and leaf senescence under drought conditions has also been previously reported (Ziyomo and Bernardo, 2012). However, to increase selection efficiency and to achieve a greater breeding progress in a drought or low N breeding program, Edmeades *et al.* (1998) proposed that secondary traits should be (i) genetically variable and genetically associated with grain yield in the target stress environment, (ii) highly heritable and stable within the measurement period, (iii) easy and cheap to measure, (iv) observed at or before flowering so that desirable parents can be selected for crossing, (v) provide an estimate of yield potential reliably before final harvest and (vi) not be associated with a yield penalty under non-stress conditions.

2.8.2 Relationship among stress and non-stress environments

Most farmers' fields are frequently subjected to a combination of stresses. Understanding the relationships among stress and non-stress environments, and their genetic basis would aid the development of effective breeding strategies to improve maize genotypes for multiple stress tolerance. Gains in yield under low N due to selection for drought tolerance has been reported (Ertiro *et al.*, 2017a; Lafitte and Banziger, 1997; Zambezi and Mwambula, 1997). Lafitte and Banziger (1997) found that maize populations improved through recurrent selection under drought combined tolerance to both drought and low N, this was observed by reduced barrenness and delayed leaf senescence under low N when cultivars selected under drought stress were used. Meseka *et al.* (2013) also found that improvement of drought tolerance in a maize population resulted in improved performance under low N stress when drought tolerant inbred lines were used in hybrid development. Meseka *et al*. (2013) thus postulated that these observations were an indication that drought tolerant inbred lines have sufficient variability that may be exploited to improve cultivar performance under both drought and low N conditions. Early investigations by Banziger *et al.* (1999) also found highly correlated responses to selection under drought for response under low-N stress, suggesting that there is a common adaptive mechanism involved for tolerance to either stress.

In breeding for drought tolerance in maize, concerns have been raised regarding selection for germplasm that performs well under drought but carries a yield penalty in well-watered conditions (Bolanos and Edmeades, 1996). Banziger *et al*. (1997) observed that the correlation between grain yield under abiotic stress and non-stress conditions generally decreases as the level of stress increases, as a result of increasing differences in mean yield between stress and non-stress environments. Cairns *et al.* (2013) reported a moderate positive genetic correlation between grain yield under well-watered with grain yield under drought stress, but correlations were not sufficient under well-watered conditions to be predictive under stress. According to Cairns *et al.* (2013), the independent genetic control could indicate that quantitative trait locus (QTLs) with large effects are involved under stress tolerance. Miti (2007) also reported low genetic correlations between optimum and drought (0.03) and a correlation of 0.45 between optimum and low N, indicating that indirect selection under optimum environments would not be effective in improving performance under stress environments. Weber *et al.* (2012) reported a strong correlation (0.83 - 0.86) between random abiotic stress and optimal conditions. Falconer and Mackay (1996) as cited by Weber *et al*. (2012) elaborated that when the same selection intensity is assumed under both stress and non-stress environments, the efficiency of indirect selection for grain yield under stress is a function of the broad sense heritability under stress and non-stress environments and high genetic correlation between yield under stress and non-stress environments. Therefore, to achieve high efficiency of indirect than direct selection, broad sense heritability (H) should be higher in the indirect test environment, and additionally, genetic correlation between the indirect and direct selection environment should be large.

Ertiro *et al*. (2017a) reported a correlation of 0.64 between optimum and low nitrogen, 0.55 between optimum and drought stress and a low correlation (0.30) between low nitrogen and drought stress. The study showed that even though some higher yielding cultivars carry yield penalties under non-stress environments, it is possible to obtain high yielding hybrids under stress environments with no yield penalties when conditions are optimal. The identified good hybrids under multiple stress suggest that tolerance to drought is also translated into spillover effects under low N and drought conditions. The success observed in obtaining both drought and low nitrogen tolerant cultivars was attributed to several cycles of simultaneous selection for multiple stresses. Therefore, the different results of the studies mentioned on the efficiency of indirect selection under non-stress environments to improve performance under stress environments are possibly associated with the variations in grain yield reduction due to stress. Selection under both stress and non-stress conditions therefore may need to be considered jointly in breeding for stress tolerance to reduce yield penalties under non-stress conditions.

2.9 Advances and gains in breeding for drought and low nitrogen stress

Substantial advances have been made by CIMMYT in collaboration with the National Agricultural Research Systems in breeding maize germplasm with tolerance to drought and improved nitrogen use efficiency (Banziger *et al.*, 2000), for deployment to smallholder farmers in Southern Africa. The success has been achieved through the private-public partnership collaboration in the Water Efficient Maize for Africa (WEMA), Improved Maize for African Soils (IMAS), and Drought Tolerant Maize for Africa (DTMA) projects (Setimela *et al*., 2017). The process has been accelerated by combining conventional breeding methods with modern breeding techniques (Beyene *et al.*, 2016). Yields of new cultivars are superior to the commercial maize cultivars under both stress and non-stress environments; most cultivars also confer multiple tolerance to both drought and low N stress. The DTMA project has released 160 drought tolerant maize cultivars between 2007 and 2013, which out yielded the commercial cultivars by an average of 25 - 56%, 24 - 47%, 83 - 137% under optimal rainfed conditions, random and managed drought stress, respectively (Fisher *et al.*, 2015; Setimela *et al*., 2017). The WEMA project in South Africa has developed 17 hybrids from 2008 to 2017 (unpublished data). These include five transgenic drought tolerant hybrids with additional protection to insect damage and an average yield of 8.7 t h^{-1} and 5.2 t h^{-1} under well-watered and drought conditions, respectively. These hybrids also exhibit tolerance to low N stress and ear rots. The conventional hybrids have an average yield of 11.9 t h^{-1}, 5.9 t h^{-1}, 2.0 t h^{-1} and 1.9 t h^{-1} under non-stress, random drought, managed drought and low N stress, respectively. All hybrids also show superior agronomic characters such as good standability and ear formation. The IMAS project registered and released eight superior hybrids between 2010 and 2016, with mean grain yields of 11.8 t h^{-1}, 1.6 t h^{-1}, 5.7 t h^{-1} and 2.0 t h^{-1} under non-stress, low N, random drought and managed drought stress, respectively (unpublished data).

Due to the polygenic inheritance and therefore complexity of grain yield and related traits, breeding for these stresses has proven to be difficult. The traits associated with tolerance are usually constitutive, and the whole-plant response to stress is complex because it is determined by interactive traits and differences in response of genotypes (Witcombe *et al.*, 2008). Different cultivars thus perform differently in specific environments due to genetic differences, intensity and duration of stress. Apart from the WEMA and IMAS partnerships, studies documented on breeding for drought and low N tolerance in South Africa are scanty.

2.10 **Heritability**

Heritability is a measure of the proportion of the phenotypic variance that is a result of genetic factors (Hartl and Jones, 1997). Heritability is partitioned into the broad sense (H) and narrow sense (h^2) heritability. Broad sense heritability is the ratio of total genetic variance to total phenotypic variance, whereas narrow sense heritability is the ratio of additive (transmissible) variance to the total phenotypic variance (Acquaah, 2007). The difference between h^2 and H is that H includes all of the genetic contributions to the variation, whereas h^2 includes only the additive effects of alleles (Hartl and Jones, 1997). Additive component of genetic variance determines the response to selection, therefore h^2 is generally more useful than H because it is a reliable measure based on breeding value and thus, can be used to predict changes in population mean with individual selection (Acquaah, 2007). Heritability estimates serve as a useful guide to the breeder because it gives an idea about the response of various characters to selection pressure, through whether the proportion of variation is due to genotypic or additive effects. Heritability is therefore useful in predicting the effectiveness of selection and hence, trait and population improvement (Hartl and Jones, 1997). For low heritable traits with h^2 below 0.15, the individual performance is of low breeding value, individuals with best genes for the trait cannot be easily identified as compared to moderate (h^2 = 0.15 to 0.40) to high (h^2 > 0.40) heritable traits (Virginia Tech, 2009).

2.10.1 Heritability under stress and non-stress environments

Differences between genotypes are usually smaller under stress conditions, and superior genotypes are therefore more difficult to detect (Banziger *et al.*, 2000), therefore, heritability of grain yield is relatively low under stress environments whereas the heritability of some secondary traits remains high. Conversely, under non-stress environments differences between individuals are large and therefore high heritability for grain yield (Banziger *et al.*, 2000). Variable ranges of heritability for grain yield under stress and non-stress conditions are reported in the literature. Zimoyo and Bernardo (2012) evaluated testcrosses of 238 recombinant inbreds; the observed h^2 for grain yield was 0.37 under managed drought and 0.60 under optimum moisture conditions. The decrease in heritability for grain yield under drought corresponded to the decrease in mean grain yields, other traits including plant height and stalk lodging also showed high heritability under non-stress than drought conditions. Heritability and genetic variance for ASI and leaf senescence was high under drought stress underlining the usefulness of these secondary traits in drought tolerance. Cairns *et al.* (2013) reported grain yield H estimates of 0.84 under well-watered conditions and 0.64 under drought stress.

Derera (2005) reported a decline in grain yield heritability from 60% under optimum to 19% under drought conditions, while heritability for other traits including ASI remained moderate to high (32 to 49%) under drought stress. In the study by Miti (2007) the heritability for grain yield under low N and drought stress was 0.38 and 0.17, respectively, whereas under optimum the heritability was 0.45. Weber *et al.* (2012) studied the performance of early and late maturing cultivars under managed drought, low N, optimum and random abiotic stress conditions. Heritability was the highest (0.65 - 0.85) under optimum, this was attributed to high genetic variance. Under managed low N stress, heritability ranged from 0.49 - 0.60, and under managed drought and random abiotic stress, heritability was 0.49 - 0.52 and 0.38 - 0.49, respectively. Ertiro *et al.* (2017a) evaluated 49 three-way cross maize hybrids under stress and non-stress environments. They reported heritability of 0.73 under optimum, 0.64 for low nitrogen and 0.54 for drought stress. High heritability observed under optimum environments was linked to high genotypic variances. However, in general, stress reduces genetic variances, increases error and genotype by environment interaction, which consequently leads to reduced heritability under drought environments (Ertiro *et al.*, 2017a). A decline in heritability under drought stress has been reported in other crops such as rice (Joseph, 2015; Sellammal *et al.*, 2014) and wheat (Eid, 2009; Hassan *et al.*, 2016). Under non-stress environments, high heritability is due to the unlimited expression of the additive variation, however under stress environments, low heritability is explained by a drastic increase in environmental variance due to stress, which also leads to high individual differences (Bubliy *et al.*, 2001). Grain yield is a complex trait controlled by polygenes (Badu-Apraku *et al.*, 2010) and therefore, its heritability is low under stress environments. Improvement of grain yield through direct selection is therefore considered inefficient, but use of secondary traits that are strongly correlated with grain yield is always recommended.

2.11 Heterosis in maize under stress and non-stress environments

The knowledge of heterosis helps in the identification of superior F_1 hybrids with potential for improving yields. Heterosis (hybrid vigor) is defined based on hybrid development, as the superiority of F_1 hybrid performance relative to that of the parents (Shull, 1952). Heterosis is explained by dominance (Davenport, 1908) and over-dominance (Shull, 1908) hypotheses. Heterosis under the dominance hypothesis is due to the masking of deleterious recessive alleles in a heterozygote by dominant alleles (Acquaah, 2007). Dominant genes in both parents thus complement each other in the hybrid, masking recessive, deleterious alleles to give a superior hybrid (Springer and Stupar, 2007). The over-dominance heterosis is due to the superiority of a heterozygote over either homozygote; heterosis is therefore proportional to the amount of heterozygosity (Acquaah, 2007). Estimates of heterosis can be based on (i) mid-parent heterosis, which is the superiority of the F_1 over the mean of the parents, (ii) better-parent heterosis calculated as the degree by which the F_1 mean exceeds the better parent in the cross and (iii) standard heterosis, defined as the superiority of the F_1 over the standard commercial check (Narayanam and Phundan, 1993). The standard heterosis has been widely accepted as the most practical and economic approach in estimating gains in a breeding program. Heterosis has been widely used in various crops to obtain steady increases in crop yields (Chigeza, 2013; Makanda *et al.*, 2009; Thomas *et al.*, 2017; Tiwari *et al.*, 2011).

Studies in the literature on maize improvement for abiotic stress tolerance report high heterosis for grain yield under drought (Makumbi *et al.*, 2011) and low N environments (Betran *et al.*, 2003b), than non-stress environments. However, the focus is on the estimates of mid-parent and better parent heterosis. Heterosis is expressed depending on the divergence and gene frequency of the parental lines (Hallauer and Miranda, 1988a). High heterosis is important for stress tolerance (Blum, 1997). However, its expression is independent of the growing conditions (Blum, 1997). Blum (1997) postulated that heterosis is a constitutive trait that does not require either stress or non-stress to be fully expressed. Thus, irrespective of the test environments, expression of heterosis is determined by the presence of genes that are expressed in hybrids. Grain yield and associated traits in maize generally show heterosis with significant variable levels in hybrids (Amiruzzaman *et al.*, 2011; Kambe-Gouda *et al.*, 2013; Uddin *et al.*, 2006; Xia *et al.*, 2005). According to Springer and Stupar (2007), these variations imply that heterotic response is not controlled by the same set of genes or by the overall genetic diversity within a hybrid; however it results from diversity at specific and important genes controlling different traits. Therefore, the magnitude of overall heterosis that might be present in a hybrid cannot be easily quantified, but maximum heterosis always occurs in crosses involving divergent parents (Hallauer and Miranda, 1988a).

Estimates based on standard heterosis in maize are rarely reported in the literature (Kumar *et al.*, 2014; Ulaganathan *et al.*, 2015). When new varieties are developed, every breeding program targets new varieties that will compete favourably with the commercial checks and give high yield advantage. Standard heterosis is therefore the most practical and economic heterosis for isolation of superior crosses. Estimates based on mid and better-parent heterosis have limitations because the estimation requires information on the perfomance of inbred lines. Inclusion of inbred lines in the experimental trials may not be always feasible especially when the number of test crosses under evaluation is too large; the application of these estimates is also practically irrelevant because farmers require hybrids instead of inbred lines (Chigeza, 2013). These estimates therefore have relatively little economic importance. The standard heterosis is the most practical estimate in plant breeding programs (Parvez, 2006). Direct comparisons of yield and associated traits of new hybrids with commercial checks provide better information about the merit of recent varieties and therefore, whether they should be considered for further testing in multi-environments.

2.12 Heterotic groups and heterotic patterns

2.12.1 Significance of heterotic grouping in maize

A heterotic group is defined as a group of related or unrelated genotypes from the same or different populations, which show similar combining ability and heterotic response when crossed with genotypes from other genetically different groups (Melchinger and Gumber, 1998). Heterotic pattern is when a cross between a pair of two heterotic groups comprising known genotypes expresses a high level of heterosis as a result of high genetic divergence and different allele frequency (Carena and Hallauer, 2001) as cited by Barata and Carena (2006). Sprague (1984) as cited by Reif *et al.* (2005) stated that “the single most important element of a breeding program is the recognition and utilisation of heterotic pattern, this recognition both simplifies and increases the efficiency of all subsequent operations”. Heterotic groups are therefore considered a backbone in plant breeding programs.

Classification of germplasm into defined heterotic groups allows breeders to obtain good results within a short timeline. This is achieved by focusing only on combinations from divergent heterotic groups, which increases the probability of obtaining superior crosses and subsequently maximum heterosis and high breeding efficiency (Melchinger and Gumber, 1998). Heterotic groups therefore reduce the number of undesirable cross combinations among intra-groups, and simplify germplasm management and improvement (Fan *et al.*, 2003). However, according to Fan *et al.* (2009), it may be possible to obtain superior crosses from cross combinations made within heterotic groups, because lines in the same heterotic group may have small differences in their alleles, which may lead to differences in their heterotic pattern. However, to obtain a large number of superior hybrids, breeders must have a good heterotic group classification, defined as “one whose classified heterotic group allow inter heterotic crosses to produce more superior hybrids than the within group crosses” (Fan *et al.*, 2009).

2.12.2 Classification of lines into heterotic groups

Available approaches for heterotic groupings include (i) pedigree analysis (Barata and Carena, 2006; Semagn *et al.*, 2012; Srdic *et al.*, 2007), classification by pedigree is considered the most reliable, however, where pedigree data is not clear or insufficient, classification of some lines may not be possible (Fan *et al.*, 2003). The second method is the use of molecular markers, which is based on analysing genetic markers and clustering inbred lines based on genetic distances; this method eases the process of identifying homozygous and heterozygous genotypes (Adetimirin *et al.*, 2008). However, for efficiency, the number of markers used should be sufficient and polymorphic (Altman and Hasegawa, 2012). Molecular markers also detect the level of genetic diversity present in the germplasm used (Adetimirin *et al.*, 2008; Aslam *et al.*, 2009; Badu-Apraku *et al.*, 2013; de Pinto *et al.*, 2003; Dhliwayo *et al.*, 2009; Nyombayire *et al.*, 2016; Qi-Lun *et al.*, 2008; Suwarno *et al.*, 2014). Another approach is heterotic grouping based on quantitative genetics; the quantitative genetics method uses heterotic performance and variance of F_1 hybrids. Classification based on specific combining ability (SCA) effects of lines is the key principle for this method, and has been used extensively in several studies (Barata and Carena, 2006; Fan *et al.*, 2003; Fan *et al.*, 2004; Fan *et al.*, 2010; Librando and Magulama, 2008; Menkir *et al.*, 2004; Parentoni *et al.*, 2001; Rajendran *et al.*, 2014; Reif *et al.*, 2005; Vasal *et al.*, 1992).

Vasal *et al.* (1992) described the procedure for grouping based on SCA effects. Lines are classified based on the direction (positive or negative) of the SCA value between two lines or between a line and tester. Lines that show negative SCA effects for grain yield when crossed together belong to the same heterotic group, these lines are expected to have similar gene frequency, whereas a positive SCA effect indicate opposite heterotic groups. The criterion thus uses the assumption that the SCA of two lines from different heterotic groups is always superior than that of lines from the same heterotic group (Fan *et al.*, 2003). Thus, when lines from the same heterotic group are mated, the SCA effects become negative (Bidhendi *et al.*, 2012). For example, the CIMMYT maize breeding program uses the A, B or AB heterotic grouping systems. In this regard, when A and B heterotic group testers are used, lines displaying negative combining ability with one tester are allocated into one heterotic group with a respective tester, or similarly; lines showing positive combining ability with one tester are allocated to the opposite tester. Whereas lines displaying positive SCA with both A and B testers are allocated to heterotic group AB and those showing negative SCA with both testers, do not belong to any heterotic group and are therefore not classified (Vasal *et al.*, 1992). The SCA effects generally show high predictive values for F_1 grain yield than heterosis (Betran *et al.*, 2003b), it is therefore regarded as the main genetic statistic for classifying maize germplasm into heterotic groups (Fan *et al.*, 2014). Another method proposed by Fan *et al.* (2008) as the most appropriate and effective approach for heterotic grouping is the heterotic groups' specific and general combining ability (HSGCA); this method combines both SCA and GCA effects.

Among various methods, classification by SCA effects, molecular markers and pedigree analysis has been widely utilised in maize heterotic groups (Aguiar *et al.*, 2008; Badu-Apraku *et al.*, 2013; Barata and Carena, 2006; de Pinto *et al.*, 2003; Fan *et al.*, 2003; Fan *et al.*, 2004; Menkir *et al.*, 2004; Parentoni *et al.*, 2001; Rajendran *et al.*, 2014). A line by tester method utilising a fewer number of divergent testers of known heterotic groups is the most practical method for determining heterotic groups and patterns of inbred lines (Hallauer and Miranda, 1988b). The line by tester procedure has been efficiently used in estimating combining ability, germplasm screening and classifying inbred lines into heterotic groups (Aguiar *et al.*, 2008; Fan *et al.*, 2010; Li *et al.*, 2007; Menkir *et al.*, 2003; Rajendran *et al.*, 2014; Vasal *et al.*, 1992), especially when the number of inbred lines to be classified is too large to be evaluated in diallel crosses (Fan *et al.*, 2010).

Different methods have been used to classify maize germplasm into heterotic groups and variable number of heterotic groups are reported. Delucchi *et al.* (2012) classified Argentine maize landraces into heterotic groups in testcrosses with five testers, representing three different heterotic groups, three heterotic groups were identified based on favorable interactions with the testers used. Vaz Patto *et al.* (2004) determined the heterotic groups of maize inbred lines using a line by tester mating scheme involving 50 inbred lines and two single-cross testers; some inbred lines were allocated to heterotic Group A, some to Group B, and others were allocated to both A and B; lines allocated to both A and B showed high heterosis for both testers. Fan *et al.* (2014) classified 12 maize inbred lines based on the SCA and grain yield data from a diallel design, three heterotic groups were identified. Fan *et al*. (2014) further observed that when inbred lines were fitted into two heterotic groups, approximately 20% of the best crosses were missed. Therefore, based on this study, the three heterotic group method is considered ideal for improving breeding efficiency (Fan *et al.*, 2008). From the observations of Fan *et al.* (2014), among the 12 inbred lines studied, some of the lines were not assigned into heterotic groups; this was attributed to the fact that heterotic groups are not naturally existing but defined by breeders. Therefore, it is always likely that there would be a difficultly in assigning some of the lines. However, because breeding efficiency is reduced with an increase in the number of intergroup crosses, resulting from many heterotic groups (Fan *et al.*, 2014; Tams *et al.*, 2006), defining the smallest number of heterotic groups is crucial for maximising breeding efficiency. The unclassified lines should therefore not be allocated into a new group but may be forced into the already defined groups that they may be more likely to conform.

The grain yield SCA effect has been used in several studies (Calvo and Magulama, 2010; Melani and Carena, 2005; Menkir *et al.*, 2004; Rajendran *et al.*, 2014). However, in some studies some lines that were previously classified into heterotic groups classified differently in another study (Badu-Apraku *et al.*, 2013; Fan *et al.*, 2008). This is due to that the SCA effects for grain yield are influenced by the interaction between two inbred lines, or the inbred line and a tester and between hybrids and environment (Fan *et al.*, 2008; Fan *et al.*, 2003). The drawback also with classification by either SCA or HSGCA is that they both use only grain yield data, which is a polygenic trait (Badu-Apraku *et al.*, 2013); this could also explain the inconsistencies in classification of the same inbred in different studies. Aguiar *et al.* (2008) also proposed that the effect of the combining ability of the tester(s) used to classify lines into heterotic groups should be considered because heterotic grouping based on the direct evaluation of each hybrid is tester-dependent. For example, when a group of testers from one heterotic group have low combining ability than the other group, most inbred lines are likely to be clustered in this group because of its low combining ability. This occurs as a result of the heterotic reaction between lines and testers (Dao *et al*., 2014), the choice of testers is therefore crucial. Hallauer and Carena (2009) thus emphasised that because heterotic groups are conceptual and not confined to a particular group or population, there will always be differences in classification of lines as a result of a particular hybrid combination. The identified heterotic groups should therefore, not be fixed or regarded as closed populations, but they must be constantly updated and extended through introduction of new lines (Delucchi *et al*., 2012). Rajendran *et al.* (2014) also suggested that updating the classification of lines is required because combining ability effects used in the allocation of lines into heterotic groups are specific to the parents under evaluation.

Some studies have used the molecular marker method alone to determine heterotic groups. Adetimirin *et al.* (2008) used Simple Sequence Repeat (SSR) markers and identified four heterotic groups from 72 maize inbred lines. In most studies, the molecular marker method has been used in conjunction with the other methods. Aguiar *et al.* (2008) used SSR markers and testcrosses from three testers to classify 16 inbred lines. Both methods grouped lines into two heterotic groups, however the groups obtained from SSR and testcrosses were distinct. Makumbi (2005) used three types of markers (SSR, restriction fragment length polymorphism and amplified fragment length polymorphism) and pedigree information to group 15 maize lines, the pooled data from all three types of markers revealed that clustering of most lines was in accordance with known pedigree data and origin. The consistency between molecular markers and other methods has also be reported in various studies (Badu-Apraku *et al.*, 2013; Fan *et al.*, 2003; Fan *et al.*, 2009; Parentoni *et al.*, 2001). Barata and Carena (2006) classified 13 inbred lines from diverse genetic backgrounds, the usefulness of markers in determining heterotic groups was observed. However, the grouping of some lines using SSR markers did not agree with the pedigree data, for example a few lines derived from the same population clustered differently; similar observations were also reported by Yu *et al.* (2001). Barata and Carena (2006) therefore, concluded that molecular markers are useful mainly for grouping unrelated lines, but when genetically similar germplasm is classified, accurate classifications are not obtained. This could be associated with a number of factors such as the presence of non-genetic variance, presence of residual heterozygosity in the original release of the inbred line, occasional mutation cases, and number of markers used (Nei, 1987; Tivang *et al*., 1994) as cited by Barata and Carena (2006). Fan *et al.* (2003) attributed the differences to the failure of primer loci of the markers to completely cover all genomes, and to that they were not all associated with loci that positively affect heterosis. In studies that did not involve use of markers in classification, it was also observed that lines originating from the same population do not always cluster together (Fan *et al.*, 2003). In this study the discrepancies were attributed to the fact that when lines used were initially grouped, grouping was mainly based on agronomic characteristics not heterosis, therefore, there could still be existing genetic differences within the same population, which result in different classifications.

2.12.3 Tropical and temperate testers in heterotic orientation

Various studies determining heterotic orientation based on SCA effects have employed either tropical (Aguiar *et al.*, 2008; Badu-Apraku *et al.*, 2011; Chandel *et al.*, 2014; Mwimali, 2014) or temperate testers (Barata and Carena, 2006; Bidhendi *et al.*, 2012; Delucchi *et al.*, 2012), depending on whether the breeding program is tropical or temperate-based. The principle used in SCA effects-based heterotic classification is that closely related lines show negative SCA effects for grain yield when crossed together, while positive SCA is an indication of opposite heterotic groups (Vasal *et al.*, 1992). It has been noted that heterotic orientation based on SCA effects is influenced by the combining ability of a tester; when two testers from opposite heterotic groups are used, most lines will tend to group with a tester with low combining ability (Aguiar *et al.*, 2008). The SCA effect is thus influenced by the interaction between two inbred lines and the environment in which hybrids are tested (Fan *et al.*, 2008). One inbred line may therefore classify differently in different studies depending on testers used (Badu-Apraku *et al.*, 2011).

With continuous germplasm improvement, the maize industry in most breeding programs have now been built on products that are derived from temperate and tropical germplasm. The combination of both has been utilised to enhance germplasm diversity and to improve performance and adaptation in hybrids (Adetimirin *et al.*, 2008; Hallauer and Carena, 2014). Within a germplasm collection, some inbred lines might be closer to the temperate or tropical material depending on the composition of the genome, this may also impact greatly on the levels of SCA effects observed, especially when only tropical or temperate testers are used to constitute heterotic groups. Studies that combine both tropical and temperate germplasm (although rare) have shown that in breeding programs that are composed of lines that were derived or introgressed with both tropical and temperate; use of temperate and tropical testers lead to the classification of lines based on relative composition of temperate or tropical germplasm in their genetic background (Adetimirin *et al.*, 2008). Lines that are genetically closer to tropical tester(s) tend to combine poorly with that group of testers, and are grouped together, and those that are more temperate than tropical will belong to a group of temperate lines. This was observed by Adetimirin *et al.* (2008) when some lines that were known to be temperate x tropical were aligned with tropical lines, because of the closest relationship with tropical than temperate material.

Furthermore, grouping of lines was confirmed by molecular marker- genotyping, where some lines clustered closest to the temperate set of testers, while others were more aligned towards the tropical set. Thus, in classifying germplasm with a history of both tropical and temperate introgression, or in cases where pedigree data is not readily available or insufficient, employment of both temperate and tropical testers will clearly discriminate temperate from tropical material. Further verification with molecular markers helps to augment pedigree data and to make conclusive decisions on the arrangement of germplasm. Among DNA markers, single nucleotide polymorphism (SNP) DNA markers have become more prominent because of their high polymorphism, flexibility, repeatability, abundance in the genomes, speed and cost-effectiveness (Thomson, 2014). Many SNPs markers, developed from the DNA sequence of known genes are now readily available in maize (Lu *et al.*, 2009).

2.13 Testers in maize breeding

A tester is a genotype that is used to identify superior germplasm based on the objectives of the breeder in a hybrid-oriented program (Hallauer and Miranda, 1988a). Testers have been used in maize breeding programs to evaluate lines for combining ability, to constitute heterotic groups and to identify superior hybrid combinations (Guimaraes *et al.*, 2012). Testers may include inbred lines, single-cross hybrids and heterogeneous material, which include open-pollinated varieties, synthetic cultivars or double cross hybrids (Acquaah, 2007). Inbred lines and single-cross hybrid testers are classified into narrow genetic base testers and the heterogeneous materials belong to the broad genetic base testers. Narrow genetic base testers are used to evaluate SCA, whereas, a broad genetic based tester is considered for GCA testing (Acquaah, 2007). However, the genetically broad-based testers have little contribution towards the line × tester interaction than narrow genetic base testers (Matzinger, 1953). The type of tester used is therefore fundamental in combining ability testing.

2.13.1 Identification of suitable testers

Maize breeders have to identify a good or convenient tester for hybrid development and evaluation of inbred lines. The definition and choice of an ideal tester depends upon the breeder’s objectives and the type of hybrids developed (Hallauer and Miranda, 1988a). Therefore, various definitions of a suitable tester are available in the literature. Allard (1960) defined an ideal tester as one that provides maximum information about the performance of a line in cross combinations under different environmental conditions. A good tester should allow the expression of greater genetic diversity among progenies or testcrosses (Russel, 1961). Abel and Pollak (1991) proposed that a good tester should be a good pollen donor to ease crossing with lines under evaluation, it should also be superior in agronomic traits such as root and stalk lodging. A good tester should be able to clearly classify germplasm into heterotic groups, therefore the tester’s genetic distance should be large and its grain yield should be different from the lines to be tested (Li *et al.*, 2007). One definition describes a good tester as a homozygous recessive line or a line with low allelic frequency, if such testers are used; lines displaying high frequency of favorable alleles can be easily identified (Hallauer and Miranda, 1988b; Smith, 1986). Hallauer (1975) coined that a suitable tester should include simplicity in use, ability to rank genotypes correctly and maximise genetic gain from selection. Matzinger (1953) described a convenient tester as one that includes simplicity in use, and also provide maximum information on the performance among inbred lines when tested in different combinations and environments. Rawlings and Thompson (1962) stated that a good tester should include the ability to discriminate among inbred lines and correctly classify the relative performance of lines. Castellanos *et al.* (1998) referred to an ideal tester as one showing positive estimates of GCA, large variation among testcrosses and acceptable *per se* performance. Therefore, additive genetic variance is emphasised during selection of a best tester (Rajendran *et al.*, 2014).

The initial tester is generally selected based on its proven performance in commercial hybrids (Acquaah, 2007). For example if the objective is to determine heterotic groups of lines with unknown origin, at least two testers from known heterotic groups are used. However, when classifying broad-based population into two heterotic groups, at least two elite lines from opposite heterotic groups may be used as testers (Acquaah, 2007). Inbred lines are identified as testers if the breeding program is focused on developing single-cross hybrids, if the aim is to develop three-way hybrids, single-cross hybrids with good combining ability are used as testers (Fasahat *et al.*, 2016). These single-cross testers usually result from two higher yielding sister lines of the same heterotic group (Fasahat *et al.*, 2016). The CIMMYT maize breeding program has identified common testers for evaluating combining ability of maize lines under non-stress, drought and low N stress conditions. These testers are widely used in most eastern and Southern African maize breeding programs. For example, CML312 and CML442 of heterotic group A, and CML444 and CML395 of heterotic group B have been used as inbred testers to develop single- cross hybrids, and to classify lines into heterotic groups, while single-cross testers mainly CML395/CML444 (B) and CML312/CML442 (A) are widely used in three-way hybrids.

The criterion based on good combining ability has been recently used to identify potential candidate testers for use under stress and non-stress environments (Ertiro *et al.*, 2017). Rajendran *et al.* (2014) identified a suitable tester based on high positive GCA effects, ability to classify lines into heterotic groups and good grain yield *per se.* Pswarayi and Vivek (2008) identified inbred and single-cross testers for use in developing early maturing single-cross and double-cross hybrids. The choice of potential lines as testers was based on display of good GCA effects for grain yield and other traits, classification of lines into heterotic groups and *per se* grain yield under different environments. Whereas, for potential single-cross testers, selection was based on good GCA effects of the inbred lines constituting the single-cross, grouping of the inbred lines constituting the single-cross to the same heterotic group, desirable *per se* performance of the inbred lines constituting the single-cross and good yield potential of the single-cross, for use as female parent in three-way and double-cross hybrids. Vasal *et al.* (1997) emphasised that an ideal tester must have high discriminating ability among genotypes for combining ability and desirable traits, simultaneously identify useful hybrid products for direct use, and must be compatible with a practical maize breeding program. Identified testers must therefore meet the objectives of a breeding program.

2.14 **Combining ability**

Combining ability is defined as the genotype's ability to combine and transmit superior characters to its crosses and thus produce superior hybrids (Sprague and Tatum, 1942). The concept is partitioned into general combining ability (GCA) and specific combining ability (SCA). General combining ability is the average performance of a line in a series of hybrid combinations, while SCA refers to those cases in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines involved (Sprague and Tatum, 1942). General combining ability indicates the presence of loci with additive effect, whereas SCA is due to non-additive or dominant genes (Falconer and Mackay, 1996). General combining ability estimates can be positive, negative or zero (Narayanam and Phundan, 1993). A zero or close to zero GCA indicates that the mean of a line is not different from the average mean of all crosses. Whereas a positive or negative GCA estimate suggests that the performance of a line is better or poorer than the other lines. A high GCA value thus shows that the parental mean is superior to the general mean, which indicates a desirable gene flow from parents to offspring (Fasahat *et al.*, 2016). Parents that would result in superior hybrids are therefore selected based on higher GCA estimates (Griffing, 1956). A high GCA estimate also indicates higher narrow sense heritability (Acquaah, 2007). However, for some traits, positive effects are not desired; for example, in traits which are indicators of the presence of diseases, positive effects for such traits would mean higher levels of disease susceptibility (Mulbah *et al.*, 2015), negative effects are also desirable for traits including days to flowering, anthesis-silking interval, plant height and ear height (Umar *et al.*, 2014).

A high SCA value mostly indicate superior crosses, hybrids are therefore selected based on the SCA effects (Griffing, 1956). Specific combining ability is therefore positively correlated with heterosis; thus, where there are no SCA estimates, heterosis may be used to select for superior crosses (Machado *et al.*, 2002). Specific combining ability indicates the presence of loci with dominance variance (non-additive effects) (Falconer and Mackay, 1996). The SCA thus statistically represents the interaction effect while GCA act as the main effect (Kulembeka *et al.*, 2012). If both GCA and SCA are non-significant, epistatic gene effects play a major role in the inheritance of such traits (Ahamed *et al.*, 2015). It has been observed in many studies that high SCA does not necessarily result from a combination of two superior parents (good general combiners). This was observed where some lines showed poor GCA effects, but when they were included in crosses, they produced high SCA effects, indicating the expression of good performance in their crosses (Bao *et al.*, 2009; Ejigu *et al.*, 2017; Ndhlela, 2012; Tyagi and Lal, 2005). High SCA effects may therefore result from crosses between (i) good GCA × good GCA, (ii) poor × poor GCA and (iii) good × poor GCA effects. High SCA effects resulting from crosses where both parents are good general combiners (good × good) may be ascribed to additive × additive gene action, the high SCA effects derived from crosses including poor x poor parents are attributed to dominance x dominance type of non-allelic gene action, which produces over-dominance in crosses, while a scenario where high SCA effects result from good x poor GCA effects may be ascribed to favorable additive effects of a good general combiner parent and non-additive effects of a poor general combiner (Fasahat *et al.*, 2016). These scenarios indicate that poor general combiners may possibly produce hybrids with high SCA effects for grain yield if the other parent in a cross is properly selected.

2.14.1 Techniques for estimation of combining ability

Combining ability information has been widely used to identify good parents that can accumulate superior genes in crosses in order to exploit maximum heterosis (Amiruzzaman *et al.*, 2010). Combining ability effects have been used also to identify heterotic pattern and heterotic groups, to understand the genetic architecture of different traits and to make inferences on the mode of gene action involved in the expression of quantitative traits (Amiruzzaman *et al.*, 2011; Bidhendi *et al.*, 2012; Galal and Mahgoub, 2011; Machikowa *et al.*, 2011). Available techniques to estimate combining ability effects include, the North Carolina mating design (Comstock *et al.*, 1949), the diallel mating design proposed by Griffing (1956) and the line by tester cross analysis (Kempthorne, 1957).

The line × tester is the most widely used mating design for hybrid development. Line x tester mating design involves crossing of 'l' lines to 't' testers to generate top crosses or testcrosses of full-sib progenies (Narayanam and Phundan, 1993). Lines and testers are different sets of genotypes, the tester acts as the common parent and is designated as a male and the lines are females. Compared to other designs, the line by tester is more flexible because it can accommodate large numbers of genotypes and is therefore appropriate for testing a large number of early generation lines (Fan *et al.*, 2010). The line by tester method has been used in many quantitative genetic studies in maize (Ertiro *et al.*, 2017; Hosana *et al.*, 2015; Meseka and Ishaaq, 2012; Narro *et al.*, 2003) and in other crops (Ahuja and Dhayal, 2007; Bao *et al.*, 2009; Fellahi *et al.*, 2013; Tyagi and Lal, 2005).

2.15 Gene action conditioning grain yield under drought and low nitrogen conditions

Gene action denotes how the inheritance of traits is affected through allelic interaction (Acquaah, 2007). The gene action influencing quantitative or polygenic traits may be classified as additive, non-additive (dominance) and epistasis (Acquaah, 2007). The effect of a gene is said to be additive when each additional gene enhances the expression of the trait by equal increments (Acquaah, 2007), the combined effects of genetic alleles at two or more gene loci are therefore equal to the sum of their individual effect. Additive gene action is linked to high heritability, indicating that selection is more effective for traits under the influence of additive gene effects (Baryshnikova *et al.*, 2013). Non-additive or dominance genetic action involve interactions between alleles at the same locus, non-additive effects are therefore deviations from additivity that make the heterozygote resemble one parent more than the other (Acquaah, 2007). Traits that are conditioned by mainly non-additive genes generally have low heritability (Acquaah, 2007). The interaction between genes at different loci is called epistasis (Hartl and Jones, 1997).

Knowledge on the type of gene action conditioning the inheritance of different traits under contrasting environments is useful for identification of superior inbred lines and devising appropriate breeding strategies for stress tolerance breeding (Badu-Apraku *et al.*, 2016). The majority of studies do not agree on the predominance of additive and non-additive gene action in the expression of GY under drought and low N environments. An increasing role of the additive type of gene action in grain yield with drought stress, with non-additive effects being more important under low N stress have has been reported in some investigations (Betran *et al.*, 2003a;

Makumbi, 2005; Meseka *et al.*, 2013; Wegary *et al.*, 2014). Similarly, some studies also observed the predominance of additive gene action in conditioning grain yield under drought conditions (Derera *et al.*, 2008) and non-additive effects under low N conditions (Medici *et al.*, 2004). In contrast, a few investigations have shown that there is an equal importance of additive gene effects in the expression of grain yield under both drought and low N environments (Annor and Badu-Apraku, 2016; Ertiro *et al.*, 2017a; Miti, 2007), while non-additive gene effects were equally important under both abiotic stresses for grain yield in another study (Ndhlela, 2012).

The contradicting findings reported in the literature imply that genotypes differ in their response to low N and drought stress environments. Ejigu *et al.* (2017) thus attributed these contrasting findings to the type of germplasm and test environments used for evaluations. In a few studies, the role of additive effects particularly under drought environments agrees. This implies that when breeding for improved hybrid performance under drought stress, acceptable tolerance could be achieved if both parents that possess alleles for drought tolerance are included (Banziger *et al.*, 2000). The type of gene action underlying grain yield under low N is still not clearly understood. The predominance of SCA (non-additive genetic effects) is a consequence of fluctuations in dominance relationships among parents (Wassimi *et al*., 1986) as cited by Ndlela (2012). Therefore, for traits indicating the predominance of non-additive effects, selection of parents for hybrid production may not be judged by high GCA effects due to dominance gene effects that may also contribute to the improvement and expression of a particular trait. However, all studies indicate that both abiotic and biotic stresses are under genetic control and therefore, it is possible to obtain drought and low N tolerant varieties.

2.16 **Genetic distances**

The genetic composition of a population is often explained in terms of the allele frequencies or relative abundances (Kalinowski, 2002). The frequency (the proportion of all alleles that are of the specified type) of each allele at each locus is usually different in each population or individuals (Dogan and Dogan, 2016). Genetic distance metrics are used to summarise the overall extent of genetic difference in a pair of populations or species (Kalinowski, 2002). Dogan and Dogan (2016) defined genetic distance as the degree of genetic differences between populations or species. The highest value of genetic distance between two populations indicate divergent populations and genetic relatedness is reflected by low values (Ren *et al*., 2014). Examples of distance coefficients employed in genetic distance estimates include Rogers' (Rogers, 1972), Nei (Nei, 1972), Gower (Gower, 1971), Jaccard's (Jaccard, 1908) and Edward's genetic distance (Edwards, 1971). Genetic distance estimates have been used widely in various crop species, in the assessment of genetic diversity and in constructing phylogenic trees and heterotic groups (Baloch *et al*., 2017, Bedoya *et al*., 2017, Ertiro *et al*., 2017b; Roy *et al*., 2015; Vikram *et al*., 2016). Genetic distances have also been utilised widely in predicting F_1 hybrid performance (Gichuru *et al*., 2017; Gupta *et al*., 2017; Olfati *et al*., 2012; Rajendran *et al*., 2014).

2.16.1 The Gower's distance and its use with SNP markers

Single nucleotide polymorphism (SNP) markers are commonly used in assessing the level of genetic diversity present in the germplasm (Dao *et al*., 2014; Nyombayire *et al*., 2016), identifying heterotic groups (Badu-Apraku *et al*., 2016; Richard *et al*., 2016) and predicting heterosis in crosses (Amuzu-Aweh *et al.,* 2013; Jan *et al*., 2016). In the application of molecular markers, several distance metrics exist for estimating genetic distances and clustering germplasm; a choice of distance measure to be used is made. The Gower's distance also known as Gower coefficient, proposed by Gower (1971) is one among the commonly used methods. Gower distance is a measure of the similarity between two individuals (Gower, 1971); similarity (s) and dissimarity (d=1-s) coefficients are employed to discriminate germplasm (Gower, 1985). The Gower method allows the simultaneous analysis of quantitative and qualitative data. Use of Gower distance and its efficiency in discriminating germplasm has been appraised in several studies on SNP's applications (Buil *et al*., 2009; Chimello *et al*., 2017; Oliveira *et al*., 2014; Wen *et al*., 2012).

2.17 Correlation between genetic distance and grain yield specific combining ability

Linear correlation between marker-estimated genetic distances (GD's) and grain yield specific combining ability or heterosis has been computed to assess the usefulness of genetic distances in predicting hybrid performance. Parentoni *et al.* (2001) reported a small correlation (r = 0.16**) between random amplified polymorphic DNA (RAPD) marker-based genetic distances and SCA for grain yield. A small correlation (r = 0.24*) between genetic distances and F_1 grain yield was also reported by Makumbi (2005), when data was pooled from three types of markers. The low correlation observed in many studies might be attributed to the markers used to compute GD's. The markers used are generally many and may not be closely linked to grain yield and associated characters, which therefore result in poor correlation between marker-estimated GD's and grain yield or heterosis (Melchinger, 1999). This suggests that information of genetic distance may be limiting because of its low predictive value in F_1 hybrid performance. Rajendran *et al.* (2014) found no correlation between SSR-estimated genetic distances and SCA for grain yield. These findings therefore emphasise that irrespective of the GD estimates and molecular makers used; use of molecular marker data alone may not be sufficient in eliminating inferior hybrids before field-testing, and thus extensive field evaluations are always crucial.

2.18 Genotype by environmental interaction

Genotype by environment interaction (G x E) has important implications for breeding. Genotype by environment interactions have been defined as the failure of genotypes to achieve consistent performance in different environments (Setimela *et al*., 2007). Fluctuations in the performance of genotypes from one environment to another and from year to year, due to environmental factors such as soil fertility levels, temperature, soil moisture content and several other environmental factors are a function of G x E (Beyene *et al.,* 2011b). When identifying superior varieties from multi-environments trials (METs), the presence of G x E complicates the selection of superior cultivars and genotype recommendation; G x E also reduces correlation of genotype and phenotypic values (Beyene *et al*., 2011a). The existence of G x E raises the need for breeders to either ignore, avoid or exploit it (Eisemann *et al*., 1990).

Genotype x environment interactions have been examined for stress and non-stress conditions. Stress environments are associated with large and significant G x E (Banziger *et al*., 2000). The performance of the maize crop can be affected by stress in various ways. As stress levels increase due to drought, low N and other abiotic and biotic stresses, genotype ranking under stress environments differ significantly from one environment to another, complicating the process of breeding and selection of superior cultivars (Banziger *et al*., 2000). Selection of stable cultivars that maintain relative good performance across a wide range of environments, and deploying different genotypes with specific adaptation in target environments have been widely used as strategies for exploiting G x E, especially when stress environments are involved (Beyene *et al*., 2011a; Ertiro *et al*., 2017a; Gasura *et al*., 2015; Makumbi *et al*., 2015; Ndhlela *et al*., 2014; Setimela *et al*., 2007; Shaibu *et al*., 2016; Sserumaga *et al*., 2016). Alternatively, when the crossover interaction is large, with repeatable G x E, the repeatable G x E pattern can be utilised to capture the effect of G x E by grouping similar environments (mega-environments) within the production region (Setimela *et al*., 2007). Gauch and Zobel (1997) defined a mega-environment as a group of homogeneous environments with the same set of good performing genotypes. This simplifies testing and facilitates selection and exchange of genotypes, by targeting appropriate genotypes for each mega-environment (Gauch and Zobel, 1997; Setimela *et al*., 2005).

Like most countries in Eastern and Southern Africa, maize growing environments of SA are characterised by multiple stresses including low soil fertility, drought and uneven rainfall distribution, which contributes to large G x E, and hence selection and recommendation of best cultivars is hampered. Identification of stable cultivars or breeding for target environments are useful strategies in dealing with G x E interactions. The genotype and genotype by environment (GGE) biplot analysis (Yan *et al*., 2000) and the additive main effect and multiplicative interaction (AMMI) analysis (Zobel *et al*., 1988; Gauch, 2013) are the two widely used statistical approaches in G x E studies. The GGE biplots are based on singular value decomposition (SVD) of environment-centred principal component analysis (PCA) (Yan *et al*., 2000) and the AMMI is based on double centred PCA (Gauch, 2006). The GGE is useful in mega-environment analysis, (ii) test environment evaluation to identify ideal environments and (iii) genotype evaluation (Yan, 2001).

2.19 **Summary of the literature**

Heterotic groupings and effective breeding strategies for improved tolerance to drought and low N stress were highlighted. Fewer heterotic groups are important for maximising breeding efficiency. Heterotic groups are not always based on historical origin, but the choice of testers used may be influential in the number of heterotic groups formed; a critical choice should therefore be made when selecting testers for use in classifying lines into heterotic groups. Heterotic orientation based on SCA effect may be influenced by the use of either temperate or tropical lines, and therefore the predominance of temperate or tropical genome in inbred lines to be classified. Inclusion of both tropical and temperate lines is therefore crucial especially when determining heterotic groups of lines that were previously introgressed with both tropical and temperate material. Classification based on molecular markers is not efficient in classifying related germplasm, incorporating both molecular markers and field evaluations may result in accurate and full classifications. Genetic distances may not always be a good predictor of heterosis in F_1 hybrids, depending on the type of germplasm, field evaluations are always essential.

Breeding for drought and low N stress tolerance requires good understanding of the mode of gene action involved and precision during hybrid evaluation and management. Mainly additive effects are in control of grain yield under drought stress; therefore, to achieve good tolerance to drought stress, both parents should be drought tolerant. Under low N stress, gene action controlling grain yield has not been fully understood. However, selection under drought tolerance generally translates into gains in low N environments; therefore, selection may be focused under drought to improve low N tolerance. This will result in the identification of favorable varieties that combine tolerance to multiple stresses, which normally occur simultaneously in most farmer's fields. The identified cultivars should however carry no yield penalties under non-stress conditions. Higher yielding cultivars under non-stress environments are not necessarily superior under stress environments, but selection gains may be maximised by simultaneous selection under stress and non-stress environments.

Heritability and genetic variances obtained under stress environments are generally low, selection for secondary characters with emphasis on traits with high heritability and strong association with grain yield is always an important approach. The degree of tolerance to abiotic stresses varies with germplasm used and test environments. Testing of new cultivars is therefore always fundamental; however, precision should be made during stress management to minimise deliberate experimental errors that may lead to biased decisions. Fewer studies on breeding for drought and low N tolerance in South Africa are reported in the literature. Therefore, the degree of standard heterosis under stress and non-stress environments is not clearly documented. However, heterosis is independent of test environments and it is largely predetermined by the presence of sufficient variability in a germplasm, and careful selection of parental lines for use in hybrid development.

References

Abdalla, A.E., F.M. Mahmoud, and A.M. El Naim. 2010. Evaluation of some maize (*Zea mays* L.) varieties in different environments of the Nuba mountain of Sudan. *Australian Journal of Basic and Applied Sciences* 4: 6605-6610.

Abel, B.C., and L.M. Pollak. 1991. Rank comparisons of unadapted maize populations by testers and per se evaluation. *Crop Science* 31: 650-656.

Acquaah, G. 2007. Principles of plant genetics and breeding. Blackwell publishing, USA, UK, Australia.

Adetimirin, V.O., I. Vroh-Bi, C. The, A. Menkir, S.E. Mitchell, and S. Kresovich. 2008. Diversity analysis of elite maize inbred lines adapted to West and Central africa using SSR markers. *Maydica* 53: 143-149.

Aguiar, C.G., I. Schuster, A.T. Amaral-Junior, C.A. Scapim, and E.S.N. Vieira. 2008. Heterotic groups in tropical maize germplasm by test crosses and simple sequence repeat markers. *Genetics and Molecular Research* 7: 1233-1244.

Ahamed, H.M., S. ul-Malook, I. Ali, M.U. Ali, Z. Aslam, B. Farid, A. Mumtaz, and M. Ahsan. 2015. Assessment of gene action to develop drought tolerance maize inbred lines. *American-Eurasian Journal of Agricultural and Environmental Science* 15: 454-461.

Ahuja, S.L., and L.S. Dhayal. 2007. Combining ability estimates for yield and fibre quality traits in 4 x 13 line x tester crosses of *Gossypium hirsutum*. *Euphytica* 153: 87-98.

Allard, R.W. 1960. Principles of plant breeding.Wiley, NY.

Altman, A., and P.M. Hasegawa. 2012. Plant Biotechnology and Agriculture: Prospects for the 21st Century. Academic Press, USA.

Amiruzzaman, M., M.A. Islam, K.V. Pixley, and M.M. Rohman. 2011. Heterosis and combining ability of CIMMYT's Tropical × Subtropical quality protein maize germplasm. *International Journal of Sustainable Agriculture* 3: 76-81.

Amiruzzaman, M., M.A. Islam, L. Hassan, and M.M. Rohman. 2010. Combining ability and heterosis for yield and component characters in maize. *Academic Journal of Plant Sciences* 3: 79-84.

Amuzu-Aweh, E.N., P. Bijma, B.P. Kinghorn, A. Vereijken, J. Visscher, J. Arendonk, and H. Bovenhuis. 2013. Prediction of heterosis using genome-wide SNP-marker data: Application to egg production traits in white Leghorn crosses. *Heredity* 111: 530–538.

Annor, B., and B. Badu-Apraku. 2016. Gene action controlling grain yield and other agronomic traits of extra-early quality protein maize under stress and non-stress conditions. *Euphytica* 212: 213–228.

Aslam, M., F.S. Awan, I.A. Khan, and A.I. Khan. 2009. Estimation of genetic distance between 10 maize accessions with varying response to different levels of soil moisture. *Genetics and Molecular Research* 4: 1459-1465.

Aslam, M., M.A. Maqbool, and R. Cengiz. 2015. Drought stress in maize (*Zea mays* L.): Effects, resistance mechanisms, global achievements and biological stratergies for imporovement. Springer, New York, London.

Badu-Apraku, B., M. Oyekunle, M.A.B. Fakorede, I. Vroh, R.O. Akinwale, and M. Aderounmu. 2013. Combining ability, heterotic patterns and genetic diversity of extra-early yellow inbreds under contrasting environments. *Euphytica* 192: 413-433.

Badu-Apraku, B., M. Oyekunle, R.O. Akinwale, and L.A. Fontem. 2011. Combining ability of early-maturing white maize inbreds under stress and nonstress environments. *Agronomy Journal* 103: 544-557.

Badu-Apraku, B., M.A.B. Fakorede, M. Gedil, B. Annor, A.O. Talabi, I.C. Akaogu, M. Oyekunle, R.O. Akinwale, and T.Y. Fasanmade. 2016. Heterotic patterns of IITA and CIMMYT early-maturing yellow maize inbreds under contrasting environments. *Agronomy Journal* 108: 1321-1336.

Badu-Apraku, B., R.O. Akinwale, and M.A.B. Fakorede. 2010. Selection of early maturing maize inbred lines for hybrid production using multiple traits under striga-infested and striga-free environments. *Maydica* 55: 261-274.

Baker, F.W.G. 1989. Drought resistance in cereals. CAB International, Wallingford, UK.

Baloch, F.S., A. Alsaleh, M.Q. Shahid, E. Cifti, L.E.S. de Miera, M. Aasim, M.A. Nadeem, H. Aktas, H. Ozkan, and R. Hatipoglu. 2017. A whole genome DArTseq and SNP analysis for genetic diversity assessment in Durum Wheat from central fertile crescent. *PLoS ONE* 12: 1-18.

Banziger, M., F.J. Betran, and H.R. Lafitte. 1997. Efficiency of high-nitrogen selection environments for improving maize for low-nitrogen target environments. *Crop Science* 37: 1103-1109.

Banziger, M., G.O. Edmeades, and H.R. Lafitte. 1999. Selection for drought tolerance increases maize yields across a range of nitrogen levels. *Crop Science* 39: 1035-1040.

Banziger, M., G.O. Edmeades, D. Beck, and M. Bellon. 2000. Breeding for drought and Nitrogen stress tolerance in maize: From theory to practice. CIMMYT, Mexico, D.F.

Banziger, M., P.S. Setimela, D. Hodson, and B. Vivek. 2004. Breeding for improved drought tolerance in maize adapted to Southern Africa. pp 1-10. In: Fischer, T., N. Turner, J. Angus, L. McIntyre, M. Robertson, A. Borrell, and D. Lloyd (eds.). 4th International Crop Science Congress, 26 Sep – 1 Oct 2004. Brisbane, Australia.

Bao, Y., S. Wang, X. Wang, Y. Wang, X. LI, L. Wang, and H. Wang. 2009. Heterosis and combining ability for major yield traits of a new wheat germplasm shannong 0095 derived from *thinopyrum intermedium*. *Agricultural Sciences in China* 8: 753-760.

Barata, C., and M. Carena. 2006. Classification of North Dakota maize inbred lines into heterotic groups based on molecular and testcross data. *Euphytica* 51: 339-349.

Baryshnikova, A., M. Costanzo, C.L. Myers, B. Andrews, and C. Boone. 2013. Genetic interaction networks: Toward an understanding of heritability. *Annual Reviews of Genomics and Human Genetics* 14: 8.1-8.23.

Bedoya, C.A., S. Dreisigacker, S. Hearne, J. Franco, C. Mir, B.M. Prasanna, S. Taba, A. Charcosset, and M.L. Warburton. 2017. Genetic diversity and population structure of native maize populations in Latin America and the Caribbean. *PLoS ONE* 12: 1-21.

Bello, O., O. Olawuyi, M. Lawal, S. Ige, J. Mahamood, M. Afolabi, M. Azeez, and S. Abdulmaliq. 2014. Genetic gains in three breeding eras of maize hybrids under low and optimum nitrogen fertilization. *Journal of Agricultural Sciences* 59: 227-242.

Bello, O.B., S.Y. Abdulmaliq, S.A. Ige, J. Mahamood, F. Oluleye, M.A. Azeez, and M.S. Afolabi. 2012. Evaluation of early and late/intermediate maize varieties for grain yield potential and adaptation to a Southern Guinea savanna agro-ecology of Nigeria. *Scholarly Journal of Agricultural Science* 2: 42-51.

Below, F.E. 1997. Growth and productivity of maize under Nitrogen stress. pp 235-240. In: Edmeades, G.O., M. Banziger, H.R. Mickelson, and C.B. Pena-Valdivia (eds.). Developing Drought- and Low N-Tolerant Maize. Proceedings of a Symposium, March 25-29,1996. CIMMYT, El Batan, Mexico D.F.

Betran, F.J., D. Beck, M. Banziger, and G.O. Edmeades. 2003a. Genetic analysis of inbred and hybrid grain yield under stress and nonstress environments in tropical maize. *Crop Science* 43: 807-817.

Betran, F.J., J.M. Ribaut, D. Beck, and D.G. de Leon. 2003b. Genetic diversity specific combining ability and heterosis in tropical maize under stress and nonstress environments. *Crop Science* 43: 797-806.

Beyene, Y., K. Semagn, J. Crossa, S. Mugo, G. Atlin, A. Tarekegne, B. Meisel, P. Sehabiague, B. Vivek, and S. Oikeh. 2016. Improving maize grain yield under drought stress and non-stress environments in sub-Saharan Africa using marker-assisted recurrent selection. *Crop Science* 56: 344-353.

Beyene, Y., S. Mugo, C. Mutinda, T. Tefera, H. Karaya, S. Ajanga, J. Shuma, R. Tende, and V. Kega. 2011a. Genotype by environment interactions and yield stability of stem borer resistant maize hybrids in Kenya. *African Journal of Biotechnology* 10: 4752-4758.

Beyene, Y., S. Mugo, K. Pillay, T. Tefera, S. Ajanga, S. Njoka, H. Karaya, and J. Gakunga. 2011b. Testcross performance of doubled haploid maize lines derived from tropical adapted backcross populations. *Maydica* 56: 351-358.

Bidhendi, M.Z., R. Choukan, F. Darvish, K. Mostafavi, and E. Majidi. 2012. Classifying of maize inbred lines into heterotic groups using diallel analysis. *World Academy of Science, Engineering and Technology* 6: 1161-1164.

Blum, A. 1997. Constitutive traits affecting plant performance under stress. pp 131–135. In: Edmeades, G.O., M. Banziger, H.R. Mickelson, and C.B. Pena-Valdivia (eds.). Developing Drought- and Low N-Tolerant Maize. Proceedings of a Symposium, March 25-29,1996. CIMMYT, El Batan, Mexico D.F.

Blum, B. 2011. Plant breeding for water-limited environments. Springer Science+Business Media, LLC., New York, USA.

Bolanos, J., and G.O. Edmeades. 1996. Eight cycles of selection for drought tolerance in lowland tropical maize. III. Responses in grain yield, biomass, and radiation utilization. *Field Crops Research* 31: 233-252.

Bubliy, O.A., V. Loeschcke, and A.G. Imasheva. 2001. Genetic variation of morphological traits in Drosophila melanogaster under poor nutrition: isofemale lines and offspring-parent regression. *Heredity* 86: 363-369.

Buil, A., A.M. Perez, A.P. Lluna, L. Rib, P. Caminal, and J.M. Soria. 2009. A new gene-based association test for genome-wide association studies. *BMC Proceedings* 3: 1-5.

Cairns, J.E., J. Crossa, P.H. Zaidi, P. Grudloyma, C. Sanchez, J.L. Araus, S. Thaitad, D. Makumbi, C. Magorokosho, M. Banziger, A. Menkir, S. Hearne, and G.N. Atlin. 2013. Identification of drought, heat, and combined drought and heat tolerant donors in maize (*Zea mays* L.). *Crop Science* 53: 1335-1346.

Calvo, C.H.R.G., and E.E. Magulama. 2010. Use of combining ability effects in assigning inbred lines into heterotic group. *USM CA research* 21: 91-100.

Carena, M.J., and A.R. Hallauer. 2001. Expression of heterosis in Leaming and Midland Corn Belt dent populations. *Journal of the Iowa Academy of Science* 108:73–78.

Castellanos, J.S., A.R. Hallauer, and H.S. Cordova. 1998. Relative performance of testers to identify elite lines of corn (*Zea mays* L.). *Maydica* 43: 217-226.

Chandel, U., B.S. Mankotia, and K.S. Thakur. 2014. Evaluation of CIMMYT maize (*Zea mays* L.) germplasm by tropical inbred testers. *Bangladesh Journal of Botany* 43: 131-139.

Chigeza, G. 2013. Genetic gain, advanced cycle pedigree breeding and correlated response to selection under varying moisture conditions in sunflower. PhD Thesis. University of KwaZulu-Natal, Republic of South Africa.

Chimello, A.M., J.G. Jesus, P.E. Teodoro, A.A.B. Rossi, K.L. Araujo, T.N. Marostega, L.G. Neves, and M.A.A. Barelli. 2017. Morphological descriptors and ISSR molecular markers in the evaluation of genetic variability of Tectona grandis genotypes. *Genetics and Molecular Research* 16: 1-16.

Comstock, R.E., H.F. Robinson, and P.H. Harvey. 1949. A breeding procedure designed to make maximum use of both general and specific combining ability. *Agronomy Journal* 41: 360-367.

Dao, A., J. Sanou, S.E. Mitchell, V. Gracen, and E.Y. Danquah. 2014. Genetic diversity among INERA maize inbred lines with single nucleotide polymorphism (SNP) markers and their relationship with CIMMYT, IITA, and temperate lines. *BMC Genetics* 15: 1-14.

Dass, S., Y.P. Dang, A.K. Dhawan, N.N. Singh, and S. Kumar. 1996. Morpho-physiological basis for breeding drought and low-N tolerant maize genotypes in India. pp 107-111. In: Edmeades, G.O., M. Banziger, H.R. Mickelson and C.B. Pena-Valdivia (eds.). Developing Drought- and Low N Tolerant Maize, Proceedings of a Symposium March 25-29, 1996. CIMMYT, El Batan, Mexico.

Davenport, C.B. 1908. Degeneration, albinism and inbreeding. *Science* 28: 454–455.

de Pinto, M.C.R., C.I. de Souza Jr, L.A. Carlini-Garcia, A.A.F. Garcia, and A.P. de Souza. 2003. Comparison between molecular markers and diallel crosses in the assignment of maize lines to heterotic groups. *Maydica* 48: 63-73.

Delucchi, C., G. Eyherabide, R.D. Lorea, D.A. Presello, M.E. Otegui, and C.G. Lopez. 2012. Classification of Argentina maize landraces in heterotic groups. *Maydica* 57: 26-33.

Derera, J. 2005. Genetic effects and associations between grain yield potential, stress tolerance and yield stability in Southern African maize (*Zea mays* L.) base germplasm. PhD Thesis. University of KwaZulu-Natal, Republic of South Africa.

Derera, J., P. Tongoona, B. Vivek, and M.D. Laing. 2008. Gene action controlling grain yield and secondary traits in Southern African maize hybrids under drought and non-drought environments. *Euphytica* 162: 411-422.

Dhliwayo, T., K. Pixley, A. Menkir, and M. Warburton. 2009. Combining ability, genetic distances and heterosis among elite CIMMYT and IITA tropical maize inbred lines. *Crop science* 49: 1201-1210.

Dogan, I., and N. Dogan. 2016. Genetic distance measures: Review. *Turkiye Klinikleri Journal of Biostatistics* 8: 87-93.

Edmeades, G.O., J. Bolanos, and H.R. Lafitte. 1992. Progress in breeding for drought tolerance in maize. pp 93-111. In: D. Wilkinson (ed.). Proceedings of the 47th annual corn and sorghum industry research conference. American Seed Trade Association, Chicago.

Edmeades, G.O., J. Bolanos, and S.C. Chapman. 1997. Value of secondary traits in selecting for drought tolerance in Tropical maize. pp 222-233. In: Edmeades, G.O., M. Banziger, H.R.

Mickelson, and C.B. Pena-Valdivia (eds.). Developing Drought- and Low N-Tolerant Maize. Proceedings of a Symposium, March 25-29,1996. CIMMYT, El Batan, Mexico, D.F.

Edmeades, G.O., J. Bolanos, M. Banziger, J.M. Ribaut, J.W. White, M.P. Reynolds, and H.R. Lafitte. 1998. Improving crop yields under water deficits in the tropics. pp 437-451. In: Chopra, V.L., R. B. Singh, and A. Varma (eds.). Crop productivity and sustainability - shaping the future. Proceedings of the 2nd International Crop Science Congress. New Delhi.

Edmeades, G.O., J. Bolanos, M. Hernandez, and S. Bello. 1993. Causes for silk delay in a lowland tropical maize population. *Crop Science* 33: 1029-1035.

Edwards, A. 1971. Distances between populations on the basis of gene frequencies. *Biometrics*: 873-881.

Eid, M.H. 2009. Estimation of heritability and genetic advance of yield traits in wheat (*Triticum aestivum* L.) under drought condition. *International Journal of Genetics and Molecular Biology* 1: 115-120.

Eisemann, R.L., M. Cooper, and D.R. Woodruff. 1990. Beyond the analytic methodology: Better interpretation and exploitation of GE interactions in plant breeding. In: Kang, M. S (ed.). Genotype by environment interactions and plant breeding. Louisiana State University Agri. Center, Baton Rouge, LA.

Ejigu, Y.G., P.B. Tongoona, and B.E. Ifie. 2017. General and specific combining ability studies of selected tropical white maize inbred lines for yield and yield related traits. *International Journal of Agricultural Science and Research* 7: 381-396.

Ertiro, B.T., K. Semagn, B. Das, M. Olsen, M. Labuschagne, M. Worku, D. Wegary, G. Azmach, V. Ogugo, T. Keno, B. Abebe, T. Chibsa, and A. Menkir. 2017b. Genetic variation and population structure of maize inbred lines adapted to the mid-altitude sub-humid maize agro-ecology of Ethiopia using single nucleotide polymorphic (SNP) markers. *BMC Genomics* 18: 1-11.

Ertiro, B.T., Y. Beyene, B. Das, S. Mugo, M. Olsen, S. Oikeh, C. Juma, M. Labuschagne, and B.M. Prasanna. 2017a. Combining ability and testcross performance of drought-tolerant maize inbred lines under stress and non-stress environments in Kenya. *Plant Breeding* 136: 197–205.

Evans, L.T., and R.A. Fisher. 1999. Yield potential: Its defination, measurement and significance. *Crop Science* 39: 1544-1551.

Falconer, D.S., and T.F.C. Mackay. 1996. Introduction to quantitative genetics., 4th edition. Longman, Essex, England.

Fan, X.M., H.M. Chen, J. Tan, C.X. Xu, Y.D. Zhang, L.M. Luo, Y.X. Huang, and M.S. Kang. 2008. Combining abilities for yield and yield components in maize. *Maydica* 53: 39-46.

Fan, X.M., J. Tan, H.M. Chen, and J.Y. Yang. 2003. Heterotic grouping for tropical and temperate maize inbreds by analysing combining ability and SSR markers. *Maydica* 48: 251-257.

Fan, X.M., J. Tan, J.Y. Yang, and H.M. Chen. 2004. Combining ability and heterotic grouping of ten temperate subtropical and tropical quality protein maize inbreds. *Maydica* 49: 267–272.

Fan, X.M., Y.D. Zhang, L. Liu, H.M. Chen, W.H. Yao, M. Kang, and J.Y. Yang. 2010. Screening tropical germplasm by temperate inbred testers. *Maydica* 55: 55-63.

Fan, X.M., Y.D. Zhang, W.H. Yao, Y.Q. Bi, L. Liu, H.M. Chen, and M.S. Kang. 2014. Reciprocal diallel crosses impact combining ability, variance estimation, and heterotic group classification. *Crop Science* 54: 89–97.

Fan, X.M., Y.M. Zhang, W.H. Yao, H.M. Chen, J. Tan, C.X. Xu, X.L. Han, L.M. Luo, and M.S. Kang. 2009. Classifying maize inbred lines into heterotic groups using a factorial mating design. *Agronomy Journal* 101: 106–112.

Fasahat, P., A. Rajabi, J.M. Rad, and J. Derera. 2016. Principles and utilization of combining ability in plant breeding. *Biometrics & Biostatistics International Journal* 4: 1-24.

Fellahi, Z.E.A., A. Hannachi, H. Bouzerzour, and A. Boutekrabt. 2013. Line by tester mating design analysis for grain yield and yield related traits in bread wheat (*Triticum aestivum* L.). *International Journal of Agronomy* 2013: 1-9.

Fisher, M., T. Abate, R.W. Lunduka, W. Asnake, Y. Alemayehu, and R.B. Madulu. 2015. Drought tolerant maize for farmer adaptation to drought in sub-Saharan Africa: Determinants of adoption in eastern and southern Africa. *Climatic Change* 133: 283-299.

Galal, M.A., and A. Mahgoub. 2011. Partitioning of general and specific combining ability effects for estimating maternal and reciprocal effects. *Agricultural Science* 3: 213-222.

Gardner, C.O. 1987. Quantitative genetic theory and integrated breeding systems for maize improvement. In Towards Self Sufficiency. A Proceedings of the Second Eastern, Centraland Southern Africa Regional Maize Workshop, Harare, Zimbabwe, March 15-21, 1987.

Gasura, E., P.S. Setimela, and C.M. Souta. 2015. Evaluation of the performance of sorghum genotypes using GGE biplot. *Canadian journal of plant science* 95: 1205-1214.

Gauch , H.G. 2006. Statistical analysis of yield trials by AMMI and GGE. *Crop Science* 46: 1488–1500.

Gauch, H.G. 2013. A simple protocol for AMMI analysis of yield trials. *Crop Science* 53: 1860-1869.

Gauch, H.G., and R.W. Zobel. 1997. Identifying mega-environments and targeting genotypes. *Crop Science* 37: 311-326.

Gichuru, L., J. Derera, P. Tongoona, and M. Mwimali. 2017. Combining ability and molecular genetic distances in tropical maize lines for grain yield and agronomic traits. *South African Journal of Plant and Soil* 34: 175-183.

Gower, J.C. 1971. A general coefficient of similarity and some of its properties. *Biometrics* 27: 857-874.

Gower, J.C. 1985. "Measures of Similarity, Dissimilarity, and Distance,". In: Kotz, S., N.L. Johnson, and C.B. Read (eds.). Encyclopedia of Statistical Sciences, Vol 5. John Wiley and Sons, New York. p. 397-405.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing system. *Australian Journal of Biological Sciences* 9: 463-493.

Guimaraes, L.J.M., G.V. Miranda, R.O. de Lima, C. Maia, L.R. de Oliveira, and L.V. de Souza. 2012. Performance of testers with different genetic structure for evaluation of maize inbred lines. *Ciência Rural* 42: 770-776.

Gupta, S.K., T. Nepolean, C.G. Shaikh, K. Rai, C.T. Hash, R.R. Das, and A. Rathore. 2017. Phenotypic and molecular diversity-based prediction of heterosis in pearl millet (*Pennisetum glaucum* L.(R.) Br). *The Crop Journal* 5: 1-11.

Hallauer, A., and M. Carena. 2014. Adaptation of tropical maize germplasm to temperate environments. *Euphytica* 196: 1-11.

Hallauer, A.R. 1975. Relation of gene action and type of tester in maize breeding procedures. *Proceeding of Annual Corn and Sorghum Research Conference* 30: 50-165.

Hallauer, A.R., and J.B. Miranda. 1988a. Quantitative genetics in maize breeding, 2nd edition. Iowa State University Press, Ames, IA.

Hallauer, A.R., and J.B. Miranda. 1988b. Testers and combining ability, in Quantitative genetics in maize breeding. In: Hallauer, A.R. and J. B. Miranda (eds.). Iowa State University Press, Ames, USA.

Hallauer, A.R., and M.J. Carena. 2009. Maize breeding. In: Carena, M.J. (ed.). Handbook of plant breeding: Cereals. Springer, New York.

Hartl, D.L., and E.W. Jones. 1997. Genetics: Principles and analysis, 4th edition. Jones and Bartlett Publishers, Sudbury, Massachusetts.

Hassan, M.I., E.A. Mohamed, M.A. El-rawy, and K.A. Amein. 2016. Evaluating interspecific wheat hybrids based on heat and drought stress tolerance. 19: 85-98.

Hosana, G.H., S. Alamerew, B. Tadesse, and T. Menamo. 2015. Test cross performance and combining ability of maize (*Zea Mays* L.) inbred lines at Bako, Western Ethiopia. *Global Journals Inc. (USA)* 15: 1-25.

Jaccard, P. 1908. Nouvelles recherche´s sur la distribution florale. *Bull Soc Vaud Sci Nat* 44: 223–270.

Jan, H.U., A. Abbadi, S. Lucke, R.A. Nichols, and R.J. Snowdon. 2016. Genomic prediction of testcross performance in canola (*Brassica napus*). *PLoS ONE* 11: 1-19.

Joseph, C.A. 2015. Genetic variation, heritability and relationship between inbred lines performances under drought stress and irrigation conditions in rice. *American Journal of Experimental Agriculture* 7: 294-307.

Kalinowski, S.T. 2002. Evolutionary and statistical properties of three genetic distances. *Molecular Ecology* 11: 1263-1273.

Kambe-Gouda, R., U. Kage, H.C. Lohithaswa, B.G. Shekara, and D. Shobha. 2013. Combining ability studies in maize (*Zea Mays* L.). *Molecular Plant Breeding* 4: 116-127.

Kempthorne, O. 1957. An introduction to genetic statistics. John Wiley and Sons. Inc., New York. U.S.A.

Khalili, M., M.R. Naghavi, A.P. Aboughadareh, and H.N. Rad. 2013. Effects of drought stress on yield and yield components in maize cultivars (*Zea mays* L.). *International Journal of Agronomy and Plant Production* 4: 809-812.

Kulembeka, H.P., M. Ferguson, L. Herselman, E. Kanju, G. Mkamilo, E. Masumba, M. Fregene, and M.T. Labuschagne. 2012. Diallel analysis of field resistance to brown streak disease in cassava (*Manihot esculenta* Crantz) landraces from Tanzania. *Euphytica* 187: 277-288.

Kumar, G.P., Y. Prashanth, V.N. Reddy, S.S. Kumar, and P.V. Rao. 2014. Heterosis for grain yield and its component traits in maize (*Zea mays* L.). *International Journal of Pure and Applied Biosciences* 2: 106-111.

Lafitte, H.R., and M. Banziger. 1997. Maize population improvement for low soil N: selection gains and the identification of secondary traits. pp 485-489. In: Edmeades, G.O., M. Banziger, H.R. Mickelson, and C.B. Pena-Valdivia (eds.). Developing Drought and Low N-Tolerant Maize. Proceedings of a Symposium, March 25-29, 1996. CIMMYT, El Batan, Mexico, D.F.

Li, M.S., X.H. Li, L.W. Deng, D.G. Zhang, L. Bai, and S.H. Zhang. 2007. Comparisons of four testers in evaluating 27 CIMMYT and Chinese maize populations. *Maydica* 52: 173-179.

Librando, R.P., and E.F. Magulama. 2008. Classifying white inbred lines into heterotic groups using yield combining ability effects. *USM R & D A Journal* 16: 99-103.

Lu, Y., J. Yan, G.T. Guimaraes, S. Taba, Z. Hao, S. Gao, S. Chen, J. Li, S. Zhang, B.S. Vivek, C. Magorokosho, S. Mugo, D. Makumbi, S.N. Parentoni, T. Shah, T. Rong, J.H. Crouch, and Y. Xu. 2009. Molecular characterization of global maize breeding germplasm based on genome-wide single nucleotide polymorphisms. *Theoretical and Applied Genetics* 120: 93–115.

Machado, F.C., J.B. dos Santos, G.H.S. Nunes, and M.A.P. Ramalho. 2002. Choice of common bean parents based on combining ability estimates. *Genetics and Molecular Biology* 25: 179-183.

Machikowa, T., C. Saetang, and K. Funpeng. 2011. General and specific combining ability for quantitative characters in sunflower. *Agricultural Science* 3: 91-95.

Magorokosho, C., K.V. Pixley, and P. Tongoona. 2003. Selection for drought tolerance in two tropical maize populations. *African Crop Science Journal* 11: 151-161.

Makanda, I., P. Tongoona, and J. Derera. 2009. Combining ability and heterosis of sorghum germplasm for stem sugar traits under off-season conditions in tropical lowland environments. *Field crops research* 114: 272-279.

Makumbi, D. 2005. Phenotypic and genotypic characterization of white maize inbreds, hybrids and synthetics under stress and non-stress environments. PhD thesis. Texas A&M University.

Makumbi, D., A. Diallo, F. Kanampiu, S. Mugo, and H. Karaya. 2015. Agronomic performance and genotype x environment interaction of herbicide-resistant maize varieties in Eastern Africa. *Crop Science* 55: 540–555.

Makumbi, D., J.F. Betran, M. Banziger, and J.M. Ribaut. 2011. Combining ability, heterosis and genetic diversity in tropical maize (*Zea mays* L.) under stress and non-stress conditions. *Euphytica* 180: 143–162.

Matzinger, D.F. 1953. Comparison of three types of testers for the evaluation of inbred lines of corn *Agronomy Journal* 45: 493-495.

Medici, L.O., M.B. Pereira, P.J. Lea, and R.A. Azevedo. 2004. Diallel analysis of maize lines with contrasting responses to applied Nitrogen. *Agricultural Sciences* 142: 535-541.

Melani, M.D., and M.J. Carena. 2005. Alternative maize heterotic patterns for the Northern corn belt. *Crop Science* 45: 2186–2194.

Melchinger, A.E. 1999. Genetic diversity and heterosis. In: Coors, J.G., and S. Pandey (eds.). The genetics and exploitation of heterosis in crops. pp 99–118. American Society of Agronomy, Madison, Wisconsin.

Melchinger, A.E., and R.K. Gumber. 1998. Overview of heterosis and heterotic groups in agronomic crops. pp 29-44. In: Lamkey, K. R., and J. E. Staub (eds.). In concepts and breeding of heterosis in crop plants. Madison, WI.

Menkir, A., A. Melake-Berhan, A.C. The, I. Ingelbrecht, and A. Adepoju. 2004. Grouping of tropical mid-altitude maize inbred lines on the basis of yield data and molecular markers. *Theoretical and Applied Genetics* 108: 1582-1590.

Menkir, A., B. Badu-Apraku, C. The, and A. Adepoju. 2003. Evaluation of heterotic patterns of IITA's lowland white maize inbred lines. *Maydica* 48: 161-170.

Meseka, S., and J. Ishaaq. 2012. Combining ability analysis among Sudanese and IITA maize germplasm at Gezira Research Station. *Journal of Applied Biosciences* 57: 4198– 4207.

Meseka, S.K., A. Menkir, A.E.S. Ibrahim, and S.O. Ajala. 2013. Genetic analysis of maize inbred lines for tolerance to drought and low Nitrogen. *Jonares* 1: 29-36.

Miti, F. 2007. Breeding investigations of maize (*Zea mays* L.) genotypes for tolerance to low Nitrogen and drought in Zambia. PhD thesis. University of KwaZulu-Natal, Republic of South Africa.

Mulbah, Q.S., H.A. Shimelis, and M.D. Laing. 2015. Combining ability and gene action of three components of horizontal resistance against rice blast. *Euphytica* 206: 805–814.

Mwimali, M.G. 2014. Genetic analysis and response to selection for resistance to two stem borers, *Busseola fusca* and *Chilo partellus*, in tropical maize germplasm. PhD Thesis. University of KwaZulu-Natal, Republic of South Africa.

Narayanam, S.S., and P. Phundan. 1993. Biometrical techniques in plant breeding. Kayani Publishers, Ludhiana.

Narro, L., S. Pandey, J. Crossa, C. de Leon, and F. Salazar. 2003. Using line x tester interaction for the formation of yellow maize synthetics tolerant to acid soils. *Crop Science* 43: 1718-1728.

Ndhlela, T. 2012. Improvement strategies for yield potential, disease resistance and drought tolerance of Zimbabwean maize inbred lines. PhD Thesis. University of the Free State, Republic of South Africa.

Ndhlela, T., L. Herselman, C. Magorokosho, P. Setimela, C. Mutimaamba, and M. Labuschagne. 2014. Genotype × environment interaction of maize grain yield using AMMI biplots. *Crop Science* 54: 1–8.

Nei, M. 1972. Genetic distance between populations. *The American Naturalist* 106: 283-292.

Njoroge, K., B. Wafula, and J.K. Ransom. 1997. Characterization of drought stress in the major maize production zones of Kenya. pp 35-38. In: Edmeades, G.O., M. Banziger, H.R. Mickelson, and C. B. Pena-Valdivia (eds.). Developing Drought- and Low N-Tolerant Maize. Proceedings of a Symposium, March 25-29, 1996. CIMMYT, El Batan, Mexico, D.F.

Nyombayire, A., J. Derera, J. Sibiya, E. Gasura, and C. Ngaboyisonga. 2016. Genetic diversity among maize inbred lines selected for the mid-altitudes and highlands of Rwanda. *Maydica* 61: 1-7.

Olfati, J., H. Samizadeh, G.H. Peyvast, B. Rabiei, and S. Khodaparast. 2012. Relationships between hybrid performance and genetic distance revealed by morphological and AFLP marker in Cucumber. *Plant Breeding and Seed Science* 65: 87-97.

Oliveira, E.J., C.F. Ferreira, V.S. Santos, and G.A.F. Oliveira. 2014. Development of a cassava core collection based on single nucleotide polymorphism markers. *Genetics and Molecular Research* 13: 6472-6485.

Parentoni, S.N., J.V. Magalhaes, C.A.P. Pacheco, M.X. Santos, T. Abadie, E.E.G. Gama, P.E.O. Guimaraes, W.F. Meirelles, M.A. Lopes, M.J.V. Vasconcelos, and E. Paiva. 2001. Heterotic groups based on yield-specific combining ability data and phylogenetic relationship determined by RAPD markers for 28 tropical maize open pollinated varieties. *Euphytica* 121: 197–208.

Parvez, S. 2006. Recent advances in understanding genetic basis of heterosis in rice (*Oriza sativa* L.). *Revista Científica UDO Agricola* 6: 1-10.

Pswarayi, A., and B. Vivek. 2008. Combining ability amongst CIMMYT's early maturing maize (*Zea mays* L.) germplasm under stress and non-stress conditions and identification of testers. *Euphytica* 162: 353-362.

Qi-Lun, Y., F. Ping, K. Ke-Cheng, and P.G. Tang. 2008. Genetic diversity based on SSR markers in maize (*Zea mays* L.) landraces from Wuling mountain region in China. *Journal of Genetics* 87: 287-291.

Rajendran, A., A. Muthiah, J. Joel, P. Shanmugasundaram, and D. Raju. 2014. Heterotic grouping and patterning of quality protein maize inbreds based on genetic and molecular marker studies. *Turkish Journal of Biology* 38: 10-20.

Rawlings, J.O., and D.L. Thompson. 1962. Performance level as criterion for the choice of maize testers. *Crop Science* 2: 217-220.

Reif, J.C., A.R. Hallauer, and A.E. Melchinger. 2005. Heterosis and heterotic patterns in maize. *Maydica* 50: 215-223.

Ren, X., H. Jiang, Z. Yan, Y. Chen, X. Zhou, L. Huang, Y. Lei, J. Huang, L. Yan, Y. Qi, W. Wei, and B. Liao. 2014. Genetic diversity and population structure of the major peanut (*Arachis hypogaea* L.) cultivars grown in China by SSR Markers. *PloS One* 9: 1-10.

Rhoads, F.M., and J. Bennett. 1990. Corn. pp 569-596. In: Stewart, B.A., and D. Nielsen (eds.). Irrigation of agricultural crops. ASA-CSSA-SSSA, Madison.

Richard, C., D.S. Osiru, M.S. Mwala, and T. Lubberstedt. 2016. Genetic diversity and heterotic grouping of the core set of southern African and temperate maize (*Zea mays* L.) inbred lines using SNP markers. *Maydica* 61: 1-9.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. In: Studies genetics VII, no. 7213. University of Texas Publication, Austin.

Roy, S., A. Banerjee, B. Mawkhlieng, A.K. Misra, A. Pattanayak, G.D. Harish, S.K. Singh, S.V. Ngachan, and K.C. Bansal. 2015. Genetic diversity and population structure in aromatic and quality Rice (*Oryza sativa* L.) landraces from North-Eastern India. *PLoS ONE* 10: 1-13.

Russell, W.A. 1961. A comparison of five types of testers in evaluating the relationship of stalk rot in corn inbred lines and stalk strength of the lines in hybrids combinations. *Crop Science* 1: 393-397.

Schussler, J.R, and M.E. Westgate. 1995. Assimilate flux determines kernel set at low water potential in maize. *Crop Science* 35:1074-1080.

Sellammal, R., S. Robin, and M. Raveendran. 2014. Association and heritability studies for drought resistance under varied moisture stress regimes in backcross inbred population of rice. *Rice Science* 21: 150-161.

Semagn, K., C. Magorokosho, B.S. Vivek, D. Makumbi, Y. Beyene, S. Mugo, B.M. Prasanna, and M.L. Warburton. 2012. Molecular characterization of diverse CIMMYT maize inbred lines from Eastern and Southern Africa using single nucleotide polymorphic markers. *BMC Genomics* 13: 1-13.

Setimela, P.S., B. Vivek, M. Banziger, J. Crossa, and F. Maideni. 2007. Evaluation of early to medium maturing open pollinated maize varieties in SADC region using GGE biplot based on the SREG model. *Field Crops Research* 103: 161–169.

Setimela, P.S., C. Magorokosho, R. Lunduka, E. Gasura, D. Makumbi, A. Tarekegne, J.E. Cairns, T. Ndhlela, O. Erenstein, and W. Mwangi. 2017. On-farm yield gains with stress-tolerant maize in Eastern and Southern Africa. *Agronomy Journal* 109: 406-417.

Setimela, P.S., Z. Chitalu, J. Jonazi, A. Mambo, D. Hodson, and M. Banziger. 2005. Environmental classification of maize testing sites in the SADC region and its implication for collaborative maize breeding strategies in the subcontinent. *Euphytica* 145: 123–132.

Shaibu, A.S., A.A. Adnan, and J.M. Jibrin. 2016. Stability performance of extra early maturing maize (*Zea mays* L.) varieties under high and low Nitrogen environments in Sudan Savanna. *Cogent Food & Agriculture* 2: 1-12.

Shaw, R. 1988. Climate requirement. pp 609-638. In: Sprague, G.F., and J.W. Dudley (eds.). Corn and Corn Improvement. American Society of Agronomy, Madison.

Shull, G.F. 1908. The composition of a field of maize. *Heredity* 4: 296–301.

Shull, G.F. 1952. Beginnings of the heterosis concept. In: Gowen, J.W (ed.) Heterosis. Iowa State College Press. Ames, IA. Iowa State College Press, Ames, IA.

Smith, O.S. 1986. Covariance between line per se and testcross performance. *Crop Science* 26: 540-543.

Sprague, G.F., and L.A. Tatum. 1942. General versus specific combining ability in single crosses of corn. *American Society of Agronomy* 34: 923-932.

Springer, N.M., and R.M. Stupar. 2007. Allelic variation and heterosis in maize: How do two halves make more than a whole? *Genome Research* 17: 264-275.

Srdic, J., S.M. Drinic, Z. Pajic, and M. Filipovic. 2007. Characterisation of maize inbred lines based on molecular markers, heterosis and pedigree data. *Genetica* 39: 355-363.

Sserumaga, J.P., S.O. Oikeh, S. Mugo, G. Asea, M. Otim, Y. Beyene, G. Abalo, and J. Kikafunda. 2016. Genotype by environment interactions and agronomic performance of doubled haploids testcross maize (Zea mays L.) hybrids. *Euphytica* 207: 353-365.

Suwarno, W.B., K.V. Pixley, N. Palacios-Rojas, S.M. Kaeppler, and R. Babu. 2014. Formation of heterotic groups and understanding genetic effects in a provitamin A biofortified maize breeding program. *Crop Science* 54: 14-24.

Tams, S.H., E. Bauer, G. Oettler, A.E. Melchinger, and C.C. Schon. 2006. Prospects for hybrid breeding in winter triticale: II. Relationship between parental genetic distance and specific combining ability. *Plant Breeding* 125: 331-336.

Thomas, N., S. Marker, G.M. Lal, and A. Dayal. 2017. Study of heterosis for grain yield and its components in wheat (*Triticum aestivum*) over normal and heat stress condition. *Pharmacognosy and Phytochemistry* 6: 824-830.

Thomson, M.J. 2014. High-Throughput SNP Genotyping to accelerate crop improvement. *Plant Breeding and Biotechnology* 2: 195-212.

Tiwari, D.K., P. Pandey, S.P. Giri, and J.L. Dwivedi. 2011. Heterosis studies for yield and its components in rice hybrids using CMS system. *Asian Journal of Plant Sciences* 10: 29-42.

Tyagi, A.P., and P. Lal. 2005. Line x tester analysis in sugar cane (*Saccharum officinarum*). *South Pacific Journal of Natural Science* 23: 30-36.

Uddin, M.S., F. Khatun, S. Ahmed, M.R. Ali, and S.A. Bagum. 2006. Heterosis and combining ability in corn (*Zea Mays* L.). *Bangladesh Journal of Botany* 35: 109-116.

Ulaganathan, V., R. Vinoth, K. Baghyalakshmi, S.R. Chimili, and A. Gurusamy. 2015. Standard heterosis for grain yield and other gronomic characters in maize (*Zea mays* L.) under normal and moisture stress conditions. *The Bioscan* 10: 1251-1253.

Umar, U.U., S.G. Ado, D.A. Aba, and S.M. Bugaje. 2014. Estimates of combining ability and gene action in maize (*Zea mays* L.) under water stress and non-stress conditions. *Journal of Biology, Agriculture and Healthcare* 4: 247-253.

Vasal, S.K., G. Srinivasan, S. Pandey, H.S. Cordova, G.C. Han, and C. Gonzalez. 1992. Heterotic patterns of ninety-two white tropical CIMMYT maize lines. *Maydica* 37: 259-270.

Vasal, S.K., H.S. Cordova, D.L. Beck, and G.O. Edmeades. 1997. Choices among breeding procedures and strategies for developing stress-tolerant maize germplasm. pp 336-347. In: Edmeades, G.O., M. Banziger, H.R. Mickelson, and C.B. Pena-Valdivia (eds.). Developing drought and low n-tolerant maize. Proceedings of a symposium, March 25-29, 1996. CIMMYT, El Batan, Mexico, D.F.

Vaz Patto, M.C., Z. Satovic, S. Pego, and P. Fevereiro. 2004. Assessing the genetic diversity of Portuguese maize germplasm using microsatellite markers. *Euphytica* 137: 63-72.

Vikram, P., J. Franco, J. Burgueno-Ferreira, H. Li, D. Sehgal, C.S. Pierre, C. Ortiz, C. Sneller, M. Tattaris, C. Guzman, C.P. Sansaloni, M. Ellis, G. Fuentes-Davila, M. Reynolds, K. Sonder, P. Singh, T. Payne, P. Wenzl, A. Sharma, N.S. Bains, G.P. Singh, J. Crossa, and S.Singh. 2016. Unlocking the genetic diversity of Creole wheats. *Scientific Reports* 6.

Virginia Tech. 2009. Using Heritability for Genetic Improvement. Communications and Marketing, College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University.

Weber, V.S., A.E. Melchinger, C. Magorokosho, D. Makumbi, M. Banziger, and G.N. Atlin. 2012. Efficiency of managed-stress screening of elite maize hybrids under drought and low Nitrogen for yield under rainfed conditions in Southern Africa. *Crop Science* 52: 1011–1020.

Wegary, D., B.S. Vivek, and M.T. Labuschagne. 2014. Combining ability of certain agronomic traits in quality protein maize under stress and non-stress environments in Eastern and Southern Africa. *Crop Science* 4: 1004–1014.

Wen, W., J. Franco, V.H. Chavez-Tovar, J. Yan, and S. Taba. 2012. Genetic characterization of a core set of a Tropical maize race Tuxpeno for further use in maize improvement. *PLoS ONE* 7.

Witcombe, J.R., P.A. Hollington, C.J. Howarth, S. Reader, and K.A. Steele. 2008. Breeding for abiotic stresses for sustainable agriculture. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 363: 703-716.

Xia, X.C., J.C. Reif, A.E. Melchinger, M. Frisch, D.A. Hoisington, D. Beck, K. Pixley, and M.L. Warburton. 2005. Genetic diversity among CIMMYT maize inbred lines investigated with SSR Markers. *Crop Science* 45: 2573-2582.

Xiong, L., R.G. Wang, G. Mao, and J.M. Koczan. 2006. Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid. *Plant physiology* 142: 1065-1074.

Yan, W. 2001. GGE Biplot- A windows application for graphical analysis of multi- environment trial data and other types of two-way data. *Agronomy Journal* 93: 1111-1118.

Yan, W., L.A. Hunt, Q. Sheng, and Z. Szlavnics. 2000. Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Science* 40: 597–605.

Yu, J., H. Lu, and R. Bernardo. 2001. Inconsistencies between SSR grouping and genetic background of white corn inbreds. *Maydica* 46: 133-139.

Zambezi, B.T., and C. Mwambula. 1997. The impact of drought and low soil Nitrogen on maize production in the SADC region. pp 29-33. In: Edmeades, G.O., M. Banziger, H.R. Mickelson, and C.B. Pena-Valvidia (eds.). Developing drought- and Low N-tolerant maize. Proceedings of a Symposium, March 25 – 29, 1996. CIMMYT, El Batan, Mexico, D.F.

Ziyomo, C., and R. Bernardo. 2012. Drought tolerance in maize: Indirect selection through secondary traits versus genomewide selection. *Crop Science* 52: 1269–1275.

Zobel, R.W., M.J. Wright, and H.G. Gauch. 1988. Statistical analysis of a yield trial. *Agronomy Journal* 80: 388-393.

CHAPTER 3

Heterotic orientation of South African public maize inbred lines towards tropical CIMMYT and temperate USA-Corn Belt testers

Abstract

An efficient heterosis breeding program defines and utilises few heterotic groups. Currently, a public maize breeding program in South Africa uses seven heterotic groups, a fairly large number. Furthermore, South Africa falls within subtropical and near warm temperate environments, hence both tropical and temperate maize germplasm is utilised. This study aimed at determining the alignment of South African public maize inbred lines to the public inbred lines testers from the tropical International Maize and Wheat Improvement Center (CIMMYT) and the temperate USA Corn Belt sources. A total of 42 inbred lines were selected by randomly picking six inbred lines from each of the seven heterotic groups from the Agricultural Research Council (ARC) of South Africa. The 42 inbred lines were genotyped with 56110 single nucleotide polymorphism (SNP) markers, using the Illumina MaizeSNP50 Bead chip in the ARC laboratory. The SNP data were used to calculate Gower's genetic distance and hierarchical clustering was done using the average linkage algorithm. The 42 lines were also crossed to four inbred line testers. Testers represented groups A and B for tropical CIMMYT (CML312 and CML444, respectively) and temperate USA Corn Belt (B73 and MO17, respectively). The testcross hybrids were evaluated under stress and non-stress conditions during the 2014/15 and 2015/16 summer seasons in South Africa. Mean squares for grain yield for genotypes and specific combining ability (SCA) were significantly different ($P<0.05$) in 2015/16 season. A poor correlation between genetic distance with grain yield and specific combining ability indicated that productivity of lines could not be predetermined based on genetic distances. The SCA effects, classified inbred lines into heterotic groups A, B and AB in addition to those that could not be grouped. Meanwhile SNP marker data divided inbred lines into 11 clusters that could be simplified into three groups of normal maize endosperm and two groups of quality protein maize. This information is useful in reorganising the ARC breeding germplasm from seven to two major heterotic groups based on biochemical composition of the grain, thereby making the breeding program more efficient.

Keywords: Heterotic groups; maize inbred lines; single nucleotide polymorphism; specific combining ability; Temperate and Tropical maize testers

3.1 Introduction

Maize is the principal crop in sub-Saharan Africa, including South Africa. It serves as both the major feed grain and the staple food for the majority of the South African population (DAFF, 2014). The crop is produced from the equator within and across the tropics of Cancer and Capricorn in Africa. This suggests that germplasm with adaption to tropical and subtropical conditions would be viable for developing productive hybrids under challenging conditions of heat and drought stress among many other factors that impact on yield. The challenges of climate change that modify these factors call for continuous development of new maize hybrids. However, breeding programs across Africa are not churning new hybrids at the rate that would improve food security in the region. This could be attributed partly to limited knowledge of the germplasm and haphazard use of local and external germplasm in breeding programs. There is limited literature that dwells on heterotic groups and patterns that can be utilised in Africa and other tropical environments elsewhere.

Advances in maize breeding have an important role in continually developing and deploying new maize cultivars, exhibiting improved grain yield and desirable agronomic characters. The strength and success of any breeding program depends on a well-established and properly arranged germplasm (Rajendran *et al.*, 2014), which provides potential exploitation of maximum heterosis in crosses (Bidhendi *et al.*, 2012; Librando and Magulama, 2008). Identification of heterotic groups (HGs) and heterotic patterns is the principal step towards developing a well-established germplasm (Barata and Carena, 2006; Delucchi *et al.*, 2012). Melchinger and Gumber (1998) defined a heterotic group as a group of related or unrelated genotypes from the same or different populations, which show similar combining ability and heterotic response when crossed with genotypes from other genetically different groups, whereas heterotic pattern is observed when a cross between a specific pair of two heterotic groups expresses high level of heterosis.

Classification of germplasm into heterotic groups offers several advantages in most breeding programs. When inbred lines from different known and unknown origins are used, a large number of hybrid combinations are made which are later evaluated in multi-environments (Bidhendi *et al.*, 2012). However, this is labour-intensive, lengthy and costly (Aguiar *et al.*, 2008) to the programs with limited funding especially in Africa, as many undesirable crosses are discarded. However, when heterotic groups are formed, undesirable combinations are avoided by crossing inbred lines from the existing and divergent groups (Aguiar *et al.*, 2008). This allows the exploitation of maximum heterosis (Melchinger and Gumber, 1998) among selected lines in hybrid combination, reduces the chance of missing superior hybrids and subsequently improves breeding efficiency. According to Fan *et al.* (2009) breeding efficiency is the percentage of superior hybrids, which are obtained relative to the total number of crosses made between lines from distinct heterotic groups in a breeding program. The employment of simple heterotic groups would simplify germplasm management and improvement (Fan *et al.*, 2003). Selecting parental lines from defined heterotic groups is rapid and efficient (Kanyamasoro *et al.*, 2012). The information on simplified heterotic orientation is therefore crucial for an efficient breeding program.

Given the foregoing, several methods have been employed in determining heterotic groupings. These include pedigree analysis (Barata and Carena, 2006; Semagn *et al.*, 2012; Srdic *et al.*, 2007), which is regarded as the most reliable method. The limitation in Africa and other developing regions with weak plant breeding informatics is that the data is not always available for breeders to use. Alternatively, the pedigree data is not clear and is often insufficient, and at worst the germplasm gets mixed up over the years. Laboratory methods such as DNA markers can be utilised to augment pedigree data where it exists or to establish genetic clusters quickly when pedigree data does not exist or it is just “messy”. This is reliable provided the number of markers used is sufficient and polymorphic with a wide coverage of the genome. Additionally, molecular markers would help in determining the level of genetic diversity in the germplasm. Many previous researchers have employed DNA molecular markers in studying maize genetic clusters (Adetimirin *et al.*, 2008; Aslam *et al.*, 2009; Badu-Apraku *et al.*, 2013; de Pinto *et al.*, 2003; Dhliwayo *et al.*, 2009; Nyombayire *et al.*, 2016; Qi-Lun *et al.*, 2008; Suwarno *et al.*, 2014). Thirdly, heterotic orientation of maize germplasm has been established by studying heterotic performance and variance of F_1 hybrids. This includes use of cluster analysis which is based on specific combining ability (SCA) effects data of a set of lines which is drawn from a population or different populations (Barata and Carena, 2006; Fan *et al.*, 2003; Fan *et al.*, 2004; Fan *et al.*, 2010; Librando and Magulama, 2008; Menkir *et al.*, 2004; Parentoni *et al.*, 2001; Rajendran *et al.*, 2014; Reif *et al.*, 2005; Vasal *et al.*, 1992). In this case, the lines are classified based on the direction (which could be positive or negative) of the SCA value between two lines or between a line and the tester. In principle, lines that show negative SCA effects for grain yield when crossed together belong to the same heterotic group, whereas lines that have positive SCA in hybrid combination would be considered to belong to different heterotic groups. The lines in the same group are expected to have similar gene frequencies (Vasal *et al.*, 1992), while those with positive SCA have different gene frequencies. Consequently, programs keep maize germplasm in different groups to maximize SCA effects in hybrid combinations. The underlying assumption is that the SCA of two lines from distinct heterotic groups is always superior to that of lines from the same heterotic group (Fan *et al.*, 2003). Whereas, germplasm has been maintained in a few distinct heterotic groups with a positive impact on productivity of hybrids in the USA Corn Belt, this has not been the case in other regions where the maize industry is young, non-existent or is still evolving from the open-pollinated to the three-way crosses era and is yet to reach the ultimate single-cross hybrid technology.

Knowledge of combining ability among lines in the program is paramount in breeding programs. Two types of combining ability, general combining ability (GCA) and specific combining ability (SCA) are used to designate the ability of parents to combine amongst each other during hybridization, such that desirable genes are transmitted to their progenies (Fasahat *et al*., 2016). General combining ability denotes the average performance of an inbred line in a series of hybrid combinations, while SCA refers to those cases in which certain hybrid combinations perform relatively better or worse than would be expected on the basis of the average performance of the parental lines involved (Sprague and Tatum, 1942). A survey of literature indicates that SCA data is an important statistical tool for classifying maize germplasm into heterotic groups. The SCA data has a high predictive value for F_1 grain yield than heterosis data (Betran *et al.*, 2003). However, the SCA data can be used together with other methods that are cheaper and even more precise. The other methods, for use in classification of germplasm lines including genotyping molecular markers and pedigree analysis has been widely reported in literature (Aguiar *et al.*, 2008; Badu-Apraku *et al.*, 2013; Barata and Carena, 2006; de Pinto *et al.*, 2003; Fan *et al.*, 2003; Fan *et al.*, 2004; Menkir *et al.*, 2004; Parentoni *et al.*, 2001; Rajendran *et al.*, 2014). Whereas, there are many methods for estimating SCA, the line by tester method utilising a fewer number of divergent testers of known heterotic groups has been the most widely used (Aguiar *et al.*, 2008; Fan *et al.*, 2010; Li *et al.*, 2007; Menkir *et al.*, 2003; Rajendran *et al.*, 2014; Vasal *et al.*, 1992). This is because it can be easily and cheaply used to test the combining ability of many lines in large programs. The use of different tools in studying combining ability has been reviewed in detail by Fasahat *et al*. (2016).

The maize industry is built on products that are derived from temperate and tropical germplasm. A combination of both can be utilised to enhance diversity in the program. Most of the world's maize breeding programs have exploited the well-known heterotic groups, Iowa Stiff Stalk Synthetic (BSSS) and Lancaster Sure Crop (LSC) of the US Corn Belt (Melani and Carena, 2005). This is partly attributed to the fact that the leading maize producing countries such as the USA, China and Argentina are located in the temperate environment. In the tropics, use of CIMMYT germplasm has undoubtedly become one of the best sources of increasing genetic diversity (Aguiar *et al.*, 2008; Fan *et al.*, 2003). In the Eastern and Southern Africa region, large proportion of the germplasm used in hybrid development programs is introduced from CIMMYT. The CIMMYT germplasm have broad genetic base, thus potential hybrids with wide adaptation are developed (Han *et al*., 1991; Vasal and Srinivasan, 1991) as cited by Vasal *et al*. (1992). The CIMMYT program uses a well-defined and narrow heterotic group pool, which classifies germplasm into A, or B and AB heterotic groups. Due to the large amount of CIMMYT germplasm which is used in Eastern and Southern African breeding programs, and for simplicity, most private and public breeding programs have adopted the CIMMYT heterotic group scheme. The fewer number of heterotic groups is more desirable and easy to work with compared to many heterotic groups that are employed in the region.

The public maize program in South Africa, which is based at the Agricultural Research Council (ARC) of South Africa works with seven heterotic groups which have been designated as the F, I, K, L, M, P and R since the Saunders and Gevers era (Gevers and Lake, 1998). These heterotic groups have been utilised extensively in different combinations to develop commercial maize hybrids (Fourie and Gevers, 1987; Gevers and Whythe, 1987). This has been criticised because use of a large number of heterotic groups from different populations complicates the breeding process and decision making during selection of parental lines. The employment of many heterotic groups is also associated with several major drawbacks including high costs of making and evaluating hybrids, extended timeline to obtain results (Aguiar *et al.*, 2008; Bidhendi *et al*., 2012; de Pinto *et al.*, 2003) and subsequent reduction in breeding efficiency (Fan *et al*., 2014). Therefore, the objectives of the study were to check whether there could be an easy simplification of the heterotic grouping of public maize germplasm in South Africa on the basis of their orientation towards tropical CIMMYT and USA temperate Corn Belt testers, given that South Africa falls within subtropical to warm temperate conditions, which are comparable to Argentina in the Southern hemisphere.

3.2 Materials and Methods

3.2.1 Germplasm

A set of 42 maize inbred lines were selected from the Agricultural Research Council maize germplasm, to represent the seven known heterotic groups which are currently utilised by the public program in South Africa. Six representative lines were randomly selected from each heterotic group. The seven heterotic groups were described as in Table 3.1 by forebearers of the public maize programs in the region, such as Saunders, Gevers, Olver, Fourie and others (Gevers and Lake, 1998; Gevers and Whythe, 1987; Olver, 1998) and Fourie (2017, personal communication). According to the literature, the inbred lines used were developed from germplasm which was introduced from the USA, Australia and also mainly from the local open-pollinated varieties which mostly originated from the USA-Corn Belt (Saunders, 1942) as cited by Gevers (1997), suggesting possibility of infusion of temperate and tropical genetic backgrounds. Therefore, it was prudent that the 42 lines were crossed with four testers, representing the heterotic Groups A and B of both tropical and temperate origin. The heterotic Group A was represented by the CIMMYT tropical Group A tester (CML312), and the temperate Group A (B73) tester of the USA Corn Belt, and their heterotic Group B counterparts (CML444 and MO17, respectively). These testers and their derivatives have been widely used worldwide. The pedigrees of all inbred lines used in the study have been analysed and the data are shown in Tables 3.1 and 3.2.

Table 3.1: Description of the South African, CIMMYT and USA Corn Belt heterotic groups

Source	**Heterotic group**	**Original germplasm code**	**Pedigree analysis**
	South African public germplasm classification, since the Saunders and Gevers era (Gevers and Lake, 1998)		
South Africa[a]	F	F2834T	Derivatives from a yellow South African open-pollinated variety (Teko Yellow).
South Africa[a]	I	I37TN	Yellow inbred line, derived from a yellow South African open-pollinated variety (Teko Yellow).
South Africa[a]	K	K64R/M162W	A US Kansas State University release.
South Africa[a]	L	Lancaster	MO17 derivative, A University of Missouri inbred line derived from Lancaster Surecrop, an old US open-pollinated variety.
South Africa[a]	M	M37W	M37W white derivative from 21A.Jellicorse, a yellow Australian inbred.
South Africa[a]	P	Natal Potchefstroom Pearl (NPP)	An old open-pollinated South African variety.
South Africa[a]	R	Reid	Reid's Yellow Dent, a US Corn Belt open-pollinated variety, from which the US inbred lines B73 and B37 were developed.
CIMMYT tropical inbred line tester[b]	A	CML312	According to CIMMYT this cluster corresponds to the Tuxpeno, Kitale, BSSS and N3* germplasm types, which is of more dent grain texture.

Source	**Heterotic group**	**Original germplasm code**	**Pedigree analysis**
CIMMYT tropical inbred line tester[b]	B	CML444	This is the counterpart of the cluster described above, which corresponds to the ETO, Ecuador 573, Lancaster and the SC* germplasm, which is more of a flint type texture
USA Corn Belt temperate (University of Missouri) tester[c]	A	B73	Stiff Stalk Synthetic (BSSS)
USA Corn Belt temperate (Iowa State University) tester[c]	B	MO17	Lancaster Sure Crop

The N3 and SC germplasm and heterotic pattern has been reviewed and discussed in detail by Derera and Musimwa (2015) and Musimwa and Derera (2015);** ([a]Gevers and Lake, 1998; [b]CIMMYT, 2001; [c]Gerdes et al., 1993)*

The B73 and MO17 maize testers are temperate inbred lines representing the US Reid and Lancaster heterotic groups, respectively. These testers were developed by the University of Missouri and Iowa State University, respectively. The CML312 and CML444 are CIMMYT tropical inbred lines that were developed by the CIMMYT breeding program in Mexico and Harare in Zimbabwe, respectively. The CML312 is an intermediate maturing and subtropical line that is drought tolerant, resistant to grey leaf spot (GLS), turcicum leaf blight (TLB) and *Exerohilum turcicum* (E.Turc), but it is susceptible to maize streak virus (MSV). The CML444 is a late maturing and mid-altitude or subtropical adapted inbred line. It is drought and low nitrogen tolerant, but MSV and TLB susceptible (CIMMYT, 2001). The seed used was obtained from the ARC germplasm bank, but some lines were originally introduced from the CIMMYT - Zimbabwe breeding program. The F_1 testcrosses were made at the Makhathini Research Station, in South Africa during winter (April/May) season of 2014 and 2015.

Table 3.2: Description of maize lines and testers used and their respective heterotic groups

Entry code	Inbred line name	Source	Pedigree	SA Heterotic group
1	B1138T	*ARC, South Africa	TEKOYELLOW	F
2	BO394Y	ARC, South Africa	F2834T.4O2	F
3	E30Y	ARC, South Africa	B390YxM136Y	F
4	RO544W	ARC, South Africa	BO160W.3J400W	F
5	UO705Y	ARC, South Africa	YOFE1(S4)	F
6	VO430Y	ARC, South Africa	(HO466Y.1BO310Y)	F
7	I-16	ARC, South Africa	I-16	I
8	I-20	ARC, South Africa	I-20	I
9	I-34	ARC, South Africa	I-34	I
10	I-39	ARC, South Africa	I-39	I
11	I-41	ARC, South Africa	I-41	I
12	I-42	ARC, South Africa	I-42	I
13	K64	ARC, South Africa	Pride off Saline	K
14	K64R-22	ARC, South Africa	K64R-22	K
15	M162W	ARC, South Africa	K64R2.B1138T	K
16	R2565Y	ARC, South Africa	K64R2(I137TN1.K64R)	K
17	U127Y	ARC, South Africa	M162W.1KO326Y	K
18	U2540W	ARC, South Africa	M162W1.DO940Y-J34	K
19	MO17HtHtN	ARC, South Africa	MO17HtHtN	L
20	NC258	ARC, South Africa	NC258	L
21	P588MSV	ARC, South Africa	MRSxVHMO17	L
22	P590MSV	ARC, South Africa	MRSxVHMO17	L
23	P594MSV	ARC, South Africa	MLSxVHMO17	L
24	P598MSV	ARC, South Africa	21A-6xVHMO17	L
25	J80W	ARC, South Africa	D800W2.HtN	M
26	RO421W	ARC, South Africa	DO940Y-11.O2(W)	M
27	RO452W	ARC, South Africa	DO940Y-13.NHK	M
28	SO181Y	ARC, South Africa	KO326Y2.NPPES1	M
29	SO503W	ARC, South Africa	KO315Y2.NPPES1	M
30	VO617Y	ARC, South Africa	(1)"M37W.TE/TEO"	M
31	FO215W	ARC, South Africa	NPPES14.O2S14	P
32	SO607W	ARC, South Africa	POWS1(S4)	P
33	SO713W	ARC, South Africa	POWS1(S4)	P
34	VO495Y	ARC, South Africa	POWS12.Y	P
35	VO500Y	ARC, South Africa	POWS12.Y	P
36	VO501Y	ARC, South Africa	POWS12.Y	P
37	P28	ARC, South Africa	P28	R

Entry code	Inbred line name	Source	Pedigree	SA Heterotic group
38	P612MSV	ARC, South Africa	B73xVHKG/C1	R
39	P614MSV	ARC, South Africa	B73xVHKG/C1	R
40	S198Y	ARC, South Africa	M28Y1.DO620Y	R
41	SO1224Y	ARC, South Africa	M28Y1.KO288Y	R
42	U71Y	ARC, South Africa	M28Y2.NP	R
	Inbred line testers			
1	B73	Iowa State University-USA	BSSS C5 (Iowa Stiff Stalk Synthetic)	Temperate A
2	MO17	University of Missouri-USA	(CL.187-2 x C103)	Temperate B
3	CML312	CIMMYT-Zimbabwe	S89500-F2-2-2-1-1-B	Tropical A
4	CML444	CIMMYT-Zimbabwe	P43-C9-1-1-1-1-1-B	Tropical B

**ARC – Agricultural Research Council of South Africa (SA); USA – United States of America*

3.2.2 Experimental design and management

The field experiments were conducted under drought, low N stress and non-stress environments in South Africa, during 2014/15 and 2015/16 growing seasons. Locations used, their long-term weather data and growing seasons data are shown in Table 3.3. The long-term weather data shown is for 29 growing seasons, except Makhathini with 26 seasons. All stress and non-stress trials were laid out in a (0, 1) α-lattice design with two replicates. Each entry was a two-row plot of 4 m length, and the intra- and inter-row spacings were 0.25 m and 0.75 m, respectively. Thirty-four seeds (two per hill) were initially sown and later thinned to 17 vigorous plants per row to achieve 53 333 plants ha^{-1}. Non-stress trials received optimal fertilisation and supplementary irrigation. The sprinkler irrigation system was used in all experimental sites except Cedara which was under rain-fed conditions. Each trial received irrigation at the interval of 7 days for four hours (5 mm hr^{-1}) until physiological maturity, except under drought environments. A compound fertiliser (NPK, 3:2:1) was applied as a basal fertiliser prior to sowing, at a rate of 25 kg N ha^{-1}, 17 kg P ha^{-1} and 8 kg K ha^{-1}. Lime ammonium nitrate (33% N) was applied as a top dressing at a rate of 150 kg ha^{-1}, at four weeks after crop emergence.

Screening for drought tolerance was achieved under random and managed drought stress conditions; random drought trials received adequate fertilisation as applied in non-stress blocks but no supplementary irrigation during the growing season, irrigation was only applied at the beginning of the season to establish a good plant stand. Drought stress was managed as proposed by CIMMYT (Banziger *et al.*, 2000). Trials were conducted during a rain-free period, with irrigation applied adequately until it was withheld from two weeks before expected flowering, so that the crop suffered drought stress during the most critical flowering and grain-filling stages. In the 2015/16 season, there were irrigation problems that coincided with flowering and grain filling periods at Vaalharts, the site was therefore treated as combined low N plus drought stress.

Low N stress trials were conducted in fields that had been previously depleted of N by growing unfertilised, non-leguminous crops (oats, wheat and sorghum) at high density for several seasons and removing the crop biomass after each season, except Vaalharts site, with sandy soil texture and thus, naturally depleted. For low N stress experiments, triple super phosphate (46% P_2O_5) and potassium chloride (61% K_2O) were applied at planting at a rate of 25 kg P_2O_5 ha^{-1} and 25 kg KCL ha^{-1} with no further top dressing. Weeds were controlled using pre-emergence herbicide, Bateleur Gold 650EC (Flumetsulam (sulfonanilide), s – metalachlor), at 1.3 L ha^{-1} and post-emergence herbicide, Basagran (480 g/L bendioxide (thiadiazine)) at 2.5 L ha^{-1} herbicides, and augmented by occasional manual weeding when needed. Insecticides were controlled using Karate (50g/L lambda-cyhalothrin) at 70 ml ha^{-1}.

Table 3.3: Weather data and geographic information for the study locations used during 2014/15 and 2015/16 growing seasons

			Geographical position			**Annual Rainfall (mm)**			***Temperature (^{0}C)***					
Environment	**Site**	**Season**	Latitude	Longitude	Altitude (m.a.s.l.)	Long-term	2014/15	2015/16	Min			Max		
									Long-term	2014/15	2015/16	Long-term	2014/15	2015/16
NS and DT	Potchefstroom	Summer	26.74^{0} S	27.08^{0} E	1349	541	519	364	15	14	16	29	29	31
NS and LN	Cedara	Summer	29.54^{0} S	30.26^{0} E	1068	662	619	521	14	13	14	25	25	27
Combined LN+DT	Vaalharts/Taung	Summer	27.95^{0} S	24.84^{0} E	1180	356	214	239	15	15	16	32	34	35
DT	Makhathini	Winter	27.39^{0} S	32.18^{0} E	77	153	127		14	9		28	29	

masl - metres above sea level (m); NS - non-stress; DT- drought stress; LN - Low nitrogen stress

3.2.3 SNP Genotyping

The 42 maize inbred lines were planted in the glasshouse for DNA sampling. Leaf samples bulked from four plants of each inbred were taken at two weeks after planting. Genomic DNA was extracted at the Agricultural Research Council-Grain Crops, following a modified CTAB procedure (Doyle, 1987). DNA samples were sent to the Agricultural Research Council-Biotechnology platform in South Africa for genotyping using the single nucleotide polymorphism (SNP) markers. Genotyping was carried out using the Illumina MaizeSNP50 Bead chip, which was derived from the B73 reference genome (www.illumina.com). The protocol provided 56110 SNP markers, which were densely covering all the ten chromosomes of the maize genome. The genetic distance (dissimilarity) estimates among inbred lines were calculated based on the Gower's distance (Gower, 1971). Genotypes were subsequently grouped by the Unweighted Pair Group Method using Arithmetic average (UPGMA) algorithm, using the R studio Software version 3.3 (RStudio Team, 2015). The inbred lines I-20 and M162W representing heterotic group I and K of South Africa, respectively, had contaminated DNA and therefore were not included in the SNP analyses.

3.2.4 Data Collection and statistical analyses

Data on grain yield and associated agronomic traits were recorded. Grain yield per plot was estimated and converted to tonnes ha^{-1} at 12.5 % moisture content. Analysis of variance (ANOVA) was performed on plot means for grain yield across sites for each season. Lines that did not cross successfully with all testers were not included in the analysis; 13 lines by three testers were extracted in 2014/15 and 18 lines with all 4 testers were analysed in 2015/16. Specific combining ability effects for grain yield were estimated using the line by tester procedure embedded in the Analyses of Genetic Designs software with 'R' (AGD-R, version 3.0) (Rodriguez *et al.*, 2015). Lines and testers were treated as random effects, the replications, genotypes and sites were treated as fixed effects. The statistical model used for the combined analysis is as follows:

$$Y_{ijkl}=\mu + L_i + T_j + (L\times T)_{ij} + E_l + (L\times E)_{il} + (T\times E)_{jl} + R_k(E_l) + (L\times T\times E)_{ijl} + e_{ijkl}$$

Where Y_{ijkl} is the measured trait of the testcross of the i^{th} line crossed to j^{th} tester in the l^{th} environment and k^{th} replicate; μ is the grand mean; L_i is the effect of the i^{th} line; T_j is the effect of the j^{th} tester; E_l is the effect of the l^{th} environment; $(L\times T)_{ij}$ is the interaction effect of the cross between the i^{th} line and the j^{th} tester; $(L\times E)_{il}$ is the interaction effect of the i^{th} line and the l^{th} environment; $(T\times E)_{jl}$ is the interaction effect of the j^{th} tester and the l^{th} environment; R_k (E_l) is the effect of the k^{th} replicate nested in the l^{th} environment; $(L\times T\times E)_{ijl}$ is the interaction effect of the i^{th} line, j^{th} tester and the l^{th} environment and E_{ijkl} is the random error term.

Classification of lines into heterotic groups was based on the specific combining ability (SCA) value of a line and a tester for grain yield as proposed by Vasal *et al*. (1992), where lines that show negative SCA effects for grain yield when crossed together belong to the same heterotic group and positive SCA effects with one tester indicate that lines belong to the opposite heterotic group. The lines displaying positive SCA with both testers (A and B) were allocated to the AB heterotic group and those displaying negative SCA with both testers could not be classified.

3.3 **Results**

3.3.1 Classification based on SNP markers

The UPGMA dendogram of 40 maize inbred lines and four testers is shown in Figure 3.1. The SNP markers divided the South African germplasm into 11 clusters. Clustering of germplasm was based into two principal clusters of USA Corn Belt temperate and the South African maize lines, with the South African lines more inclined towards the tropical CIMMYT than the USA temperate testers. The 11 clusters are summarised in Table 3.4.

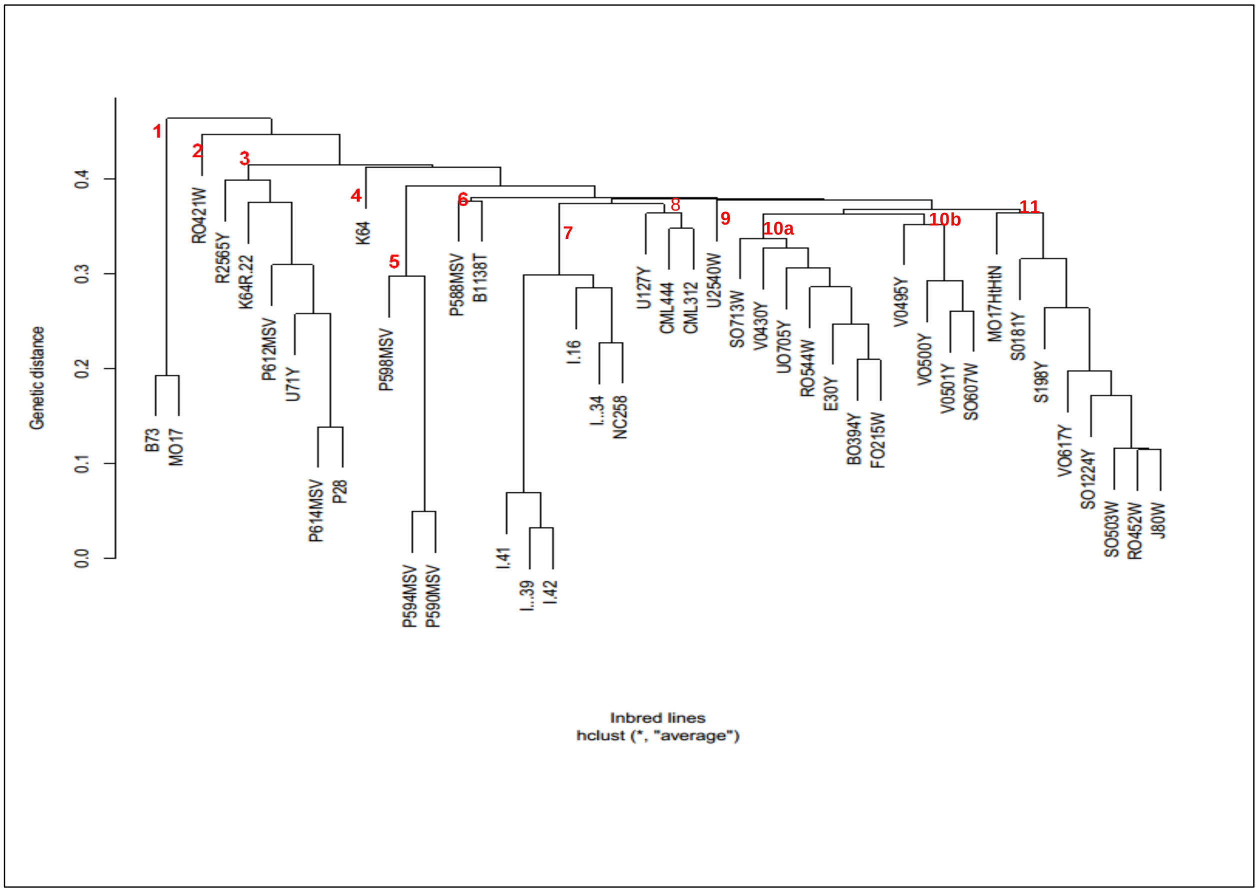

Figure 3.1: UPGMA dendogram based on the analysis of 40 maize inbred lines and four testers using the Gower's distance (Gower, 1971)

Table 3.4: Summary of the dendogram clusters and mean genetic distance between the clusters and testers

			Average genetic distance with testers			
Cluster	**Inbred lines within a cluster**	**General description of the cluster**	**B73**	**MO17**	**CML444**	**CML312**
1	B73 (A) and MO17 (B)	Temperate testers	-	-	-	-
2	RO421W (M)	A QPM line derivative of M37W, singleton cluster	0.81	0.83	0.77	0.76
3	K64R.22 (K), R2565Y (K), J80W (M), P28 (R), P612MSV (R), P614MSV (R), U71Y (R	Mainly B73 introgressed into South African lines with BC to South Africa	0.91	0.98	0.70	0.71
4	K64 (K)	Kansas inbred line, singleton cluster	0.81	0.87	0.61	0.61
5	P590MSV (L), P594MSV (L), P598MSV (L)	Derivatives of Lancaster Sure crop through introgression of MO17 into South African lines via a BC to the South African lines	0.74	0.86	0.68	0.67
6	B1138T (F), P588MSV(L)	Purely South African germplasm, with no known temperate alignment	0.83	0.92	0.55	0.55
7	I-16 (I), I-34 (I), I-39 (I), I-41 (I), I-42 (I), NC258 (L)	South African inbred lines, of mainly the I-heterotic group	0.97	1.07	0.66	0.64
8	U127Y (K), CML312 (A), CML444 (B)	Tropical lines from CIMMYT and one South African line (U127Y)	0.83	0.92	0.52	0.52
9	U2540W (K)	K64R/M162W derivative, singleton cluster	0.83	0.91	0.55	0.57
10a	BO394Y (F), E30Y (F), RO544W (F), UO705Y (F), V0430Y (F), FO215W (P), SO713W (P)	QPM lines, which were derived from the F2834T and Natal Potchefstroom Pearl (NPP)	0.91	1.01	0.60	0.61
10b	FO215W (P), SO713W (P), SO607W (P)V0495Y (P), VO500Y (P), V0501Y (P)	QPM lines, which are derivatives of the Natal Potchefstroom Pearl (NPP)	0.88	0.97	0.59	0.59
11	MO17HtHtN (L), RO452W (M), SO503W (M), VO617Y (M), S0181Y (M), S198Y R), SO1224Y R)	Reid derivatives and QPM inbred lines from predominantly the South African M-group (M37W)	0.97	1.07	0.70	0.70

The average genetic distances between lines and different testers are shown in Table 3.5. The widest (0.95) average genetic distance was between lines and the USA Corn Belt testers. The distance ranged from 0.68 and 1.19, with the minimum distance between P598MSV and B73 and the maximum between J80W and MO17. The mean genetic distance within the tropical testers was 0.80, ranging from 0.52 to 0.80. The lowest distance was estimated between U127Y and both CIMMYT testers, CML312 and CML444, while the highest was between J80W with both tropical testers, and between RO452W and CML444.

Table 3.5: Genetic distances between the maize lines and the tropical CIMMYT and USA temperate testers

Inbred line	**Current South African heterotic group**	**Pedigree**	**Average genetic distance with testers**			
			B73	**MO17**	**CML444**	**CML312**
B1138T	F	TEKO YELLOW	0.822	0.908	0.545	0.547
BO394Y	F	F2834T.4O2	0.976	1.085	0.640	0.647
E30Y	F	B390YxM136Y	0.932	1.038	0.588	0.595
RO544W	F	BO160W.3J400W	0.905	1.005	0.609	0.609
UO705Y	F	YOFE1(S4)	0.890	0.983	0.546	0.553
V0430Y	F	(HO466Y.1BO310Y)	0.885	0.990	0.575	0.577
I-16	I	I-16	0.880	0.975	0.577	0.555
I-34	I	I-34	0.909	1.007	0.594	0.575
I-39	I	I-39	1.033	1.139	0.733	0.712
I-41	I	I-41	1.011	1.109	0.708	0.688
I-42	I	I-42	1.029	1.134	0.729	0.709
K64	K	Pride off Saline	0.814	0.874	0.614	0.613
K64R.22	K	K64R-22	0.849	0.935	0.569	0.572
R2565Y	K	K64R2(I137TN1.K64R)	0.790	0.823	0.779	0.794
U127Y	K	M162W.1KO326Y	0.832	0.923	0.516	0.521
U2540W	K	M162W1.DO940Y-J34	0.834	0.908	0.554	0.565
MO17HtHtN	L	MO17HtHtN	0.826	0.922	0.564	0.569
NC258	L	NC258	0.943	1.048	0.614	0.604
P588MSV	L	MRSxVHMO17	0.838	0.925	0.555	0.558
P590MSV	L	MRSxVHMO17	0.774	0.889	0.713	0.701
P594MSV	L	MLSxVHMO17	0.778	0.893	0.709	0.698
P598MSV	L	21A-6xVHMO17	0.678	0.799	0.621	0.613
J80W	M	D800W2.HtN	1.083	1.191	0.796	0.798
RO421W	M	DO940Y-11.O2(W)	0.811	0.831	0.768	0.764

Inbred line	Current South African heterotic group	Pedigree	Average genetic distance with testers			
			B73	**MO17**	**CML444**	**CML312**
RO452W	M	DO940Y-13.NHK	1.044	1.147	0.798	0.797
SO503W	M	KO315Y2.NPPES1	1.036	1.136	0.790	0.788
VO617Y	M	(1)"M37W.TE/TEO"	1.013	1.125	0.724	0.723
S0181Y	M	KO326Y2.NPPES1	0.919	1.020	0.619	0.620
FO215W	P	NPPES14.O2S14	0.956	1.061	0.617	0.625
SO607W	P	POWS1(S4)	0.874	0.978	0.593	0.587
SO713W	P	POWS1(S4)	0.852	0.927	0.633	0.634
V0495Y	P	POWS12.Y	0.812	0.893	0.584	0.587
VO500Y	P	POWS12.Y	0.910	1.012	0.603	0.600
V0501Y	P	POWS12.Y	0.904	1.012	0.590	0.586
P28	R	P28	0.912	0.967	0.714	0.742
P612MSV	R	B73xVHKG/C1	0.878	0.962	0.613	0.631
P614MSV	R	B73xVHKG/C1	0.949	1.008	0.730	0.758
S198Y	R	M28Y1.DO620Y	0.902	1.000	0.658	0.654
SO1224Y	R	M28Y1.KO288Y	1.036	1.142	0.745	0.751
U71Y	R	M28Y2.NP	0.930	1.005	0.671	0.698
Average			**0.901**	**0.993**	**0.647**	**0.648**
Min			**0.678**	**0.799**	**0.516**	**0.521**
Max			**1.083**	**1.191**	**0.798**	**0.798**

There were generally large average genetic distances between lines within clusters, ranging from 0.31 to 0.58 (Table 3.6). The minimum distance was between the temperate inbred lines in cluster 1, whereas the maximum was in clusters 3 and 6. The distance between lines within cluster 11 showed the widest range (0.17 to 0.70). The average genetic distance between clusters ranged from 0.70 to 0.86, the minimum distance was recorded between clusters 6 and 8 and clusters 8 and 9; the maximum was between clusters 1 and 12.

The average distances among the South African heterotic groups and among tester lines are shown in Table 3.7. The average distance between the South African heterotic groups ranged from 0.74 (L and K) to 0.86 (I and M as well as M and R). The average genetic distance between the South African heterotic groups and the tester lines was the highest (0.97) between group R and temperate testers, while the minimum distance (0.59) was between heterotic group F and the tropical testers. The distance between testers was generally large, ranging from 0.31 (B73 and MO17) to 0.91 (MO17 and CML444).

Table 3.6: Average genetic distances (GD) between maize germplasm clusters and within clusters

Cluster	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10a**	**10b**	**11**	Average GD between lines within clusters	GD Range within a cluster
1	-												0.31	
2	0.83	-											Singleton	
3	0.84	0.81	-										0.54	0.45-1.06
4	0.79	0.76	0.78	-									Singleton	
5	0.80	0.77	0.78	0.73	-								0.40	0.07-0.51
6	0.79	0.75	0.77	0.72	0.73	-							0.54	
7	0.85	0.81	0.83	0.78	0.79	0.77	-						0.42	0.05-0.59
8	0.78	0.74	0.76	0.71	0.72	0.70	0.76	-					0.51	0.49-0.52
9	0.79	0.75	0.77	0.72	0.73	0.71	0.77	0.70	-				Singleton	
10a	0.82	0.79	0.80	0.76	0.76	0.75	0.81	0.74	0.75	-			0.45	0.31-0.58
10b	0.81	0.78	0.79	0.74	0.75	0.74	0.80	0.73	0.74	0.77	-		0.48	0.38-0.54
11	0.86	0.83	0.84	0.79	0.80	0.79	0.85	0.78	0.79	0.82	0.81		0.48	0.17-0.70

Table 3.7: Average genetic distances between the current South African heterotic groups and between testers

	F	I	K	L	M	P	R	**B73**	**MO17**	**CML444**	**CML312**
F	-										
I	0.80	-									
K	0.75	0.79	-								
L	0.76	0.79	0.74	-							
M	0.83	0.86	0.81	0.82	-						
P	0.77	0.80	0.75	0.76	0.83	-					
R	0.80	0.84	0.78	0.79	0.86	0.80	-				
B73	***0.90***	***0.97***	***0.82***	***0.81***	***0.98***	***0.88***	***0.93***	***-***			
MO17	***1.00***	***1.07***	***0.89***	***0.91***	***1.08***	***0.98***	***1.01***	***0.31***	***-***		
CML444	***0.58***	***0.67***	***0.61***	***0.63***	***0.75***	***0.60***	***0.69***	***0.83***	***0.91***	***-***	
CML312	***0.59***	***0.65***	***0.61***	***0.62***	***0.75***	***0.60***	***0.71***	***0.81***	***0.90***	***0.49***	***-***

3.3.2 Analyses of variance

The analysis of variance results of yield in 2014/15 and 2015/16 are presented in Table 3.8. During the 2014/15 season, the variance associated with sites was highly significant ($P<0.001$). The results also showed significant differences due to lines ($P<0.05$) and testers ($P<0.001$) main effects. Although the genotype x site interaction effects were not significant, the site and tester interaction effect was highly significant ($P<0.001$). The mean square associated with line (L) x tester (T) interaction effects was not significant for grain yield, and site x line and site x line x tester interaction effects were not significant.

There were no significant differences between sites, testers and tester x sites during the 2015/16 season. The variances associated with genotypes, lines and the interaction between site x line were highly significant ($P<0.001$). The interactions between lines x testers, sites x genotypes were also significant ($P<0.01$). The variance associated with site x line x tester was also significant ($P<0.05$). The results indicated the presence of sufficient variability among lines to be classified into heterotic groups.

Table 3.8: Analysis of variance of yield for two non-stress locations in 2014/15 and 2015/16

Source	**DF**	**MS**	**DF**	**MS**
	2014/15		**2015/16**	
Site	1	35.81***	1	0.09
Rep(site)	2	5.03	2	10.24
Genotypes	41	4.90**	71	5.71***
Line	13	5.22*	17	7.61***
Tester	2[!]	26.85***	3	0.32
Line × tester	26	3.05	51	5.40**
Site × genotypes	41	3.28	71	5.20**
Site × line	13	3.15	17	7.93***
Site × tester	2	29.06***	3	2.81
Site × line × tester	26	1.36	51	4.44*
Residuals	82	2.38	142	3.09

****, **, * Data significant at the 0.001, 0.01 and 0.05 probability levels, respectively; DF - degree of freedom; [!]Testers excluding MO17*

The results indicated lack of association between genetic distance with both grain yield and specific combining ability (Tables 3.9 - 3.17).

3.3.3 Heterotic groupings based on SCA effects under stress and non-stress environments

Heterotic orientation of 14 inbred lines with tropical inbred testers, using SCA effects for grain yield under non-stress, drought and low N stress during the 2014/15 is presented in Tables 3.9 to 3.11. Across non-stress environments, five inbred lines that belong to four heterotic groups (M, K, L, and I) were allocated into heterotic group A; group A inbred lines were, I-39 (I), K64 (K), K64R-22 (K), MO17HtHtN (L) and P594MSV (L). Heterotic group B consisted of five inbred lines from heterotic group F, K, M, P and R; lines allocated to group B were B1138T (F), M162W (K), SO181Y (M), SO713W (P) and SO1224Y (R). Three inbred lines from heterotic group P (VO500Y) and R (U71Y and P612MSV) were classified into AB while one inbred line, U2540W (K) did not belong to any heterotic group.

Classification under drought stress allocated four inbred lines into CIMMYT group A and B, each; three were assigned into AB while three could not be assigned into heterotic groups. Heterotic orientation under low N stress placed six inbred lines into group A, four into B, one into AB and three could not be classified. The classification under non-stress and stress conditions did not follow a similar trend.

Table 3.9: Heterotic orientation, SCA effects, genetic distance and mean yield of South African maize inbred lines with tropical inbred testers across non-stress environments during the 2014/15 season

Inbred line	Current South African heterotic group	SCA effects		Heterotic orientation with tropical testers	Genetic distances with tropical testers		Mean yield ($t\ ha^{-1}$) in crosses with tropical testers	
		CML312 (A)	CML444 (B)		CML312	CML444	CML312	CML444
B1138T	F	0.88	-1.47*	B	0.547	0.545	6.65	4.26
I-39	I	-1.04	1.12	A	0.712	0.733	5.84	7.96
U2540W	K	-0.11	-1.03	-	0.565	0.554	7.09	6.13
M162W	K	0.22	-0.15	B	-	-	7.7	7.28
K64	K	-0.98	0.87	A	0.613	0.614	5.52	7.32
K64R-22	K	-0.15	0.89	A	0.572	0.569	5.42	6.41
MO17HtHtN	L	-0.31	0.09	A	0.569	0.564	7.09	7.44
P594MSV	L	-0.75	0.01	A	0.698	0.709	5.82	6.53
SO181Y	M	0.20	-0.18	B	0.620	0.619	7.47	7.05
SO713W	P	0.23	-1.14	B	0.634	0.633	7.26	5.83
VO500Y	P	0.20	0.05	AB	0.600	0.603	6.56	6.37
SO1224Y	R	0.44	-0.14	B	0.751	0.745	6.21	5.59
U71Y	R	0.06	0.88	AB	0.698	0.671	5.92	6.7
P612MSV	R	1.11	0.21	AB	0.631	0.613	7.35	6.41
Correlation with GD		**-0.21**	**0.39**				**-0.34**	**0.26**
Grand mean ($t\ ha^{-1}$)		**6.14**						
Standard error (SCA)		**0.69**						

**Significant at the 0.05 probability level; SCA: Specific combining ability; GD: Genetic distance*

Table 3.10: Heterotic orientation, SCA effects, genetic distance and mean yield of South African maize inbred lines with tropical inbred testers, across drought stress conditions during the 2014/15 season

Inbred line	Current South African heterotic group	SCA effects		Heterotic orientation with tropical testers	Genetic Distances with tropical testers		Mean yield ($t\ ha^{-1}$) in crosses with tropical testers	
		CML312 (A)	CML444 (B)		CML312	CML444	CML312	CML444
B1138T	F	-0.12	0.003	A	0.547	0.545	3.13	3.33
I-39	I	0.36	-0.25	B	0.712	0.733	3.79	3.26
U2540W	K	-0.60	-0.13	-	0.565	0.554	2.88	3.42
M162W	K	0.54	-0.45	B	-	-	4.15	3.23
K64	K	0.42	-0.06	B	0.613	0.614	3.67	3.26
K64R-22	K	-0.50	0.59	A	0.572	0.569	2.61	3.77
MO17HtHtN	L	0.62	-0.62	B	0.569	0.564	4.77	3.61
P594MSV	L	0.43	0.25	AB	0.698	0.709	3.35	3.24
SO181Y	M	-0.61	0.61	A	0.62	0.619	2.32	3.62
SO713W	P	-0.71	-0.50	-	0.634	0.633	2.95	3.24
VO500Y	P	-0.10	0.10	A	0.6	0.603	2.75	3.02
SO1224Y	R	-0.14	-0.39	-	0.751	0.745	3.12	2.95
U71Y	R	0.15	0.38	AB	0.698	0.671	2.81	3.11
P612MSV	R	0.26	0.48	AB	0.631	0.613	3.58	3.87
Correlation with GD		**0.26**	**-0.11**				**0.003**	**-0.53**
Grand mean ($t\ ha^{-1}$)		**3.12**						
Standard error (SCA)		**0.46**						

SCA: Specific combining ability; GD: Genetic distance

Table 3.11: Heterotic orientation, SCA effects, genetic distance and mean yield of South African maize inbred lines with tropical inbred testers, across low Nitrogen stress conditions during the 2014/15 season

Inbred line	Current South African Heterotic group	SCA effects		Heterotic orientation with tropical testers	Genetic distances with tropical testers		Mean yield (t ha^{-1}) in crosses with tropical testers	
		CML312 (A)	CML444 (B)		CML312	CML444	CML312	CML444
B1138T	F	0.83	-0.14	B	0.547	0.545	3.91	3.29
I-39	I	0.74	0.08	AB	0.712	0.733	3.86	3.56
U2540W	K	-0.31	-0.20	-	0.565	0.554	2.88	3.35
M162W	K	-0.47	-0.88	-	-	-	1.95	1.90
K64	K	-0.48	0.12	A	0.613	0.614	1.87	2.82
K64R-22	K	-0.06	0.72	A	0.572	0.569	2.34	3.48
MO17HtHtN	L	-0.77	0.28	A	0.569	0.564	2.66	4.06
P594MSV	L	-0.08	0.12	A	0.698	0.709	2.50	3.06
SO181Y	M	-0.09	-0.10	-	0.62	0.619	2.98	3.33
SO713W	P	0.24	-0.61	B	0.634	0.633	2.81	2.31
VO500Y	P	-0.20	0.33	A	0.600	0.603	2.12	3.00
SO1224Y	R	0.19	-0.53	B	0.751	0.745	3.06	2.69
U71Y	R	-0.21	1.06*	A	0.698	0.671	2.83	4.45
P612MSV	R	0.67	-0.26	B	0.631	0.613	3.26	2.68
Correlation with GD		**0.22**	**-0.11**				**0.15**	**-0.12**
Grand mean (t ha^{-1})		**3.03**						
Standard error (SCA)		**0.53**						

**Significant at the 0.05 probability level; SCA: Specific combining ability: GD: Genetic distance*

Heterotic orientation of inbred lines in season two (2015/16), towards CIMMYT tropical testers and USA Corn Belt temperate testers, under non-stress, drought and low N stress is presented in Tables 3.12 to 3.17. Heterotic orientation based on CIMMYT testers under non-stress environments allocated six inbred lines into heterotic group A, nine into group B and three from heterotic group K (K64R), M (RO421W) and R (S198Y) did not belong to any group. Heterotic group A inbred lines were from the I-group (I-16 and I-42), K (M162W), M (J80W), P (FO215W) and R (U71Y). Group B consisted of F (E30Y and RO544W), K (U127Y and U2540W), L (MO17HtHtN), M (RO452W and SO181Y), P (VO495Y) and R (P614MSV).

Seven inbred lines were placed into heterotic groups A and B under drought stress, three into AB and one inbred line could not be classified. Grouping under low N stress assigned six inbred lines into A and B, two into AB and four inbred lines were not assigned. Most inbred lines did not classify consistently under non-stress and stress environments, however, the heterotic orientation of some lines particularly under drought and low N stress did not vary.

Heterotic grouping by Corn Belt testers classified five inbred lines into heterotic group A, six into B, three into AB and four did not belong to any heterotic group under non-stress conditions. Heterotic group A inbred lines were E30Y (F), MO17HtHtN (L), J80W (M) and S198Y (R) and U71Y (R). Group B inbred lines were RO544W (F), K group lines (U127Y and U2540W), M lines (RO452W and SO181Y) and P614MSV (R). Lines K64 (K), M162W (K) and RO421W (M) were classified into AB. Four inbred lines from group I (I-16 and I-42) and P (FO215W and VO495Y) could not be clearly classified by temperate testers. The heterotic grouping under drought stress allocated four inbred lines into A, seven into B, four into AB and three were not classified. Grouping under low N stress placed four lines into group A, five into B and five did not belong to any heterotic group.

Table 3.12: Heterotic orientation, SCA effects, genetic distance and mean yield of South African maize inbred lines with tropical inbred testers across non-stress environments during the 2015/16 season

Inbred Line	Current South African heterotic group	SCA effects		Heterotic orientation with tropical testers	Genetic Distances with tropical testers		Mean yield (t ha^{-1}) in crosses with tropical testers	
		CML312 (A)	CML444 (B)		CML312	CML444	CML312	CML444
E30Y	F	0.90	-0.82	B	0.595	0.588	6.91	5.34
RO544W	F	2.05*	-1.3	B	0.609	0.609	7.72	4.51
I-16	I	-0.10	1.58	A	0.555	0.577	5.98	7.82
I-42	I	-0.20	0.75	A	0.709	0.729	4.96	6.05
K64	K	-1.32	-0.44	-	0.613	0.614	4.04	5.06
M162W	K	-2.32*	0.72	A			3.39	6.57
U127Y	K	0.18	-0.68	B	0.521	0.516	4.86	4.14
U2540W	K	0.02	-0.15	B	0.565	0.554	5.92	5.9
MO17HtHtN	L	0.69	-1.16	B	0.569	0.564	6.06	4.35

Inbred Line	Current South African heterotic group	SCA effects		Heterotic orientation with tropical testers	Genetic Distances with tropical testers		Mean yield (t ha^{-1}) in crosses with tropical testers	
		CML312 (A)	CML444 (B)		CML312	CML444	CML312	CML444
J80W	M	-0.55	0.96	A	0.798	0.796	4.58	6.23
RO421W	M	-1.90*	-0.76	-	0.764	0.768	5.76	7.05
RO452W	M	0.22	-0.17	B	0.797	0.798	5.92	5.67
SO181Y	M	0.80	-0.77	B			6.76	5.34
FO215W	P	-0.78	3.01**	A	0.625	0.617	6.19	10.13
VO495Y	P	1.40	-0.29	B	0.587	0.584	7.58	6.04
P614MSV	R	1.40	-0.83	B	0.758	0.73	7.16	5.08
S198Y	R	-0.29	-0.47	-	0.654	0.658	5.92	5.89
U71Y	R	-0.20	0.83	A	0.698	0.671	6.2	7.38
Correlation with GD		**-0.26**	**0.1**				**-0.13**	**0.16**
Grand mean (t ha^{-1})		**5.98**						
Standard error (SCA)		**0.98**						

**Significant at the 0.05 probability level; SCA: Specific combining ability; GD: Genetic distance*

Table 3.13: Heterotic orientation, SCA effects, genetic distance and mean yield of South African maize inbred lines with temperate inbred testers across non-stress environments during the 2015/16 season

Inbred Line	Current South African Heterotic group	SCA effects		Heterotic orientation with temperate testers	Genetic distances with temperate testers		Mean yield (t ha^{-1}) in crosses with temperate testers	
		B73(A)	MO17(B)		B73	MO17	B73	MO17
E30Y	F	-0.88	0.8	A	0.932	1.038	5.27	6.92
RO544W	F	0.66	-1.41	B	0.905	1.005	6.47	4.36
I-16	I	-0.9	-0.59	-	0.88	0.975	5.32	5.6
I-42	I	-0.33	-0.22	-	1.029	1.134	4.97	5.04
K64	K	0.83	0.93	AB	0.814	0.874	6.33	6.39
M162W	K	0.72	0.89	AB			6.57	6.7
U127Y	K	1.2	-0.7	B	0.832	0.923	6.02	4.08
U2540W	K	1.13	-0.99	B	0.834	0.908	7.17	5.01
MO17HtHtN	L	-0.57	1.05	A	0.826	0.922	4.94	6.52
J80W	M	-0.45	0.05	A	1.083	1.191	4.8	5.27
RO421W	M	1.79	0.86	AB	0.811	0.831	9.58	8.63
RO452W	M	0.08	-0.13	B	1.044	1.147	5.91	5.67
SO181Y	M	0.36	-0.38	B			6.46	5.68
FO215W	P	-1.98*	-0.25	-	0.956	1.061	5.12	6.82
VO495Y	P	-0.7	-0.41	-	0.812	0.893	5.62	5.87
P614MSV	R	0.31	-0.88	B	0.949	1.008	6.2	4.98
S198Y	R	-0.09	0.84	A	0.902	1	6.26	7.16
U71Y	R	-1.19	0.55	A	0.93	1.005	5.34	7.05
Correlation with GD		**-0.4**	**-0.15**				**-0.48**	**-0.28**
Grand mean (t ha^{-1})		**5.98**						
Standard error (SCA)		**0.98**						

**Significant at the 0.05 probability level; SCA: Specific combining ability; GD: Genetic distance*

Table 3.14: Heterotic orientation, SCA effects, genetic distance and mean yield of South African maize inbred lines with tropical inbred testers, across drought stress conditions during the 2015/16 season

Inbred line	Current South African heterotic group	SCA effects		Heterotic orientation with tropical testers	Genetic Distances with tropical testers		Mean yield (t ha^{-1}) in crosses with tropical testers	
		CML312 (A)	CML444 (B)		CML312	CML444	CML312	CML444
E30Y	F	-0.88	0.32	A	0.595	0.588	3.08	4.16
RO544W	F	-0.82	0.92	A	0.609	0.609	4.11	5.74
I-16	I	-0.97	0.24	A	0.555	0.577	4.10	5.19
I-42	I	-1.16	1.84**	A	0.709	0.729	4.82	7.70
K64	K	0.61	0.11	AB	0.613	0.614	6.54	5.92
M162W	K	-0.4	-1.11	NONE			4.05	3.23
U127Y	K	0.64	-0.22	B	0.521	0.516	4.86	3.88
U2540W	K	0.11	-0.81	B	0.565	0.554	4.87	3.84
MO17HtHtN	L	0.71	-0.66	B	0.569	0.564	5.54	4.04
J80W	M	0.46	-0.09	B	0.798	0.796	6.24	5.57
RO421W	M	1.26	-1.67*	B	0.764	0.768	6.63	3.58
RO452W	M	0.42	-0.42	B	0.797	0.798	6.28	5.31
SO181Y	M	-0.89	0.78	A			4.27	5.83
FO215W	P	0.76	0.3	AB	0.625	0.617	6.40	5.83
VO495Y	P	0.84	-1.39*	B	0.587	0.584	5.61	3.26
P614MSV	R	0.69	0.85	AB	0.758	0.730	5.69	5.74
S198Y	R	-0.72	0.39	A	0.654	0.658	3.78	4.77
U71Y	R	-0.64	0.62	A	0.698	0.671	4.24	5.39
Correlation with GD		**0.16**	**0.1**				**0.44**	**0.42**
Grand mean (t ha^{-1})		**4.52**						
Standard error (SCA)		**0.71**						

**, **Significant at the 0.05 and 0.01 probability levels, respectively; SCA: Specific combining ability; GD: Genetic distance*

Table 3.15: Heterotic orientation, SCA effects, genetic distance and mean yield of South African maize inbred lines with temperate inbred testers, across drought stress conditions during the 2015/16 season

Inbred Line	Current South African Heterotic group	SCA effects		Heterotic orientation with temperate testers	Genetic distances with temperate testers		Mean yield ($t\ ha^{-1}$) in crosses with temperate testers	
		B73(A)	MO17(B)		B73	MO17	B73	MO17
E30Y	F	0.72	-0.15	B	0.932	1.038	3.72	2.73
RO544W	F	0.27	-0.38	B	0.905	1.005	4.24	3.47
I-16	I	1.04	-0.3	B	0.880	0.975	5.17	3.69
I-42	I	-0.61	-0.07	NONE	1.029	1.134	4.42	4.83
K64	K	-0.32	-0.39	NONE	0.814	0.874	4.66	4.45
M162W	K	1.18	0.32	AB			4.68	3.69
U127Y	K	0.31	-0.73	B	0.832	0.923	3.58	2.40
U2540W	K	0.75	-0.04	B	0.834	0.908	4.55	3.63
MO17HtHtN	L	0.002	-0.05	B	0.826	0.922	3.87	3.69
J80W	M	0.10	-0.48	B	1.083	1.191	4.92	4.22
RO421W	M	0.25	0.15	AB	0.811	0.831	4.67	4.44
RO452W	M	-0.32	0.32	A	1.044	1.147	4.59	5.10
SO181Y	M	-0.86	0.97	A			3.35	5.06
FO215W	P	-1.21	0.16	A	0.956	1.061	3.48	4.72
VO495Y	P	0.38	0.16	AB	0.812	0.893	4.20	3.84
P614MSV	R	-0.91	-0.63	NONE	0.949	1.008	3.14	3.30
S198Y	R	0.003	0.33	AB	0.902	1.00	3.55	3.75
U71Y	R	-0.77	0.8	A	0.930	1.005	3.16	4.60
Correlation with GD		**-0.40**	**0.03**				**0.04**	**0.32**
Grand mean ($t\ ha^{-1}$)		**4.52**						
Standard error (SCA)		**0.71**						

SCA: Specific combining ability; GD: Genetic distance

Table 3.16: Heterotic orientation, SCA effects, genetic distance and mean yield of South African maize inbred lines with tropical inbred testers, across low nitrogen stress conditions during the 2015/16 season

Inbred Line	Current South African heterotic group	SCA effects		Heterotic orientation with tropical testers	Genetic Distances with tropical testers		Mean yield (t ha^{-1}) in crosses with tropical testers	
		CML312 (A)	CML444 (B)		CML312	CML444	CML312	CML444
E30Y	F	-0.48	-0.05	NONE	0.595	0.588	2.71	3.30
RO544W	F	-0.17	0.62	A	0.609	0.609	3.88	4.83
I-16	I	-0.38	-0.04	NONE	0.555	0.577	3.30	3.81
I-42	I	-0.42	1.48**	A	0.709	0.729	3.93	5.99
K64	K	0.41	-0.18	B	0.613	0.614	4.11	3.68
M162W	K	-0.13	-0.37	NONE			2.96	2.89
U127Y	K	-0.33	0.53	A	0.521	0.516	2.93	3.95
U2540W	K	0.1	-1.18*	B	0.565	0.554	3.26	2.14
MO17HtHtN	L	0.35	-0.45	B	0.569	0.564	4.02	3.38
J80W	M	-0.58	-0.59	NONE	0.798	0.796	3.16	3.32
RO421W	M	0.56	-0.82	B	0.764	0.768	4.42	3.21
RO452W	M	0.17	0.07	AB	0.797	0.798	4.22	4.29
SO181Y	M	-0.23	0.03	A			3.66	4.09
FO215W	P	0.3	-0.07	B	0.625	0.617	4.52	4.31
VO495Y	P	0.51	-0.76	B	0.587	0.584	3.79	2.69
P614MSV	R	0.4	0.29	AB	0.758	0.730	4.09	4.14
S198Y	R	-0.03	0.46	A	0.654	0.658	3.21	3.87
U71Y	R	-0.05	1.03*	A	0.698	0.671	3.83	5.07
Correlations with GD		**0.07**	**0.12**				**0.39**	**0.30**
Grand mean (t ha^{-1})		**3.45**						
Standard error (SCA)		**0.53**						

**, **Significant at the 0.05 and 0.01 probability levels, respectively; SCA: Specific combining ability; GD: Genetic distance*

Table 3.17: Heterotic orientation, SCA effects, genetic distance and mean yield of South African maize inbred lines with temperate inbred testers, across low Nitrogen stress conditions during the 2015/16 season

Inbred Line	Current South African Heterotic group	SCA effects		Heterotic orientation with temperate testers	Genetic distance with temperate testers		Mean yield ($t\ ha^{-1}$) in crosses with temperate testers	
		B73(A)	MO17(B)		B73	MO17	B73	MO17
E30Y	F	0.49	0.03	AB	0.932	1.038	3.21	2.66
RO544W	F	0.3	-0.75	B	0.905	1.005	3.87	2.73
I-16	I	0.2	0.22	AB	0.88	0.975	3.42	3.34
I-42	I	-0.56	-0.5	NONE	1.029	1.134	3.32	3.28
K64	K	-0.44	0.2	A	0.814	0.874	2.79	3.34
M162W	K	1.01	-0.51	B			3.63	2.02
U127Y	K	-0.16	-0.04	NONE	0.832	0.923	2.62	2.65
U2540W	K	0.12	0.97	AB	0.834	0.908	2.81	3.57
MO17HtHtN	L	0.5	-0.4	B	0.826	0.922	3.70	2.71
J80W	M	0.77	0.39	AB	1.083	1.191	4.04	3.57
RO421W	M	0.55	-0.29	B	0.811	0.831	3.93	3.00
RO452W	M	-0.38	0.14	A	1.044	1.147	3.20	3.63
SO181Y	M	-1.07*	1.27*	A			2.35	4.60
FO215W	P	-0.58	0.35	A	0.956	1.061	3.17	4.01
VO495Y	P	0.57	-0.31	B	0.812	0.893	3.38	2.41
P614MSV	R	-0.46	-0.23	NONE	0.949	1.008	2.76	2.90
S198Y	R	-0.23	-0.2	NONE	0.902	1	2.54	2.48
U71Y	R	-0.63	-0.34	NONE	0.93	1.005	2.78	2.98
Correlation with GD		**-0.22**	**0.06**				**0.17**	**0.42**
Grand mean ($t\ ha^{-1}$)		**3.45**						
Standard error (SCA)		**0.53**						

**Significant at the 0.05 probability level; SCA: Specific combining ability; GD: Genetic distance*

3.4 Discussion

3.4.1 Classification by SNP markers

The SNP markers genotyping was highly effective in confirming classification of the germplasm collection in South Africa. As expected, the SNP markers divided the germplasm lines into two principal clusters of USA Corn Belt temperate and the South African maize lines. The South African lines were more inclined towards the tropical CIMMYT than the USA temperate testers. However, there were 11 sub-clusters within the South African germplasm with only the lines from the I heterotic group placed in the closest cluster with both CIMMYT testers, CML444 and CML312, indicating that these lines have never been introgressed with temperate germplasm, and were more pro-tropical germplasm than the rest of the 40 lines. Based on the pedigree data, lines with a history of temperate introgression such as P614MSV and P612MSV with B73 introgression and P590MSV and P594MSV with MO17 were placed in clusters 3 and 5, near the temperate cluster (cluster 1) on the dendogram, indicating their orientation towards the temperate material. Clusters 10 and 11, furthest away from the temperate cluster on the dendogram were formed by a group of quality protein maize lines. These lines resulted from the opaque-2 breeding program, which led to the release of yellow and white opaque-2 modified inbred lines (Gevers, 1972). This therefore confirmed the power of SNP’s markers in discriminating inbred lines. However, within the QPM group, the inbred line MO17HtHtN from the L heterotic group was non-QPM by origin. Clustering of this line within the QPM collection is an implication of more QPM alleles than normal maize in its genetic background, which could have been introduced along with Northern corn leaf blight (Ht) resistance.

Although genotypic classification by SNP markers was closely linked with the origin of inbred lines and pedigree records, there were deviations that were observed. The lines NC258 and U127Y which are classified as L and K heterotic groups, respectively, in South Africa, were actually placed in the same sub-cluster with the I and CIMMYT testers, respectively. This indicates that the classification of South African lines in the whole collection requires confirmation with SNP genotyping. In the same vein, lines RO421W (M), K64 (K) and U2540W (K) which were expected to cluster with the M and K heterotic groups of South Africa, formed singleton clusters, and were not associated with any of the South African lines. They have also displayed a lack of association with the USA temperate and tropical CIMMYT lines, qualifying them as representing distinct germplasm groups within the South African inbred lines collection.

3.4.2 Heterotic orientation based on SCA effects under non-stress environments

Based on yield specific combining ability effects, inbred lines were grouped into three heterotic groups by both temperate and tropical testers. The SCA value reveals the genetic relationship between two parents or between a line and a tester (Vasal *et al*., 1992). High SCA value (+ve) between two parents indicates a distant relationship and low (-ve) value is an indication of close relationship (Fan *et al*., 2003). Testers used in the study were tropical heterotic Group A of CIMMYT, CML312 and the temperate group A of the USA Corn Belt, B73, and their heterotic Group B counterparts CML444 and MO17, respectively. When tropical testers were used to classify inbred lines into heterotic groups, six lines were allocated into group A, nine into B and three could not be classified. Classification by the temperate Corn Belt testers allocated five lines into group A, six into B; three were classified as AB, while four lines could not be classified. Both tropical and temperate testers were consistent in assigning at least 44% of the inbred lines studied, where two lines were classified into group A and six were assigned into B by both testers, indicating some genetic similarities in the background of the A-testers and within the B-testers.

3.4.3 Heterotic orientation under stress environments

The heterotic grouping of inbred lines under stress environments did not show consistency in the placement of most inbred lines into heterotic groups, using both tropical and temperate testers. However, there were still some lines that showed consistency in their orientation especially when tropical testers were used under drought and low N stress environments. Grouping of most inbred lines thus varied greatly under stress than non-stress environments. Hence, the study indicated that the placement of inbred lines into heterotic groups using SCA effects for grain yield would vary in different test environments. The assignment of inbred lines into heterotic groups using SCA effects for grain yield is largely influenced by the interaction between two inbred lines and the environments in which hybrids are evaluated (Fan *et al*., 2008). The heterotic orientation of inbred lines in this study showed that it was largely based on the reaction of the inbred lines to stress conditions and to a small extent on their pedigree, this was observed by the grouping of similar inbred lines differently under different growing conditions. This has also been observed in previous studies where the same inbred line was placed into different heterotic groups under different test environments (Badu-Apraku *et al*., 2013; Badu-Apraku *et al*., 2016; Mwimali, 2014). Heterotic grouping involving stress environments, and using only SCA effects for grain yield may therefore not result in precise and conclusive heterotic groups. Grain yield is a polygenic trait and is influenced largely by the presence of genotype by environment interaction; its heritability also declines in the presence of stress, as yield levels decrease (Banziger *et al*., 2000). This often leads to different number of heterotic groups in the same set of inbred lines under different environments. In this study, the trend observed in heterotic orientation of inbred lines under stress environments did not closely correspond with the SNP-marker approach, than the grouping of lines under non-stress environments. The use of stress environments may therefore not be recommended for deducing the placement of inbred lines into heterotic groups.

3.4.4 Relationship between SNP and SCA-based classification

Heterotic orientation of inbred lines under non-stress environments corresponded with the SNP marker classification. It was noted that lines that did not belong to any heterotic group when tropical testers were used could classify clearly using temperate testers and vice versa. For example, in SNP cluster analysis, the I-group representatives were more aligned to both tropical testers. However, heterotic orientation by temperate testers did not classify them into any heterotic group, as revealed by negative SCA effects with both B73 and MO17. The grouping of the I-group lines with only tropical testers was an indication of a distant relationship between the I heterotic group and the temperate lines. This was further supported by the maximum average genetic distance between the I-group and both temperate testers, B73 (0.97) and MO17 (1.07). Therefore, delineation of inbred lines by either temperate or tropical lines is influenced by the predominance of either temperate or tropical genome in their genetic background, which was clearly confirmed by the SNPs markers. In support of the current observations, Adetimirin *et al.* (2008) also observed that markers clearly discriminated temperate from tropical germplasm. They further observed that some lines that were known to be temperate x tropical were aligned with the tropical lines, indicating that they were more closely related to the tropical than temperate material. In this study, some lines were consistent in their heterotic orientation by both USA temperate and CIMMYT tropical lines. However, the strongest relationship towards either temperate or tropical lines was observed from the magnitude of SCA effects and their placement in the dendogram.

The inbred lines including RO421W, K64 and U254OW formed distinct singleton clusters; use of the SCA heterotic orientation approach did not clearly classify these lines. For example, RO421W did not belong to any heterotic group under tropical classification, while classification based on temperate testers allocated this line into heterotic group AB. The inbred line K64 was assigned into AB group by temperate testers and into heterotic group A by tropical testers; however, in another season tropical testers did not assign the same inbred line into heterotic group. The line U2540W could not be classified by tropical testers in one season, while in another season; both tropical and temperate testers allocated it into heterotic group B. These deviations could therefore be an explanation of the independent grouping of these lines in marker-based clustering, and the non-alignment with both the USA temperate and tropical CIMMYT lines.

Grouping by SCA effects therefore revealed that when inbred lines are classified into heterotic groups using two sets of testers, failure of one set of testers to classify lines could be an indication of distant relationship between a line and a particular set of testers. Alternatively, classification of some lines might not be clear with both sets, such lines could represent distinct germplasm groups within the germplasm collection. Therefore, when the molecular data is not available for validation, there should be no preference for any set of testers over another. However, including two sets of testers could be a more accurate approach, especially in distinguishing germplasm like the South African germplasm with a history of both tropical and temperate introgression.

The inbred lines used in the study were pooled from seven known South African heterotic groups to minimise the number of heterotic groups for improved breeding efficiency. The 11 clusters obtained could be simplified into a broader group of normal endosperm maize and quality protein maize lines. Normal maize endosperm lines may be grouped into three groups, with one group forming temperate germplasm (clusters 1 and 3) with pro MO17 and B73. The second group is a group of South African lines in clusters five and six, with no temperate or tropical alignment, while the I-group lines and those lines with tropical alignment formed the third group. The QPM lines on the far right of the dendogram could be split into two groups (clusters 10 and 11). Intra-group combinations among the normal maize groups and between the QPM groups are expected to give high heterosis in crosses. Cross combinations between normal maize and some lines in the QPM group, particularly the white versions are also recommended to maximise heterosis. The heterotic potential of these groups was shown by a cross between FO215W from the QPM cluster and the tropical tester, CML444 (data not presented). Lines that represented distinct germplasm should also be considered for utilisation in crosses; for example in the current study, high heterosis was revealed by crosses RO421W x B73 and RO421W x MO17. Previous investigations revealed high heterosis in crosses including F and I, M and K, K and P, M and P and crosses between M and F and R and L group combinations (Gevers and Whythe, 1987). Based on the new clusters formed, these combinations also included temperate x tropical lines. The identified groups will likely result in superior tropical × temperate combinations with new favourable alleles, wider genetic base, and consequently improvement in agronomic performance and adaptation of local varieties.

From the seven heterotic groups of South Africa studied, some groups clearly clustered into the identified clusters. The study therefore indicated that the established patterns would be beneficial in inferring the classification of lines that were not included in the study, but represented in the seven heterotic groups. The F, P and M group lines and the rest of the Opaque-2 modified lines from other South African heterotic groups belong to the QPM group. Lines from the L- group form a distinct group of normal maize lines; the I-group lines form a distinct group closest to the CIMMYT lines, while the temperate group include the R lines and K-group lines with a normal maize endosperm. However, classification of some South African heterotic groups, particularly the K-group lines may not be straightforward as lines from this group were distributed in clusters. Such deviations may be an indication of the presence of diversity within the K-group; this also suggests that inbred lines extracted from the same population do not always cluster together. This has also been observed in many previous investigations (Barata and Carena, 2006; Fan *et al*., 2003; Vasal *et al*., 1992; Yu *et al*., 2001), where inbred lines derived from the same population classified differently. Therefore, for such groups, the inbred-inbred classification procedure with SNP markers may be essential for full and accurate classification.

3.4.5 Genetic distances

Genetic distances summarise the extent of genetic differences between populations or species (Dogan and Dogan, 2016). Genetic distances were estimated to determine the relatedness among inbred lines studied. The average genetic distances computed between clusters, South African heterotic groups, inbred lines within clusters and among tester inbred lines indicated the presence of sufficient variability between the identified clusters and therefore the possibility of obtaining superior hybrid combinations from inter-cluster crosses. The average genetic distances between lines within clusters clearly indicated that lines that formed the same cluster were genetically related by distance, as the average genetic distance ranged from 0.31 to 0.58 between clusters. However, for some clusters, the genetic distance range of inbred lines showed a considerable diversity within a cluster. For example, the genetic distance in cluster 11 ranged from 0.17 to 0.70. This therefore showed that the collection of QPM lines that formed cluster 11 were derived from distinct genetic backgrounds. The maximum average genetic distance between clusters 1 and 11, further demonstrated the reliability of SNP markers in distinguishing between inbred lines. Genetic distances between testers clearly revealed that both temperate Corn Belt testers, MO17 and B73 are closely related. The closest relationship was further confirmed by the grouping together of these inbred testers by the SNP markers. Similarly, the close genetic relationship between tropical CIMMYT testers, CML312 and CML444 was confirmed by the minimum genetic distance between them, and their placement within the same cluster.

The genetic differences among the current South African heterotic groups were generally large, indicating the presence of diversity within the South African germplasm. The largest genetic distance was between group R and M, and I and M, indicating genetic dissimilarity between these South African heterotic groups. The R group lines were grouped in cluster 3, with temperate alignment, whereas the M group lines were mainly grouped along with the QPM lines in cluster 11; the M group also showed the highest genetic dissimilarity with the I group. Inbred lines from heterotic group I formed a distinct group in cluster seven, that was closest to the tropical testers, and were thus not closely associated with the M -group lines. The highest average genetic distance between these heterotic crops thus indicated that they are the most distinct heterotic groups within the seven South African heterotic groups. The minimum distance between the South African heterotic groups K and L was an indication of genetic similarity. Although these groups did not cluster together, however their placement by the SNP markers in closest clusters; for example allocation of the K lines in clusters 3 and 4 and the L-group lines in cluster 5 could be an explanation of the minimum genetic distance estimates between them. Overall, the average genetic distance was generally large between inbred lines and temperate testers than with tropical testers, indicating that although some lines may be aligned towards temperate than tropical testers, the lines are of South African origin, therefore the general genetic relationship with temperate material is small.

3.4.6 Correlation of genetic distance with grain yield and specific combining ability

Marker-based prediction of hybrid performance can accelerate hybrid selection and subsequent operations in a hybrid breeding program. Correlation of SNP-based genetic distance with grain yield and specific combining were computed to assess the usefulness of genotype’s genetic distances in predicting the performance of F_1 hybrids. Low estimates of correlations were detected between genetic distance with grain yield and specific combining ability. The observed poor relationship between genetic distance with grain yield and SCA suggested that hybrid performance was not efficiently predicted using molecular marker-based genetic distances. The low predictive value of genetic distance was also confirmed by the degree of heterosis observed in crosses (data not shown). For example, a pair of lines that showed the widest range of genetic distances did not necessarily show maximum heterosis in their testcrosses. These observations are in agreement with previous studies where poor (Makumbi, 2005; Parentoni *et al*., 2001; Xu *et al*., 2004) to no (Rajendran *et al*., 2014) correlation was demonstrated between genetic distance with grain yield and SCA. The low correlation observed in several studies has been associated with the markers used to compute genetic distances. The markers used are generally many, evenly distributed on the genome, and may not be closely linked to grain yield and associated quantitative characters (Melchinger, 1999). This therefore reduces the association between genetic distances and heterosis. Furthermore, the genetic differences between parents are due to many traits and loci which may not all be correlated with yield. Zhang *et al*. (2010) thus suggested the use of only yield-related markers to analyse genetic differences, and subsequently to reliably predict hybrid performance.

In contrast, Schrag *et al*. (2010) and Gichuru *et al*. (2017) reported high predictive value of molecular maker genetic distances in heterosis; the observed degree of correlation was associated with the genetic differences and complementary traits of lines used. The ability of genetic distance to predict heterosis in crosses therefore varies with germplasm used (Betran *et al*., 2003). In the current study, the observed levels of correlations therefore underlines the need for conducting field trials to evaluate hybrid performance, irrespective of known genetic distances between inbred lines. However, despite the inability of genetic distances to reliably predict hybrid performance, their usefulness as a guide during selection of inbred lines for hybrid make-ups is underscored.

Conclusion

The SNP markers genotyping was highly effective in confirming classification of the germplasm collection in South Africa. The SNP genotyping data enabled the division of the 40 inbred lines into 11 genetic clusters. As expected, the SNPs data divided the lines into two principal clusters of USA Corn Belt temperate and the South African maize lines with CIMMYT tropical testers. However, the observed sub-clusters could be summarised into two broader groups based on biochemical composition of the grain. The first group is a group of quality protein maize lines and the second group is normal endosperm maize lines. Normal endosperm maize lines formed (i) a temperate group consisting of B73 and MO17, (ii) the I-group lines of South Africa closest to the tropical CIMMYT tester lines, (iii) and a group of pure South African lines. The QPM lines were further divided into two groups indicating that hybrids could be created among lines from the two clusters. Based on the identified groups, it would be possible to deduce or make inferences of the classification of the South African inbred lines that were not included in the study, because clustering of lines using the 56110 SNPs was generally consistent with pedigree data, except in only a few cases. The identified groups will be useful in the South African maize breeding programs in predetermining the base materials for use in both pedigree and F_1 cross end products. Stress environments were not efficient in assigning inbred lines into heterotic groups as many inconsistencies were observed. The identified heterotic groups, based on biochemical composition of the grain should be adopted and maintained constantly and separately to ensure they remain distinct, and should be extended by continually introducing new germplasm. However, productivity of the lines could not be predetermined on the basis of genetic distance data, because of poor correlation between genetic distance with grain yield and specific combining ability.

References

Adetimirin, V.O., I. Vroh-Bi, C. The, A. Menkir, S.E. Mitchell, and S. Kresovich. 2008. Diversity analysis of elite maize inbred lines adapted to West and Central africa using SSR markers. *Maydica* 53: 143-149.

Aguiar, C.G., I. Schuster, A.T. Amaral-Junior, C.A. Scapim, and E.S.N. Vieira. 2008. Heterotic groups in tropical maize germplasm by test crosses and simple sequence repeat markers. *Genetics and Molecular Research* 7: 1233-1244.

Aslam, M., F.S. Awan, I.A. Khan, and A.I. Khan. 2009. Estimation of genetic distance between 10 maize accessions with varying response to different levels of soil moisture. *Genetics and Molecular Research* 4: 1459-1465.

Badu-Apraku, B., M. Oyekunle, M.A.B. Fakorede, I. Vroh, R.O. Akinwale, and M. Aderounmu. 2013. Combining ability, heterotic patterns and genetic diversity of extra-early yellow inbreds under contrasting environments. *Euphytica* 192: 413-433.

Badu-Apraku, B., M.A.B. Fakorede, M. Gedil, B. Annor, A.O. Talabi, I.C. Akaogu, M. Oyekunle, R.O. Akinwale, and T.Y. Fasanmade. 2016. Heterotic patterns of IITA and CIMMYT early-maturing yellow maize inbreds under contrasting environments. *Agronomy Journal* 108: 1321-1336.

Banziger, M., G.O. Edmeades, D. Beck, and M. Bellon. 2000. Breeding for drought and Nitrogen stress tolerance in maize: From theory to practice. CIMMYT, Mexico, D.F.

Barata, C., and M. Carena. 2006. Classification of North Dakota maize inbred lines into heterotic groups based on molecular and testcross data. *Euphytica* 51: 339-349.

Betran, F.J., J.M. Ribaut, D. Beck, and D.G. de Leon. 2003. Genetic diversity specific combining ability and heterosis in tropical maize under stress and nonstress environments. *Crop Science* 43: 797-806.

Bidhendi, M.Z., R. Choukan, F. Darvish, K. Mostafavi, and E. Majidi. 2012. Classifying of maize inbred lines into heterotic groups using diallel analysis. *World Academy of Science, Engineering and Technology* 6: 1161-1164.

CIMMYT. 2001. Maize inbred lines released by CIMMYT: A Compilation of 454 CIMMYT Maize Lines (CMLs), CML1-CML454, August 2001, Second draft. CIMMYT, Mexico, D.F.

DAFF. 2014. Maize market value chain profile. Department of Agriculture, Forestry and Fisheries, Directorate Plant Production. Pretoria, South Africa.

http://www.nda.agric.za/doaDev/sideMenu/Marketing/Annual%20Publications/Commodity%20Profiles/field%20crops/Maize%20Market%20value%20Chain%20Profile%202014.pdf (Accessed 15/09/2016).

de Pinto, M.C.R., C.I. de Souza Jr, L.A. Carlini-Garcia, A.A.F. Garcia, and A.P. de Souza. 2003. Comparison between molecular markers and diallel crosses in the assignment of maize lines to heterotic groups. *Maydica* 48: 63-73.

Delucchi, C., G. Eyherabide, R.D. Lorea, D.A. Presello, M.E. Otegui, and C.G. Lopez. 2012. Classification of Argentina maize landraces in heterotic groups. *Maydica* 57: 26-33.

Derera, J., and T.R. Musimwa. 2015. Why SR52 is such a great maize hybrid? I. Heterosis and generation mean analysis. *Euphytica* 205: 121-135.

Dhliwayo, T., K. Pixley, A. Menkir, and M. Warburton. 2009. Combining ability, genetic distances and heterosis among elite CIMMYT and IITA tropical maize inbred lines. *Crop science* 49: 1201-1210.

Dogan, I., and N. Dogan. 2016. Genetic distance measures: Review. *Turkiye Klinikleri Journal of Biostatistics* 8: 87-93.

Doyle, J.J. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11-15.

Fan, X.M., H.M. Chen, J. Tan, C.X. Xu, Y.D. Zhang, L.M. Luo, Y.X. Huang, and M.S. Kang. 2008. Combining abilities for yield and yield components in maize. *Maydica* 53: 39-46.

Fan, X.M., J. Tan, H.M. Chen, and J.Y. Yang. 2003. Heterotic grouping for tropical and temperate maize inbreds by analysing combining ability and SSR markers. *Maydica* 48: 251-257.

Fan, X.M., J. Tan, J.Y. Yang, and H.M. Chen. 2004. Combining ability and heterotic grouping of ten temperate subtropical and tropical quality protein maize inbreds. *Maydica* 49: 267–272.

Fan, X.M., Y.D. Zhang, L. Liu, H.M. Chen, W.H. Yao, M. Kang, and J.Y. Yang. 2010. Screening tropical germplasm by temperate inbred testers. *Maydica* 55: 55-63.

Fan, X.M., Y.D. Zhang, W.H. Yao, Y.Q. Bi, L. Liu, H.M. Chen, and M.S. Kang. 2014. Reciprocal diallel crosses impact combining ability, variance estimation, and heterotic group classification. *Crop Science* 54: 89–97.

Fan, X.M., Y.M. Zhang, W.H. Yao, H.M. Chen, J. Tan, C.X. Xu, X.L. Han, L.M. Luo, and M.S. Kang. 2009. Classifying maize inbred lines into heterotic groups using a factorial mating design. *Agronomy Journal* 101: 106–112.

Fasahat, P., A. Rajabi, J.M. Rad, and J. Derera. 2016. Principles and utilization of combining ability in plant breeding. *Biometrics & Biostatistics International Journal* 4: 1-24.

Fourie, A.P., and H.O. Gevers. 1987. Adaptation patterns in crosses between South African and US Corn Belt maize breeding material. pp 17-21. In: Fourie, A.P., and J.D. Duplessis (eds.). Proceedings of the seventh South African maize breeding Symposium, 1986. Summer Grain Centre, Grain Crops Research Institute, Potchefstroom.

Gerdes, J.T., C.F. Behr, J.G. Coors, and W.F. Tracy. 1993. Compilation of North American Breeding Germplasm. In: W. F. Tracy, J. G. Coors and J. L. Geadelmann (eds.). Crop Science Society of America, Madison, USA.

Gevers, H.O. 1972. Breeding for improved protein quality in maize. *Transactions of the Royal Society of South Africa*: 81-92.

Gevers, H.O. 1997. Patterns of heterosis in South Africa maize breeding. pp 102-103. In: Listman, M (ed.). Book of abstracts. The genetics and exploitation of heterosis in crops. An international symposium, 17-22 August 1997. CIMMYT, Mexico, D.F.

Gevers, H.O., and I.V. Whythe. 1987. Patterns of heterosis in South African maize breeding material. pp 21-26. In: Fourie, A.P., and J.G. Duplessis (eds.). Proceedings of the Seventh South African Maize Breeding Symposium 1986. Summer Grain Centre, Grain Crops Research Institute, Potchefstroom.

Gevers, H.O., and J.K. Lake. 1998. The Natal maize breeding programme: 1948 -1993. pp 16 – 22. In: Gevers, H.O (ed.). Proceedings of eleventh South African maize breeding symposium, 1994. Cedara Agricultural development Institute, Summer Grain Sub-Centre, Pietermaritzburg, KZN.

Gichuru, L., J. Derera, P. Tongoona, and M. Mwimali. 2017. Combining ability and molecular genetic distances in tropical maize lines for grain yield and agronomic traits. *South African Journal of Plant and Soil* 34: 175-183.

Gower, J.C. 1971. A general coefficient of similarity and some of its properties. *Biometrics* 27: 857-874.

Kanyamasoro, M.G., J. Karung, G. Asea, and P. Gibson. 2012. Determination of the heterotic groups of maize inbred lines and the inheritance of their resistance to the maize weevil. *African Crop Science Journal* 20: 99-104.

Li, M.S., X.H. Li, L.W. Deng, D.G. Zhang, L. Bai, and S.H. Zhang. 2007. Comparisons of four testers in evaluating 27 CIMMYT and Chinese maize populations. *Maydica* 52: 173-179.

Librando, R.P., and E.F. Magulama. 2008. Classifying white inbred lines into heterotic groups using yield combining ability effects. *USM R & D A Journal* 16: 99-103.

Makumbi, D. 2005. Phenotypic and genotypic characterization of white maize inbreds, hybrids and synthetics under stress and non-stress environments. PhD thesis. Texas A&M University.

Melani, M.D., and M.J. Carena. 2005. Alternative maize heterotic patterns for the Northern corn belt. *Crop Science* 45: 2186–2194.

Melchinger, A.E. 1999. Genetic diversity and heterosis. In: Coors, J.G. and S. Pandey (eds.). The genetics and exploitation of heterosis in crops. pp 99–118. American Society of Agronomy, Madison, Wisconsin.

Melchinger, A.E., and R.K. Gumber. 1998. Overview of heterosis and heterotic groups in agronomic crops. pp 29-44. In: K. R. Lamkey and J. E. Staub (eds.). In concepts and breeding of heterosis in crop plants. Madison, WI.

Menkir, A., A. Melake-Berhan, A.C. The, I. Ingelbrecht, and A. Adepoju. 2004. Grouping of tropical mid-altitude maize inbred lines on the basis of yield data and molecular markers. *Theoretical and Applied Genetics* 108: 1582-1590.

Menkir, A., B. Badu-Apraku, C. The, and A. Adepoju. 2003. Evaluation of heterotic patterns of IITA's lowland white maize inbred lines. *Maydica* 48: 161-170.

Musimwa, T.R., and J. Derera. 2017. Why SR52 is such a great maize hybrid? II. Heritability, correlation and path-coefficient analyses. *Euphytica* 213: 1-14.

Mwimali, M.G. 2014. Genetic analysis and response to selection for resistance to two stem borers, Busseola fusca and Chilo partellus, in tropical maize germplasm. PhD Thesis. University of KwaZulu-Natal, Pietermaritzburg.

Nyombayire, A., J. Derera, J. Sibiya, E. Gasura, and C. Ngaboyisonga. 2016. Genetic diversity among maize inbred lines selected for the midaltitudes and highlands of Rwanda. *Maydica* 61: 1-7.

Olver, R.C. 1998. Maize breeding in Zimbabwe. pp 22-27. In: H. O. Geverseditor (eds.). Proceedings of Eleventh South African Maize Breeding Symposium, 1994. Cedara Agricultural development Institute, Summer grain Sub-Centre, Pietermaritzburg, KZN.

Parentoni, S.N., J.V. Magalhaes, C.A.P. Pacheco, M.X. Santos, T. Abadie, E.E.G. Gama, P.E.O. Guimaraes, W.F. Meirelles, M.A. Lopes, M.J.V. Vasconcelos, and E. Paiva. 2001. Heterotic groups based on yield-specific combining ability data and phylogenetic relationship determined by RAPD markers for 28 tropical maize open pollinated varieties. *Euphytica* 121: 197–208.

Qi-Lun, Y., F. Ping, K. Ke-Cheng, and P.G. Tang. 2008. Genetic diversity based on SSR markers in maize (*Zea mays* L.) landraces from Wuling mountain region in China. *Journal of Genetics* 87: 287-291.

Rajendran, A., A. Muthiah, J. Joel, P. Shanmugasundaram, and D. Raju. 2014. Heterotic grouping and patterning of quality protein maize inbreds based on genetic and molecular marker studies. *Turkish Journal of Biology* 38: 10-20.

Reif, J.C., A.R. Hallauer, and A.E. Melchinger. 2005. Heterosis and heterotic patterns in maize. *Maydica* 50: 215-223.

Rodriguez, F., G. Alvarado, A. Pacheco, J. Crossa, and J. Burgue. 2015. “AGD-R (Analysis of Genetic Designs with R for Windows) Version 3.0”, http://hdl.handle.net/11529/10202 International Maize and Wheat Improvement Center [Distributor] V11 [Version].

RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

Schrag, T.A., J. Mohring, A. Melchinger, B. Kusterer, B.S. Dhillon, H.P. Piepho, and M. Frisch. 2010. Prediction of hybrid performance in maize using molecular markers and joint analyses of hybrids and parental inbreds. *Theoretical and applied genetics* 120: 451-461.

Semagn, K., C. Magorokosho, B.S. Vivek, D. Makumbi, Y. Beyene, S. Mugo, B.M. Prasanna, and M.L. Warburton. 2012. Molecular characterization of diverse CIMMYT maize inbred lines from Eastern and Southern Africa using single nucleotide polymorphic markers. *BMC Genomics* 13: 1-13.

Sprague, G.F., and L.A. Tatum. 1942. General versus specific combining ability in single crosses of corn. *American Society of Agronomy* 34: 923-932.

Srdic, J., S.M. Drinic, Z. Pajic, and M. Filipovic. 2007. Characterisation of maize inbred lines based on molecular markers, heterosis and pedigree data. *Genetica* 39: 355-363.

Suwarno, W.B., K.V. Pixley, N. Palacios-Rojas, S.M. Kaeppler, and R. Babu. 2014. Formation of heterotic groups and understanding genetic effects in a provitamin A biofortified maize breeding program. *Crop Science* 54: 14-24.

Vasal, S.K., G. Srinivasan, S. Pandey, H.S. Cordova, G.C. Han, and C. Gonzalez. 1992. Heterotic patterns of ninety-two white tropical CIMMYT maize lines. *Maydica* 37: 259-270.

XU, S., J. Liu, and G.S. Liu. 2004. The use of SSRs for predicting the hybrid yield and yield heterosis in 15 key inbred lines of Chinese maize. *Hereditas* 141: 207-215.

Yu, J., H. Lu, and R. Bernardo. 2001. Inconsistencies between SSR grouping and genetic background of white corn inbreds. *Maydica* 46: 133-139.

Zhang, T., X.L. Ni, K.F. Jiang, Q.H. Yang, L. Yang, X.Q. Wan, Y.J. Cao, and J. Zheng. 2010. Correlation between heterosis and genetic distance based on molecular markers of functional genes in rice. *Rice Science* 17: 1-7.

CHAPTER 4

Combining ability and gene action for major traits in maize under stress and non-stress environments

Abstract

The frequent occurrence of drought combined with low levels of soil nitrogen (N) limit maize production in South Africa. Maize breeding efforts targeting varieties with combined tolerance to drought and low soil N are crucial for improved yields. Understanding the combining ability and mode of gene action conferring the inheritance of grain yield under these conditions is also important in selecting suitable germplasm and in predicting breeding progress. The objectives of this study were to (i) asses yield potential of single-cross experimental hybrids under non-stress, drought and low N stress conditions, (ii) estimate combining ability of inbred lines for grain yield and associated secondary traits under stress and non-stress environments and (iii) determine the mode of gene action involved in the expression of yield under stress and non-stress environments. Seventy-two experimental hybrids generated from a 18 × 4 line by tester mating design were evaluated along with four commercial checks under non-stress, drought and low N stress environments in South Africa, during the 2015/16 season. All trials were designed in an (0, 1) α-lattice design with two replications. Low N and drought stress reduced grain yield by 43% and 25%, respectively, relative a mean yield of 6.03 t ha^{-1} under non-stress environments. Furthermore, drought and low N stress significantly reduced plant and ear height. Low N stress increased anthesis-silking interval than drought stress. The hybrids FO215W x CML444 and I-42 x CML444 ranked best across all environments. Among the best hybrids, some contained temperate material; incorporating temperate germplasm into the South African maize program will therefore improve yields. The lines FO215W, I-16, I-42 and K64 displayed desirable general combining ability (GCA) effects for grain yield and several agronomic traits across stress and non-stress environments. Contribution of GCA due to lines and testers varied depending on the trait and environmental conditions. Additive gene effects were more important in controlling grain yield under drought, while non-additive were predominant under low N and non-stress environments. The findings indicate the possibility of obtaining potential hybrids with improved performance under stress and non-stress environments.

Keywords: Gene action; grain yield; combining ability; non-stress and stress environments

4.1 Introduction

South Africa is the largest maize producer in Southern Africa, accounting for 17% of the total maize crop produced in Africa (Agri SA, 2016). South African maize production has undergone significant fluctuations due to drought and low natural soil fertility (WWF-SA, 2011). In this country, a considerable proportion of maize is grown under low nitrogen (N) conditions due to limited fertile land and only approximately 3% of the country agricultural land is considered as "truly fertile" (WWF-SA, 2011). Use of fertilisation is a common solution to the problem; however, due to high input costs, adequate use of fertiliser is impossible for many South African farmers. Low N stress combined with drought effects has posed serious risks in maize production. The country is also characterised by extremely variable and below average rainfall patterns and is classified as one of the most water scarce countries in the world (Agri SA, 2016). An estimated 83% of the SA maize crop is cultivated under dryland conditions; the recent *El Nino* phenomenon has further enhanced the country's vulnerability to drought (Agri SA, 2016). The estimated area planted with maize during the driest growing seasons, 2014/15 and 2015/16 was 43% lower than the 10-year average, coupled by 30 to 35% yield losses (Agri SA, 2016). The effect of drought is probable to increase with negative long-term climate change, which is associated with irregular rainfall patterns and high temperatures coupled with dry soils (WWF-SA, 2011). In farmers' fields, drought and low N stress generally occur simultaneously during the same growing season, this raises the need for selecting varieties with multiple stress tolerance.

Through private-public partnership breeding approaches, progress in improving varieties for adaptation to drought and low N stress has been made in the Water Efficient Maize for Africa (WEMA) and Improved Maize for African soils (IMAS) projects. Relying on the same and few varieties may be risky because failure of these varieties will greatly affect the maize industry. More breeding programs aimed at developing and selecting germplasm that perform efficiently under these two limiting constraints will reduce the impact of drought and low N stress on maize production. However, the success depends on the choice of available superior parents that give good hybrid combinations under both conditions. Knowledge of the combining ability of the available inbred lines in the germplasm is important in determining the breeding value of lines in a hybrid breeding program (Sprague and Tatum, 1942). The total combining ability of inbred lines is partitioned into general (GCA) and specific combining ability (SCA) effects. Sprague and Tatum (1942) defined the GCA as the average performance of a line in a series of cross combinations and SCA as a deviation in performance of a specific cross in relation to the performance of all cross combinations. The GCA predicts the potential benefits of using a parent and is associated with additive gene effects, while SCA is attributed to non-additive gene action for the trait (Haley, 1996). Additive gene effects are reliably fixable and therefore are a good measure of response to selection than non-additive gene effects (Xiang and Li, 2001; Yan and Hunt, 2002; Iqbal *et al*., 2007) as cited by Fellahi *et al.* (2013).

Several approaches were suggested based on breeder's objectives for the estimation of combining ability effects. These include; the line by tester (Kempthorne, 1957), North Carolina design II (Comstock *et al.*, 1949) and the diallel mating design proposed by Griffing (1956). The effective use of a line x tester approach to evaluate the combining ability of a large number of inbred lines and understanding the mode of gene action involved in the expression of grain yield and secondary traits has been reported (Singh and Chaudhary, 1985). Assessing the combining ability of inbred lines and understanding the mode of gene action conditioning grain yield and secondary traits under stress and non-stress conditions is crucial for designing appropriate breeding programs for developing drought and low N tolerant hybrids. Evaluating the combining ability of lines and subsequently the mode of gene action controlling major traits is therefore essential for a breeding program.

Contrasting findings on whether additive or non-additive gene effects condition grain yield and agronomic traits under stress and non-stress have been reported in previous studies. Predominance of additive gene effects for grain yield under drought environments were reported (Betran *et al.*, 2003b; Derera *et al.*, 2008; Medici *et al.*, 2004; Meseka *et al.*, 2013). However, on the contrary, Ndlela (2012) observed the importance of non-additive effects as opposed to additive effects under drought and low N environments. Predominance of non-additive gene effects for grain yield under low N environments were also reported by Betran *et al.* (2003a), Maseka *et al.* (2006) and Meseka *et al.* (2013). Gissa *et al.* (2007) contrarily reported the importance of additive effects for grain yield under low N conditions. However, in some studies (Annor and Badu-Apraku, 2016; Ertiro *et al.*, 2017), the predominance of additive gene effects across non-stress, drought and low N environments has been reported. Meseka and Ishaaq (2012) reported the importance of non-additive gene effects for inheritance of grain yield and most traits under non-stress environments. Both additive and non-additive gene effects were reported by Betran *et al*. (2003b) under non-stress environments conditions; similarly, Derera *et al.* (2008) also found the operation of both additive and non-additive gene action under non-stress conditions. Therefore, the nature of gene action controlling the inheritance of grain yield and other traits under stress and non-stress conditions is not properly understood.

According to Hallauer and Miranda (1988), estimated GCA and SCA effects are only applicable and relative to the sets of inbred lines used in hybrid combinations under test. Information on estimated GCA and SCA effects therefore cannot be generalised. Variations reported in the nature of gene action controlling grain yield under different management conditions indicate complexity in the inheritance of low N and drought tolerant genes. Therefore, studying gene action in control of grain yield and related agronomic traits under different conditions is important for every germplasm. The study was conducted to (i) evaluate the performance of experimental maize hybrids under non-stress, drought and low N stress conditions, (ii) determine the combining ability of inbred lines involved in hybrid development, and (iii) asses the mode of gene action conditioning the expression of grain yield under non-stress, drought and low N conditions.

4.2 **Materials and Methods**

4.2.1 Experimental materials

A total of 18 maize inbred lines were used to generate testcross hybrids using four inbred testers during the 2015/16 season. Inbred lines and testers used are presented in Table 4.1. The inbred lines used were selected to represent the South African maize heterotic groups, which form the basis of a hybrid breeding program. Inbred lines used were developed from germplasm introduced from the United States of America, Australia and mainly from the local open pollinated varieties which mostly originated from the Corn Belt (Saunders, 1942; Van Schaik *et al.,* 1958) as cited by CIMMYT (1997). Details on line development were previously reported (Gevers and Lake, 1998; Gevers and Whythe, 1987; Olver, 1998) and Fourie (2017, personal communication). The four testers were representing known CIMMYT heterotic group A (CML312) and B (CML444) and temperate Corn Belt inbred lines, B73 (group A) and MO17 (group B). The B73 and MO17 testers represent Reid and Lancaster heterotic groups, respectively, and they were developed by the University of Missouri and Iowa State University, respectively. CML312 and CML444 are CIMMYT lines developed by CIMMYT; CML312 is an intermediate maturing line, drought tolerant, subtropical, and resistant to grey leaf (GLS), *Turcicum leaf blight* (TLB) and *Exerohilum turcicum* and is *Maize streak virus* (MSV) susceptible. CML444 is a late maturing inbred line, drought and low N tolerant, mid-altitude or subtropical, but susceptible to MSV and TLB (CIMMYT, 2001). The CIMMYT- derived testers are well adapted and their utility in hybrid breeding programs has been proven for sub-tropical and mid-altitude environments (Ertiro *et al.*, 2017). The seeds used were all obtained from the Agricultural Research Council (ARC) germplasm; however, some inbred lines were originally introduced from the CIMMYT- Zimbabwe breeding program.

Table 4.1: Pedigree and description of materials used in the study

Line Code	**Name**	**Pedigree**	**Description**
L1	E30Y	B390YxM136Y	Line
L2	FO215W	NPPES14.O2S14	Line
L3	I-16	I-16	Line
L4	I-42	I-42	Line
L5	J80W	D800W2.HtN	Line
L6	K64	Pride off Saline	Line
L7	M162W	K64R2.B1138T	Line
L8	MO17HtHtN	MO17HtHtN	Line
L9	P614MSV	B73xVHKG/C1	Line
L10	RO421W	DO940Y-11.O2(W)	Line
L11	RO452W	DO940Y-13.NHK	Line
L12	RO544W	BO160W.3J400W	Line
L13	S198Y	M28Y1.DO620Y	Line
L14	SO181Y	KO326Y2.NPPES1	Line
L15	U127Y	M162W.1KO326Y	Line
L16	U2540W	M162W1.DO940Y-J34	Line
L17	U71Y	M28Y2.NP	Line
L18	VO495Y	POWS12.Y	Line
T1	MO17	(CL.187-2 x C103)	Tester
T2	B73	BSSS C5 (Iowa Stiff Stalk Synthetic)	Tester
T3	CML312	S89500-F2-2-2-1-1-B	Tester
T4	CML444	P43-C9-1-1-1-1-1-B	Tester
Check1	CAP9004	Capstone	Check
Check2	PAN6479	Pannar	Check
Check3	SNK2147	Sensako	Check
Check4	WE3127	WEMA	Check

4.2.2 Experimental design and management

Seventy-two F_1 hybrids derived from the 2015/16 winter nurseries at Makhathini Research Station were evaluated with four commercial checks during the 2015/16 summer season. Evaluations were done across seven experimental environments, which were two non-stress environments, three low nitrogen environments and two drought environments (random and managed drought stress). Experimental sites used, weather data and geographical information is presented in Table 4.2.

Table 4.2: Weather data and geographic information for the study locations used during the 2015/16 growing season

Environment	**Site**	**Season**	**Geographical position**			**Annual Rainfall (mm)**			***Temperature (^0C)***					
									Min			Max		
			Latitude	Longitude	Altitude (m.a.s.l.)	Long-term	2014/15	2015/16	Long-term	2014/15	2015/16	Long-term	2014/15	2015/16
NS and DT	Potchefstroom	Summer	26.74^0 S	27.08^0 E	1349	541	519	364	15	14	16	29	29	31
NS and LN	Cedara	Summer	29.54^0 S	30.26^0 E	1068	662	619	521	14	13	14	25	25	27
Combined LN+DT	Vaalharts	Summer	27.95^0 S	24.84^0 E	1180	356	214	239	15	15	16	32	34	35
DT	Makhathini	Winter	27.39^0 S	32.18^0 E	77	153	127		14	9		28	29	

masl- metres above sea level (m); NS-non-stress; DT-drought stress; LN-Low nitrogen stress

Under non-stress conditions, trials received optimal fertilisation and supplementary irrigation. Basal fertiliser NPK (3:2:1) was applied prior to planting, at 25 kg N ha^{-1}, 17 kg P ha^{-1} and 8 kg K kg ha^{-1}. At four weeks after seedling emergence, trials were top-dressed with 150 kg ha^{-1} of Lime ammonium nitrate (33% N). Phenotyping for drought and low N stress was carried out according to procedure used at CIMMYT (Banziger *et al*., 2000). Screening for low N tolerance was achieved in fields that had been previously depleted of N by growing unfertilised, non-leguminous crops (oats, wheat and sorghum) at high density for several seasons and removing the crop biomass after each season.

For managed low N blocks, basal fertilisers, triple super phosphate (46% P_2O_5) and potassium chloride (61% K_2O) were applied at planting, at a rate of 25 kg P_2O_5 ha^{-1} and 25 kg KCL ha^{-1}. Moisture stress trials were conducted under random drought and managed drought stress conditions. Random drought received sufficient fertilisation as applied in non-stress blocks, with irrigation only applied at the beginning of the season to establish a good plant stand. Managed drought trials were carried out during a rain-free period; trials were adequately irrigated from planting until two weeks before expected flowering. At Vaalharts, there were irrigation problems that coincided with flowering and grain-filling periods, the stress type and duration corresponded with the drought management protocol described by CIMMYT (Banziger *et al*., 2000). Hence, Vaalharts was treated as a site that combined both low N and drought stress.

All trials were arranged in a (0, 1) α-lattice design with two-row plot and two replicates. Two seeds per genotype were initially hand planted per hill, and at 3 weeks after planting; seedlings were thinned to attain a plant population of 53 333 plants ha^{-1}. The rows were 4 m long, with a spacing of 0.75 m apart and 0.25 intra-row spacing. To control weeds, Bateleur Gold 650EC (1.3 L ha^{-1}) was used as a pre-emergence herbicide and Basagran was applied as the post-emergence herbicide at 2.5 L ha^{-1}; subsequent manual weeding was also done to ensure clean fields. Karate (50g/L lambda-cyhalothrin), at 70 ml ha^{-1} was used to control insecticides. Recommended practices for maize production at the Agricultural research council- Grain Crops (ARC-GC) were followed in the execution of all trials. The sprinkler irrigation system was used in all experimental sites except for Cedara where all trials were carried out under rain-fed conditions. Each trial received irrigation at the interval of 7 days for four hours (5 mm hr^{-1}) until physiological maturity, except under drought environments. Data for grain yield (GY) and secondary traits were recorded in all environments. Measurements of traits are described in Table 4.3 (Banziger *et al.*, 2000).

Table 4.3: Description of traits recorded from the experimental trials

Trait	Description and measurement
Anthesis date-AD	Measured as number of days after planting when 50% of the plants shed pollen.
Silking date-SD	Measured as number of days after planting when 50% of the plants show emerged silks.
Anthesis-silking interval-ASI	Determined by calculating the difference between silking date and anthesis date: ASI = SD - AD. Under drought or low N stress, small or negative ASI values indicate stress tolerance.
Plant Height-PH	Measured as height (cm) between the base of a plant to the insertion of the first tassel branch of the same plant.
Ear Height-EH	Measured as height (cm) between the base of a plant to the insertion of the top ear of the same plant.
Ear position-EPO	A ratio of ear height to plant height: EPO=EH/PH. Small values indicate low ear position; large values indicate high ear position.
Number of ears per plant-EPP	Counted as number of ears (NE) with at least one fully developed grain divided by the number of harvested plants (NP): EPP=NE/NP. Values below 1.0 indicates partial barrenness; values above 1.0 indicates prolificacy. Under drought or N stress, values of greater or equal to 1.0 indicate stress tolerance.
Leaf senescence-SEN	Scored under drought and low N conditions on a scale from 1 to 10, 1=10% dead leaf area; 10=100% dead leaf area. Small scores indicate stress tolerance. Drought trials: First score (SEN1) measured at flowering and second score (SEN2) at 7-10 days interval after flowering, average score used. Low N trials: SEN1 measured at 2 weeks before flowering (8-9 leaf stage) and SEN2 at 3 weeks after flowering, average score used.
Plant aspect-PA	Scored on a scale from 1-5 at physiological maturity; include characteristics such as plant and ear height, uniformity of plants, disease and insect damage and lodging; 1 = excellent overall phenotypic appeal, 5 = poor overall phenotypic appeal.

4.2.4 Data analysis

Combined analysis of variance were performed for GY and secondary traits of hybrids including commercial checks, using Genstat 18th edition (VSN International, 2016). Analysis of variance of a line by tester pooled across environments were performed for all traits after omitting the checks. The line by tester procedure embedded in the analyses of genetic designs software with 'R' (AGD-R), (Rodriguez *et al.*, 2015) was used for analysis of variance and to estimate combining ability effects (Singh and Chaudhary, 1985). The following models were used to determine the contribution of lines, testers and their interaction with the hybrids:

$Y_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ij}$

Where; Y_{ijk} = performance of the hybrid when i^{th} line is crossed to j^{th} tester, μ is the overall mean, g_i is the GCA of i^{th} line, g_j is the GCA of the j^{th} tester, s_{ij} is the SCA when i^{th} line is crossed to j^{th} tester and e_{ij} is the random error term for ijk^{th} observation.

$Y_{ijk} = \mu + E_d + REPk\ (E_d) + L_i + T_j + L_i{*}T_j + E_d{*}Li + E_d{*}T_j + E_d{*}L_i{*}T_j + e_{ijkd}$

Y_{ijk} is the k^{th} observation on the i^{th} in the j^{th} progeny, μ is the grand mean, E_d is the environmental effect (d = 1; 2; :::; s), REP_k (E_d) is the effect of replicate k nested in environment d (k = 1; 2; :::; r), L_i is the effects of the i^{th} line, T_j is the effect of the j^{th} tester and $L_i{*}T_j$ is the interaction effect of the cross between the i^{th} line and the j^{th} tester.

The GCA effects of lines (g_i) and testers (g_j) and SCA effects of crosses were estimated as GCA effects:

$$\text{Lines: } g_i = \frac{x.i..}{tr} - \frac{x\ldots}{ltr}$$

$$\text{Testers: } g_j = \frac{x.j..}{lr} - \frac{x\ldots}{ltr}$$

SCA effects:

$$s_{ij} = \frac{x.ij.}{r} - \frac{x.i..}{tr} - \frac{x.j..}{lr} - \frac{x\ldots}{ltr}$$

Where:

g_i and g_j are the GCA effects for ith line and jth tester, respectively; $X_{.j..}$ and $X_{.i..}$ are the sum of the jth tester and the ith line, respectively; $X_{...}$ is the grand total; $X_{.i.j.}$ is the i × j cross sum; l, t and r are the number of lines, testers and replications, respectively, and S_{ij} is the SCA effect of the ijth crosses.

Proportional contribution of lines, testers and their interaction to total variance were estimated as follows: (Singh and Chaudhary, 1977).

$$\text{Contribution of lines} = \frac{SS_l \times 100}{SS_{crosses}}$$

$$\text{Contribution of testers} = \frac{SS_t \times 100}{SS_{crosses}}$$

$$\text{Contribution of l x t} = \frac{SS_{l\times t} \times 100}{SS_{crosses}}$$

Where SS=sums of squares due to lines (l), testers (t), line × tester (l×t) and genotypes (crosses).

Standard Errors for combining ability effects were estimated as:

SE (GCA for line) = $(MSe/rt)^{½}$

SE (GCA of tester) = $(MSe/rl)^{½}$

SE (SCA effects) = $(MSe/r)^{½}$

Standard error of difference between GCA effects of lines and testers were:

SE (g_i-g_j) lines = $(2MSe/rt)^{½}$

SE (g_i-g_j) testers = $(2MSe/rl)^{½}$

Standard error of the difference between SCA effects was:

SE (S_{ji}-S_{ki}) = $(2MSe/r)^{½}$

Where: MSe is the error mean squares from the analysis of variance table, r is the number of replicates, l and t are the number of lines and testers, respectively.

The significance of GCA and SCA effects were tested using t-test; respective GCA and SCA degrees of freedom were used:

$$t = \frac{gca\ or\ sca - 0}{S.E_{gca\ or\ sca}}$$

The hybrid component of the variation for different traits were partitioned into variation to lines (females), testers (male) and line × tester interaction. The main effects of testers and lines represents GCA effects while their interaction (line × tester) represents SCA effects (Hallauer and Miranda, 1988). Lines and testers were treated as random effects. The variances of general (σ^2gca) and specific combining ability (σ^2sca) were tested against their respective error variances derived from the analysis of variance of different traits as follows:

Combining ability variances:

Variance of GCA = [(MSl-MSlxt) + (MSt-MSlxt)]/[r*e (l+t)]
Variance of SCA = [MSlxt-MSe]/r*e

Where: MSl, MSt, MSe and MSlxt are the mean squares for lines, testers, error and line by tester interaction, respectively, l is the number of lines, t is the number of testers, r and e is the number of replications and environments, respectively.

4.3 Results

4.3.1 Analysis of variance for grain yield and selected agronomic traits

Mean squares for genotypes were highly significant ($P<0.01$ to $P<0.001$) for all traits across all environments (Table 4.4). The genotype mean squares were also significant for most traits under non-stress, drought and low N conditions except ASI and PA under non-stress stress (Table 4.5) and drought conditions (Table 4.6), ASI and SP under low N (Table 4.7). Mean squares for both males and females were highly significant ($P<0.001$) for all traits across environments. Female mean squares were significant for most traits in all environmental conditions except PH, EPP and PA under non-stress, ASI across drought and EPP and PA across low N conditions. Mean squares for males were not significant for some traits (GY, ASI, EH, PH, PA and SP) across non-stress conditions. However, under drought and low N conditions, the general combining ability due to testers (GCAt) for most traits was significant ($P<0.05$ to $P<0.001$), except for SEN under drought and SP under low N conditions.

The SCA mean squares were only significant (P<0.05) for AD, EH, EPO and MOI across environments. Under non-stress conditions, the SCA mean squares were significant (P<0.05 to P<0.01) for most traits except DA, DS, ASI and PA, however, none of the traits showed significance under drought conditions, whereas under low N stress conditions only AD and SEN were significant (P<0.05 and P<0.01, respectively). The environment by genotype interaction was significant (P<0.05 and P<0.001) for GY, EH, PH, EPP and MOI across environments, GY, PH, MOI and SP across non-stress and GY, PA and SEN across low N conditions; whereas across drought environments only MOI was significant (P<0.01). The environment, GCAl and GCAt interaction effect was only significant for GY (P<0.01) and EPP (P<0.05) across environments, GY (P<0.05) under non-stress and EPP and SEN (P<0.05) under drought environments. The environment x GCAl interaction was significant (P<0.05 to P<0.001) for some traits across environments (GY, EPP, MOI and SP), non-stress (GY, PH, EPP, MOI and SP), drought (MOI and SP) and low N (GY, SD, EPP, SP and SEN) conditions. Across environments (Table 4.4), the interaction of environment × GCAt was highly significant (P<0.05 to P<0.001) for most traits except DA, DS and SP, whereas under non-stress (Table 4.5) only a few traits (ASI, EH, EPO, MOI and PA) were significant (P<0.05 to P<0.01). The SD, EPP, MOI and SP for environment x GCAt were the only traits that were not significant under low N conditions. The interaction of environments × SCA were highly significant (P<0.05) for GY and EPP across environments, GY across non-stress and EPP and SEN across drought conditions but non-significant across low N (Table 4.7).

Table 4.4: Combined analysis of variance for grain yield and other agronomic traits across seven environments in 2015/16

		Mean Squares										
Source	**df**	**GY**	**AD**	**SD**	**ASI**	**EH**	**PH**	**EPO**	**EPP**	**MOI**	**PA**	**SP**
Environments	6	336.16***	8044.09***	8335.20***	103.36***	29802.79***	68933.89***	0.376***	0.61***	222.98***	17.94***	0.069***
REP(Environment)	7	12.16***	180.94***	200.13***	1.33	1443.33***	5595.55***	0.053***	0.22***	7.70***	0.99**	0.005**
Genotype	71	4.03***	41.91***	52.67***	4.12**	566.38***	936.37***	0.010***	0.09***	4.21***	0.49**	0.003**
GCAl	17	6.08***	58.54***	88.96***	7.45***	839.38***	2028.08***	0.014***	0.07*	5.64***	0.77**	0.003*
GCAt	3	9.93**	513.54***	526.79***	11.38**	5690.20***	5124.58***	0.106***	1.07***	42.94***	1.87***	0.007**
SCA	51	3.00	8.63*	12.72	2.58	173.97*	326.10	0.003*	0.05	1.46*	0.31	0.002
Environment*Genotype	426	3.48***	5.71	9.03	2.98	146.54*	350.21*	0.002	0.05***	1.55***	0.37	0.002
Environment*GCAl	102	4.30***	6.88	10.98	3.06	104.30	362.22	0.002	0.07***	1.78***	0.37	0.003***
Environment*GCAt	18	4.80*	7.22	11.40	7.24***	648.32***	881.13***	0.007***	0.13***	6.47***	1.25***	0.002
Environment*GCAl*GCAt	306	3.13**	5.23	8.25	2.71	131.10	314.97	0.002	0.04*	1.19	0.32	0.002
Error	497	2.42	5.71	9.42	2.81	124.04	284.47	0.002	0.04	1.06	0.33	0.002

****significant at P<0.001; **P<0.01; *P<0.05; GCAl and GCAt=General combining ability due to lines and testers, respectively; SCA=Specific combining ability; df=Degrees of freedom; GY=Grain yield; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; EH=Ear height; PH=Plant height; EPO=Ear position; EPP=Ears per plant; MOI=Grain moisture; PA=Plant aspect; SP=Shelling percentage*

Table 4.5: Combined analysis of variance for grain yield and other agronomic traits across two non-stress environments in 2015/16

		Mean Squares										
Source	**df**	**GY**	**AD**	**SD**	**ASI**	**EH**	**PH**	**EPO**	**EPP**	**MOI**	**PA**	**SP**
Environments	1	0.09	4270.42***	4984.61***	20.60**	61012.04***	31097.41***	0.620***	0.32***	0.25	3.23**	0.015***
REP(Environment)	2	10.24*	456.96***	496.97***	1.96	3359.33***	12026.16***	0.138***	0.08*	11.92***	2.05**	0.001
Genotype	71	5.71***	17.98***	21.67***	2.08	201.19**	444.36*	0.004***	0.04**	2.72***	0.27	0.002***
GCAl	17	7.61**	22.64***	29.38***	3.51*	265.01**	473.83	0.006***	0.03	3.10***	0.39	0.003**
GCAt	3	0.32	174.97***	209.74***	3.66	162.31	538.67	0.012***	0.11**	23.47***	0.49	0.002
SCA	51	5.40**	7.19	8.11	1.51	182.21*	428.99*	0.003**	0.04**	1.38*	0.22	0.002**
Environment*Genotype	71	5.20***	8.24	10.15	2.21	144.39	404.60*	0.002	0.03	2.06***	0.37	0.002*

Source	df	Mean Squares										
		GY	AD	SD	ASI	EH	PH	EPO	EPP	MOI	PA	SP
Environment*GCAl	17	7.93***	9.93	9.07	1.28	88.21	686.66**	0.002	0.05**	2.41***	0.44	0.003***
Environment*GCAt	3	2.81	7.07	6.80	9.35**	625.27**	568.72	0.013***	0.04	20.73***	1.42**	0.001
Environment*GCAl*GCAt	51	4.44*	7.74	10.71	2.11	134.83	300.92	0.002	0.02	0.85	0.28	0.001
Error	142	3.09	7.74	10.09	2.00	124.48	294.78	0.002	0.02	0.96	0.32	0.001

****significant at P<0.001; **P<0.01; *P<0.05; GCAl and GCAt=General combining ability due to lines and testers, respectively; SCA=Specific combining ability; df=Degrees of freedom; GY=Grain yield; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; EH=Ear height; PH=Plant height; EPO=Ear position; EPP=Ears per plant; MOI=Grain moisture; PA=Plant aspect; SP=Shelling percentage*

Table 4.6: Combined analysis of variance for grain yield and other agronomic traits across two drought environments in 2015/16

Source	Mean Squares												
	Df	GY	AD	SD	ASI	EH	PH	EPO	EPP	MOI	PA	SP	SEN
Environments	1	17.91*	4560.12***	3770.01***	37.56***	13945.89***	2098.44**	0.280***	1.86***	267.77***	15.36***	0.064***	30.03***
REP (Environment)	2	24.33***	64.17***	79.76***	0.85	773.71**	3252.45***	0.004*	0.52***	1.04	0.59	0.008*	4.30**
Genotype	71	4.35*	18.62***	21.42***	2.51	384.27***	766.03***	0.006***	0.13***	2.30*	0.59	0.003*	1.02*
GCAl	17	5.72*	33.56***	43.16***	2.58	448.47***	1406.67***	0.007***	0.16***	4.14**	0.93**	0.003*	1.43**
GCAt	3	22.47***	144.03***	130.86***	9.56*	4263.48***	5115.64***	0.069***	1.10***	5.63*	2.75***	0.011**	1.62
SCA	51	2.83	6.27	7.73	2.07	134.68	296.62	0.001	0.06	1.49	0.35	0.002	0.85
Environment*Genotype	71	3.80	6.08	8.58	2.82	136.99	338.51	0.001	0.08	2.64**	0.49	0.002	0.83
Environment*GCAl	17	2.89	7.25	7.98	2.04	78.02	188.45	0.001	0.07	3.22*	0.35	0.004*	0.22
Environment*GCAt	3	6.32	10.47	7.80	3.75	355.38*	634.51	0.003	0.03	4.72*	1.62*	0.001	0.56
Environment*GCAl*GCAt	51	3.95	5.44	8.83	3.03	143.80	371.12	0.001	0.08*	2.32	0.47	0.001	1.04*
Error	142	3.16	5.51	9.47	2.75	122.41	311.25	0.001	0.06	1.66	0.46	0.002	0.68

****significant at P<0.001; **P<0.01; *P<0.05; GCAl and GCAt=General combining ability due to lines and testers, respectively; SCA=Specific combining ability; df=Degrees of freedom; GY=Grain yield; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; EH=Ear height; PH=Plant height; EPO=Ear position; EPP=Ears per plant; MOI=Grain moisture; PA=Plant aspect; SP=Shelling percentage; SEN=leaf senescence*

Table 4.7: Combined analysis of variance for grain yield and other agronomic traits across three low N environments in 2015/16

	Mean Squares												
Source	**df**	**GY**	**AD**	**SD**	**ASI**	**EH**	**PH**	**EPO**	**EPP**	**MOI**	**PA**	**SP**	**SEN**
Environments	2	284.96***	17398.54***	17672.99***	108.69***	12424.22***	128134.41***	0.439***	0.03	202.69***	19.34***	0.010**	126.67***
REP(Environment)	3	16.77***	33.62***	38.59***	1.02	531.89**	3169.93***	0.010**	0.34***	7.49***	0.62	0.010**	2.28*
Genotype	71	3.12**	21.45***	30.97***	4.26	439.02***	694.72***	0.007***	0.06***	2.77***	0.45**	0.003	1.23***
GCAl	17	3.54*	27.05***	48.29***	6.29*	436.07***	1170.57***	0.009***	0.04	3.40***	0.45	0.004*	1.62***
GCAt	3	13.56***	243.37***	269.13***	9.88*	5799.08***	5065.47***	0.077***	0.74***	28.05***	3.15***	0.003	1.87*
SCA	51	2.36	6.54*	11.18	3.25	124.71	278.99	0.002	0.03	1.08	0.30	0.002	1.07**
Environment*Genotype	142	2.55*	4.76	9.94	3.95	130.74	265.98	0.002	0.04	0.98	0.39*	0.002	0.91**
Environment*GCAl	34	2.98*	6.11	14.35*	4.58	117.02	208.59	0.002	0.06***	1.25	0.34	0.004*	0.99*
Environment*GCAt	6	7.34***	9.53*	10.98	8.23*	487.21***	615.15*	0.005*	0.06	0.75	1.38***	0.002	2.23**
Environment*GCAl*GCAt	102	2.13	4.03	8.42	3.48	114.35	264.58	0.002	0.03	0.91	0.34	0.002	0.80
Error	213	1.84	4.42	8.98	3.55	118.40	247.75	0.002	0.03	0.87	0.30	0.002	0.57

****significant at P<0.001; **P<0.01; *P<0.05; GCAl and GCAt=General combining ability due to lines and testers, respectively; SCA=Specific combining ability; df=Degrees of freedom; GY=Grain yield; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; EH=Ear height; PH=Plant height; EPO=Ear position; EPP=Ears per plant; MOI=Grain moisture; PA=Plant aspect; SP=Shelling percentage; SEN=leaf senescence*

4.3.2 Mean performance of hybrids

The mean grain yields of hybrids varied under stress and non-stress environments. Mean grain yield of hybrids ranged from 2.98 - 6.31 t ha^{-1} across environments (Table 4.8), 3.39 - 10.13 t ha^{-1} under non-stress (Table 4.9), 2.40 - 7.70 t ha^{-1} under drought (Table 4.10) and 2.02 - 5.99 t ha^{-1} under low N stress environments (Table 4.11). The average yields were 4.46 t ha^{-1} across sites, 6.03 t ha^{-1} under non-stress, 4.54 t ha^{-1} under drought and 3.46 t ha^{-1} under low N environments. Compared to non-stress environments, 43% grain yield losses were observed under low N conditions while losses due to drought stress were 25%. Most of the experimental hybrids among the 10 superior hybrids across environments performed above the trial mean (relative yield above 100%), mean of checks and some were superior than the best check. The hybrids; L2 x T4, L4 x T4, L10 x T3, L2 x T3, L11 x T3 and L12 x T4 were common under both drought and low N environments (Table 4.10 and Table 4.11). The most superior cross that was common under all management conditions was L2 x T4 (in boldface and underlined; Table 4.8 to Table 4.11). The majority of superior hybrids had CML312 and CML444 as the male parents (Fig 4.1). Anthesis days ranged from 70 - 79 across environments (average 73), 71 - 80 under non-stress (75), 65 - 75 under drought (68) and 69 - 78 under low N (73) environments. The average for days to silking was 75, 76, 69, and 75 days across environments (Table 4.8), non-stress (Table 4.9), drought (Table 4.10) and low N environments (Table 4.11), respectively. Generally, drought stress reduced the number of days to flowering by at least 9%. Anthesis-silking interval was not significantly reduced by drought stress, while N stress increased ASI by 100%. No reductions were observed for the number of ears per plant (EPP) under drought stress, but 9% reductions were due to low N stress. On average, plant and ear height were reduced by up to 3% and 13%, respectively, under drought stress, whereas under low N stress, reductions were 9% and 17%, respectively.

Table 4.8: Performance of top 10 and bottom 10 testcrosses and commercial checks for grain yield (t ha^{-1}) and other agronomic traits across environments in 2015/16

Cross	Absolute GY	Relative yield	%Checks	%Best Check	AD	SD	ASI	EH	PH	EPO	EPP	MOI	PA	SP	SEN
L2 x T4	6.31	141	129	109	79	79	1	97.73	173.57	0.57	1.02	14.37	4.29	0.82	3.33
L4 x T4	5.88	132	120	102	75	76	2	104.79	190.85	0.55	0.97	13.38	4.13	0.85	2.52
L10 x T2	5.79	130	118	100	74	76	2	91.58	175.56	0.52	0.92	12.11	4.17	0.83	2.68
L17 x T4	5.52	124	113	96	73	74	1	111.22	191.60	0.58	0.91	12.75	4.17	0.81	4.14
L3 x T4	5.45	122	111	94	74	76	2	104.56	174.57	0.60	1.13	13.61	4.08	0.81	3.82
L9 x T3	5.38	121	110	93	73	74	1	101.81	183.63	0.55	0.96	12.26	4.29	0.84	2.85
L10 x T1	5.32	119	109	92	73	75	2	92.11	183.48	0.50	0.96	12.82	4.29	0.81	3.17
L2 x T3	5.23	117	107	91	76	76	1	98.26	183.59	0.54	0.90	15.03	4.04	0.84	3.49
L18 x T3	5.07	114	103	88	75	76	1	110.13	190.77	0.58	0.85	13.78	3.88	0.82	3.33
L10 x T3	5.03	113	103	87	76	78	3	99.17	189.90	0.53	0.84	13.62	3.96	0.81	3.01
L14 x T2	3.86	87	79	67	73	74	1	93.89	175.73	0.53	0.86	12.57	4.54	0.80	3.17
L8 x T4	3.85	86	79	67	73	75	2	105.26	185.39	0.57	0.94	13.68	4.29	0.79	3.33

Cross	Absolute GY	Relative yield	%Checks	%Best Check	AD	SD	ASI	EH	PH	EPO	EPP	MOI	PA	SP	SEN
L2 x T2	3.83	86	78	66	75	76	3	84.35	162.60	0.52	0.78	12.43	4.50	0.80	3.33
L16 x T4	3.79	85	77	66	75	76	1	100.47	173.01	0.58	1.08	13.23	4.63	0.80	3.17
L15 x T4	3.73	84	76	65	74	75	1	100.55	183.74	0.55	0.86	12.44	4.83	0.80	3.49
L17 x T2	3.73	84	76	65	72	73	1	97.73	187.38	0.52	0.81	12.02	4.50	0.79	3.33
L9 x T1	3.66	82	75	63	70	71	1	99.83	181.72	0.55	0.95	12.55	4.46	0.81	4.46
L12 x T1	3.60	81	73	62	73	75	2	81.24	161.92	0.50	0.78	12.72	4.58	0.79	2.68
L7 x T3	3.36	75	69	58	76	78	2	104.91	180.82	0.58	0.76	13.19	4.58	0.79	3.65
L15 x T1	2.98	67	61	52	72	74	2	83.17	167.17	0.50	0.77	12.10	4.54	0.80	3.17
Check 1	4.09	92	83	71	74	77	3	101.89	178.57	0.57	0.87	12.27	4.75	0.81	3.01
Check 2	4.94	111	101	86	75	77	2	97.72	172.98	0.56	0.87	12.99	4.46	0.81	3.49
Check 3	4.81	108	98	83	74	76	2	94.74	178.16	0.53	0.88	12.36	4.42	0.80	2.85
Check 4	5.77	129	118	100	75	77	2	105.67	191.41	0.55	0.95	12.79	4.04	0.83	3.01
Grand Mean	***4.46***				***73***	***75***	***1***	***97.83***	***179.95***	***0.55***	***0.91***	***12.88***	***4.38***	***0.81***	***3.24***
Checks	***4.90***														
Best Check	***5.77***														
#Locations	***6***														
Minimum	***2.98***				***70***	***71***	***1***	***81.24***	***159.75***	***0.49***	***0.76***	***12.01***	***3.79***	***0.78***	***2.36***
Maximum	***6.31***				***79***	***79***	***3***	***111.67***	***203.71***	***0.61***	***1.13***	***15.03***	***4.83***	***0.85***	***4.46***
Standard error	***0.45***				***0.82***	***1.00***	***0.49***	***3.31***	***5.37***	***0.01***	***0.05***	***0.31***	***0.17***	***0.01***	***0.24***
LSD (0.05)	***1.25***				***2.26***	***2.78***	***1.35***	***9.21***	***14.93***	***0.04***	***0.15***	***0.85***	***0.47***	***0.03***	***0.65***
CV (%)	***34.90***				***3.90***	***4.60***	***112.80***	***11.70***	***10.30***	***9.50***	***21.0***	***8.30***	***13.30***	***5.10***	***25.00***

LSD=Least significant difference; CV=coefficient of variation; GY=Grain yield; AD=Anthesis date; SD=Silking date; ASI=Anthesis-silking interval; EH=Ear height; PH=Plant height; EPO=Ear position; EPP=Ears per plant; MOI=Grain moisture; PA=Plant aspect; SP=Shelling percentage; SEN=leaf senescence

Table 4.9: Performance of top 10 and bottom 10 testcrosses and commercial checks for grain yield (t ha^{-1}) and other agronomic traits under two non-stress environments in 2015/16

Cross	Absolute GY	Relative yield	%Checks	%Best Check	AD	SD	ASI	EH	PH	EPO	EPP	MOI	PA	SP
L2 x T4	10.13	168	146	134	80	81	1	109.95	192.45	0.60	1.15	15.2	4.60	0.85
L10 x T2	9.58	159	138	127	77	79	3	103.40	184.80	0.55	0.90	12.4	4.00	0.90
L10 x T1	8.63	143	124	114	75	75	0	104.70	200.65	0.55	1.05	14.1	4.10	0.90
L3 x T4	7.82	130	112	103	76	78	2	115.05	183.10	0.65	1.15	14.6	4.10	0.80
L12 x T3	7.72	128	111	102	76	77	1	98.65	197.30	0.50	1.10	14.2	4.20	0.85
L18 x T3	7.58	126	109	100	75	77	2	117.35	199.50	0.60	1.00	13.9	4.35	0.85
L17 x T4	7.38	122	106	98	75	75	0	118.80	195.65	0.60	0.95	12.7	3.95	0.85
L16 x T2	7.17	119	103	95	72	73	1	115.80	195.35	0.60	1.00	12.9	4.20	0.80
L9 x T3	7.16	119	103	95	75	77	2	112.00	186.45	0.60	0.95	12.6	4.10	0.90
L13 x T1	7.16	119	103	95	74	75	1	107.20	205.15	0.55	1.00	13.3	4.25	0.80
L15 x T3	4.86	81	70	64	77	77	1	110.55	191.15	0.60	0.80	13.0	4.35	0.90
L5 x T2	4.81	80	69	64	75	78	2	109.00	203.85	0.55	0.90	13.1	4.50	0.85
L5 x T3	4.58	76	66	61	79	80	2	114.95	192.40	0.60	0.90	13.7	4.60	0.85
L12 x T4	4.52	75	65	60	76	78	1	95.45	169.70	0.55	0.95	14.1	4.60	0.85
L12 x T1	4.36	72	63	58	76	77	2	88.75	171.25	0.55	0.75	12.6	4.60	0.85
L8 x T4	4.35	72	63	58	75	77	3	108.90	176.45	0.65	0.80	14.5	4.35	0.85
L15 x T4	4.14	69	60	55	77	78	1	106.95	193.65	0.55	0.80	12.6	5.00	0.85
L15 x T1	4.08	68	59	54	74	75	2	90.35	169.65	0.55	0.95	13.7	4.50	0.80
L6 x T3	4.04	67	58	53	78	79	2	99.75	170.95	0.60	0.65	13.2	4.85	0.75
L7 x T3	3.39	56	49	45	79	81	2	110.15	183.55	0.60	0.65	14.5	4.85	0.80
Check 1	6.61	110	95	87	75	74	-2	109.85	194.10	0.55	1.25	13.1	4.60	0.80
Check 2	7.37	122	106	97	76	76	0	107.65	202.55	0.55	1.15	11.7	3.45	0.85
Check 3	7.56	125	109	100	74	75	1	106.55	202.45	0.55	1.00	11.3	3.45	0.80
Check 4	7.28	104	90	83	76	76	-1	115.10	205.00	0.55	1.10	12.8	3.10	0.80
Grand mean	***6.03***				***75***	***76***	***1***	***108.35***	***189.53***	***0.57***	***0.94***	***13.5***	***4.41***	***0.83***

Cross	Absolute GY	Relative yield	%Checks	%Best Check	AD	SD	ASI	EH	PH	EPO	EPP	MOI	PA	SP
Checks	***6.96***													
Best Checks	***7.56***													
#Locations	***2.00***													
Minimum	***3.39***				***71***	***72***	***-2***	***88.75***	***164.10***	***0.50***	***0.65***	***11.25***	***3.10***	***0.75***
Maximum	***10.13***				***80***	***81***	***3***	***117.35***	***200.65***	***0.65***	***1.15***	***15.20***	***4.60***	***0.90***
Standard error	***0.87***				***1.86***	***2.04***	***0.71***	***5.54***	***10.64***	***0.03***	***0.07***	***0.52***	***0.28***	***0.02***
LSD (0.05)	***2.42***				***5.1***	***5.60***	***2***	***15.34***	***29.23***	***0.07***	***0.21***	***1.5***	***0.81***	***0.05***
CV (%)	***28.7***				***4.9***	***5.30***	***142.8***	***10.1***	***11.00***	***9.4***	***16.1***	***7.8***	***13.1***	***4.1***

LSD=Least significant difference; CV=coefficient of variation; GY=Grain yield; AD=Anthesis date; SD=Silking date; ASI=Anthesis-silking interval; EH=Ear height; PH=Plant height; EPO=Ear position; EPP=Ears per plant; MOI=Grain moisture; PA=Plant aspect; SP=Shelling percentage

Table 4.10: Performance of top 10 and bottom 10 testcrosses and commercial checks for grain yield (t ha^{-1}) and other agronomic traits under two drought environments in 2015/16

Cross	Absolute GY	Relative yield	%Checks	%Best Check	AD	SD	ASI	EH	PH	EPO	EPP	MOI	PA	SP	SEN
L4 x T4	7.70	170	156	113	70	70	-1	107.75	214.65	0.50	1.05	13.58	3.38	0.86	1.75
L10 x T3	6.63	146	134	97	69	70	1	103.75	215.85	0.48	0.95	14.08	3.25	0.85	2.25
L6 x T3	6.54	144	133	96	67	68	1	107.70	196.45	0.55	1.05	13.85	3.00	0.87	3.00
L2 x T3	6.40	141	130	94	72	73	-1	94.60	186.40	0.51	0.99	16.00	3.25	0.85	2.75
L11 x T3	6.28	138	127	92	69	69	-1	107.35	205.35	0.53	0.94	13.90	4.13	0.84	2.25
L5 x T3	6.24	137	126	91	70	72	1	106.05	205.50	0.52	0.96	14.20	3.63	0.81	2.75
L6 x T4	5.92	130	120	87	67	68	1	102.35	182.45	0.56	1.46	13.50	3.63	0.83	2.25
L14 x T4	5.83	128	118	85	69	70	0	95.95	173.60	0.56	1.35	14.05	4.63	0.83	3.00
L2 x T4	5.83	128	118	85	75	74	-1	92.30	168.65	0.55	0.94	14.00	3.38	0.83	2.50
L12 x T4	5.74	126	116	84	67	68	1	90.05	162.35	0.56	1.19	13.23	4.00	0.81	2.50
L12 x T1	3.47	76	70	51	67	69	1	78.40	163.30	0.48	0.73	14.25	4.38	0.77	2.25
L14 x T2	3.35	74	68	49	67	68	1	90.70	175.20	0.51	0.87	13.38	4.00	0.84	2.25

Cross	Absolute GY	Relative yield	%Checks	%Best Check	AD	SD	ASI	EH	PH	EPO	EPP	MOI	PA	SP	SEN
L9 x T1	3.30	73	67	48	65	66	0	91.10	182.25	0.50	1.02	12.35	4.25	0.82	3.25
L18 x T4	3.26	72	66	48	64	65	1	98.00	171.75	0.57	1.27	12.50	4.13	0.75	3.50
L7 x T4	3.23	71	65	47	69	71	1	104.45	187.30	0.56	1.01	13.70	4.50	0.76	3.75
L17 x T2	3.16	70	64	46	68	69	1	88.60	185.50	0.48	0.74	12.33	4.25	0.80	3.25
L9 x T2	3.14	69	64	46	65	66	1	82.50	169.05	0.49	0.91	13.10	4.25	0.80	2.75
L1 x T3	3.08	68	62	45	69	69	1	96.40	188.35	0.51	0.96	13.15	3.75	0.80	2.50
L1 x T1	2.73	60	55	40	66	66	-1	87.40	167.85	0.52	0.74	12.68	4.50	0.77	4.25
L15 x T1	2.40	53	49	35	67	68	1	79.55	169.05	0.47	0.66	11.15	4.63	0.81	3.00
Check 1	3.62	80	73	53	72	75	3	94.85	175.25	0.54	0.80	12.18	4.75	0.82	2.00
Check 2	4.96	109	101	73	68	70	2	86.85	175.45	0.50	0.90	14.10	4.00	0.82	2.75
Check 3	4.33	95	88	63	69	71	1	91.75	189.05	0.49	0.83	13.10	3.88	0.80	2.25
Check 4	6.82	150	138	100	68	70	1	102.35	198.20	0.51	0.88	13.40	3.50	0.83	3.00
Grand Mean	***4.54***				***68***	***69***	***1.00***	***94.34***	***183.75***	***0.51***	***0.94***	***13.23***	***4.08***	***0.81***	***2.69***
Checks	***4.93***														
Best Check	***6.82***														
#Locations	***2.00***														
Minimum	***2.40***				***64***	***65***	***-1***	***72.7***	***158.7***	***0.4***	***0.6***	***11.2***	***3.0***	***0.8***	***1.5***
Maximum	***7.70***				***75***	***75***	***4***	***123.1***	***215.9***	***0.6***	***1.5***	***16.0***	***4.8***	***0.9***	***4.3***
Standard error	***0.90***				***1.15***	***1.54***	***0.89***	***5.67***	***9.08***	***0.02***	***0.12***	***0.64***	***0.02***	***0.34***	***0.41***
LSD (0.05)	***2.52***				***3.20***	***4.30***	***2.40***	***15.83***	***25.30***	***0.05***	***0.34***	***1.80***	***0.95***	***0.06***	***1.15***
CV (%)	***39.7***				***3.40***	***4.50***	***182.00***	***12.00***	***9.90***	***6.60***	***26.2***	***9.70***	***16.6***	***5.5***	***30.50***

LSD=Least significant difference; CV=coefficient of variation; GY=Grain yield; AD=Anthesis date; SD=Silking date; ASI=Anthesis-silking interval; EH=Ear height; PH=Plant height; EPO=Ear position; EPP=Ears per plant; MOI=Grain moisture; PA=Plant aspect; SP=Shelling percentage; SEN=leaf senescence

Table 4.11: Performance of top 10 and bottom 10 testcrosses and commercial checks for grain yield (t ha^{-1}) and other agronomic traits under three low N environments in 2015/16

Cross	**Absolute GY**	**Relative yield**	**%Checks**	**%Best Check**	**AD**	**SD**	**ASI**	**EH**	**PH**	**EPO**	**EPP**	**MOI**	**PA**	**SP**	**SEN**
L4 x T4	5.99	173	166	113	74	77	2	95.67	182.33	0.53	0.98	12.58	4.08	0.83	2.00
L17 x T4	5.07	147	141	96	72	73	1	104.00	189.00	0.56	0.98	12.55	4.17	0.80	2.83
L12 x T4	4.83	140	134	91	72	73	1	87.33	165.00	0.54	1.11	12.05	4.50	0.80	2.33
L14 x T1	4.60	133	128	87	71	73	1	79.00	164.67	0.49	0.86	12.43	4.33	0.84	2.50
L2 x T3	4.52	131	126	85	75	77	1	90.67	175.67	0.52	0.87	13.90	4.25	0.83	2.83
L10 x T3	4.42	128	123	83	75	79	3	90.33	179.67	0.51	0.75	12.58	4.08	0.78	2.33
L2 x T4	4.31	125	120	81	78	79	1	90.67	163.67	0.56	0.90	14.07	4.25	0.80	2.67
L11 x T4	4.29	124	119	81	72	73	1	102.67	178.67	0.58	1.09	12.87	4.33	0.75	2.33
L11 x T3	4.22	122	117	80	75	77	1	102.33	189.67	0.55	0.81	12.65	4.58	0.81	2.50
L9 x T4	4.14	120	115	78	73	73	1	101.33	179.67	0.57	0.97	12.20	4.33	0.78	2.17
L18 x T4	2.69	78	75	51	70	72	1	95.67	168.00	0.57	1.00	12.10	4.33	0.77	3.17
L1 x T1	2.66	77	74	50	71	73	1	86.33	161.00	0.55	0.90	11.87	4.83	0.80	2.50
L15 x T1	2.65	77	74	50	71	73	2	79.33	165.67	0.48	0.75	10.83	4.58	0.79	2.00
L15 x T2	2.62	76	73	49	71	72	1	80.33	162.33	0.50	0.70	11.07	4.67	0.81	2.00
L13 x T2	2.54	73	70	48	74	78	3	88.67	167.33	0.53	0.71	11.77	4.58	0.80	2.50
L13 x T1	2.48	72	69	47	75	76	2	75.33	155.33	0.49	0.82	11.50	4.92	0.77	2.00
L18 x T1	2.41	70	67	46	71	75	4	86.67	168.67	0.52	0.75	11.55	4.58	0.82	2.50
L14 x T2	2.35	68	65	44	73	76	2	78.33	160.33	0.49	0.75	12.45	4.83	0.80	2.33
L16 x T4	2.14	62	60	40	75	77	2	95.00	163.67	0.58	0.96	12.53	4.75	0.76	2.83
L7 x T1	2.02	58	56	38	73	75	2	81.00	163.67	0.51	0.71	11.77	4.83	0.78	2.67
Check 1	2.30	67	64	43	74	80	5	87.33	161.67	0.54	0.65	11.38	4.83	0.79	2.77
Check 2	3.52	102	98	66	73	76	3	91.33	170.00	0.54	0.85	12.02	4.42	0.78	3.03
Check 3	3.25	94	90	61	72	75	3	86.33	169.67	0.51	0.82	11.75	4.50	0.77	2.03
Check 4	5.30	153	147	100	74	76	2	99.67	182.67	0.55	0.90	12.07	3.83	0.82	2.53
Gran Mean	***3.46***				***73***	***75***	***2***	***89.55***	***171.80***	***0.53***	***0.86***	***12.00***	***4.48***	***0.79***	***2.46***

Cross	Absolute GY	Relative yield	%Checks	%Best Check	AD	SD	ASI	EH	PH	EPO	EPP	MOI	PA	SP	SEN
Checks	***3.60***														
Best Check	***5.30***														
#Locations	***3***														
Minimum	***2.02***	***58.41***	***56.14***	***38.11***	***69***	***70***	***1***	***70.00***	***150.00***	***0.46***	***0.65***	***10.83***	***3.67***	***0.75***	***1.83***
Maximum	***5.99***	***173.03***	***166.31***	***112.89***	***78***	***80***	***5***	***108.67***	***205.33***	***0.60***	***1.11***	***14.07***	***4.92***	***0.84***	***3.83***
Standard error	***0.56***				***0.8***	***1.22***	***0.78***	***4.45***	***6.61***	***0.02***	***0.07***	***0.39***	***0.22***	***0.37***	***0.02***
LSD (0.05)	***1.56***				***2.4***	***3.40***	***2***	***12.40***	***18.40***	***0.05***	***0.21***	***1.07***	***0.63***	***0.06***	***0.71***
CV (%)	***39.70***				***2.8***	***4.00***	***92.7***	***12.2***	***9.4***	***8.9***	***20.9***	***7.90***	***12.30***	***6.20***	***25.5***

LSD=Least significant difference; CV=coefficient of variation; GY=Grain yield; AD=Anthesis date; SD=Silking date; ASI=Anthesis-silking interval; EH=Ear height; PH=Plant height; EPO=Ear position; EPP=Ears per plant; MOI=Grain moisture; PA=Plant aspect; SP=Shelling percentage; SEN=leaf senescence

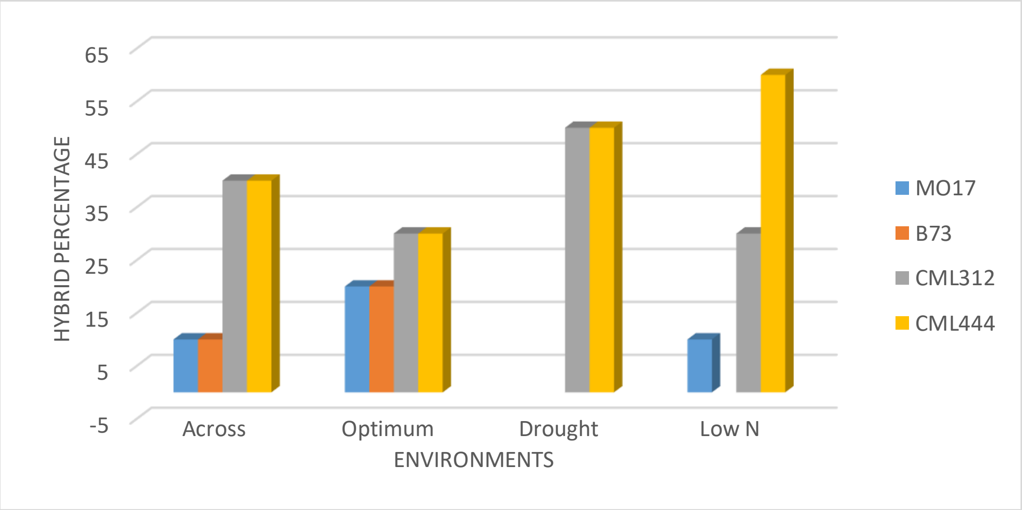

Figure 4.1: Percentage of hybrids containing MO17, B73, CML312 and CML444 across all environments, non-stress, drought and low N environments in 2015/16

4.3.3 General combining ability effects

General combining ability effects for GY and selected agronomic traits estimated across environments are presented in Table 4.12. Line 10 (RO421W) was a good general combiner (0.77; P<0.05) for grain yield. Desirable GCA effect for GY was also shown by line 2 (FO215W); however, line 2 showed a high positive and significant GCA effects for anthesis days (2.14) and grain moisture (0.95). Significant and negative GCA effects for ear height and ear position were shown by line 12 (RO544W) and line 4 (I-42); line 4 also had the highest and significant shelling percentage, while the lowest SP was shown by line 7 (M162W). Line 3 (I-16) was a good general combiner for ears per plant with the highest positive and significant GCA effect, the worst (-0.075; P<0.05) combiner for EPP was line 15 (U127Y). Among testers, CML444 was the best combiner for GY and most traits.

Under non-stress environments (Table 4.12), line 10 (RO421W) and line 2 (FO215W) were also good general combiners for GY with high positive GCA effects of 1.78 (P<0.05) and 1.09, respectively. Line 2 also had a positive and significant GCA effect for grain moisture. High, positive (1.26) and significant GCA effect for ASI was shown by Line 3 (I-16). Good, negative and significant GCA effects for ear height and ear position were recorded for line 12 (RO544W), whereas line 11 (RO452W) had a high positive and significant GCA effect for EPO.

Under drought conditions (Table 4.13), line 2 (FO215W), 4 (I-42), 5 (J80W), 6 (K64) and line 11 (RO452W) had desirable GCA effects for GY. Line 2 showed high positive and significant GCA effects for anthesis and silking days and grain moisture. High and significant GCA effects for anthesis and silking days were also shown by line 13 (S198Y). Line 4 (I-42) had good negative GCA effects for anthesis-silking interval, ear position and leaf senescence. CML444 was also the best tester for most traits under drought conditions.

Line 2, 4 and line 11 were also among good general combiners for GY under low N stress environments (Table 4.13). However, line 2 also showed a significant (P<0.001) and positive GCA effect for grain moisture content. Line 4 also showed good negative GCA for ear position and the highest GCA effect for the number of ears per plant, while line 13 (S198Y) was the poorest general combiner with high negative and significant GCA effect for EPP, line 13 (S198Y) also showed high and significant GCA effects for both anthesis and silking days.

Table 4.12: Estimates of general combining ability (GCA) effects of lines and testers for grain yield and selected agronomic traits across and under non-stress environments in 2015/16

LINE	**Name**	**GY**	**AD**	**ASI**	**EH**	**EPO**	**EPP**	**MOI**	**SP**	**GY**	**ASI**	**EH**	**EPO**	**MOI**
		Across environments								**Non-stress environments**				
1	E30Y	-0.29	-0.44	-0.45	-2.99	0.012	0.036	0.14	0.003	0.13	-0.05	-3.12	0.002	0.61
2	FO215W	0.59	2.14*	0.13	-6.09	-0.011	-0.023	0.95**	0.009	1.09	-0.17	-1.76	0.002	0.88*
3	I-16	0.21	0.16	0.71	-3.18	0.011	0.079*	0.33	0.001	0.20	1.26**	-2.56	0.01	0.88
4	I-42	0.26	-0.90	-0.08	-1.99	-0.037*	0.032	-0.22	0.017*	-0.72	-0.11	-0.49	-0.02	-0.3
5	J80W	-0.14	1.14	0.71	4.99	0.009	-0.001	-0.07	-0.005	-0.76	0.45	5.04	0.01	-0.05
6	K64	-0.05	-0.78	-0.27	-0.34	0.017	0.012	0.14	0.006	-0.52	-0.43	-1.06	0.01	-0.23
7	M162W	-0.38	0.72	0.36	5.80	0.017	-0.032	-0.01	-0.017*	-0.18	0.07	4.88	0.01	-0.09
8	MO17HtHtN	-0.22	-0.51	0.05	1.97	-0.009	-0.02	0.01	-0.006	-0.51	0.45	-0.71	-0.002	0.21
9	P614MSV	0.002	-1.80	-0.64	3.09	0.012	0.031	-0.42	0.005	-0.12	-0.43	2.71	0.02	-0.64
10	RO421W	0.77*	1.51	0.51	-1.80	-0.016	0.012	0.01	0.002	1.78*	0.20	-0.85	-0.01	0.08
11	RO452W	0.23	-0.34	-0.33	6.56	0.032	0.007	0.02	-0.007	-0.19	0.14	5.30	0.04*	-0.09
12	RO544W	0.08	-0.26	0.17	-8.36*	-0.018	0.021	0.19	0.001	-0.22	0.51	-10.95**	-0.04*	0
13	S198Y	-0.22	1.89	0.15	-0.49	0.004	-0.063	0.02	-0.008	0.33	-0.11	2.71	0.01	0.13
14	SO181Y	0.01	0.12	-0.33	-2.65	-0.013	0.028	0.167	0.003	0.08	-0.43	0.34	-0.02	-0.14
15	U127Y	-0.76*	-0.19	-0.27	-3.07	-0.023	-0.075*	-0.64	-0.005	-1.21	-0.36	-4.97	-0.03	-0.56
16	U2540W	-0.23	0.01	-0.02	1.21	0.013	-0.012	-0.23	-0.009	0.02	-0.18	0.67	0.02	-0.09
17	U71Y	0.24	-1.07	-0.43	5.05	-0.004	-0.037	-0.48	0.002	0.51	-0.80	4.74	0.002	-0.63
18	VO495Y	-0.09	-1.40	0.01	2.31	0.005	0.007	0.06	0.008	0.30	0.01	0.08	-0.02	0.02
Grand mean		**4.43**	**73.34**	**1.45**	**97.75**	**0.55**	**0.91**	**12.90**	**0.81**	**5.98**	**1.11**	**108.28**	**0.57**	**13.57**
Standard error		**0.35**	**1.07**	**0.38**	**4.06**	**0.02**	**0.04**	**0.33**	**0.01**	**0.67**	**0.46**	**3.96**	**0.02**	**0.43**
Tester														
1	MO17	-0.21	-1.48	-0.16	-5.25	-0.018	-0.02	-0.18	-0.004	0.005	-0.32	-1.74	-0.01	-0.16
2	B73	-0.15	-1.13	0.25	-3.54	-0.015	-0.04	-0.55	0.001	0.039	0.21	-0.71	-0.01	-0.73
3	CML312	0.12	1.65	0.14	4.04	0.002	-0.04	0.37	0.008	-0.096	0.08	1.56	0.003	0.58
4	CML444	0.24	0.97	-0.23	4.76	0.031	0.1	0.34	-0.005	0.052	0.04	0.89	0.02	0.31
Tester mean		**4.43**	**73.34**	**1.45**	**97.75**	**0.55**	**0.91**	**12.90**	**0.81**	**5.98**	**1.11**	**108.28**	**0.57**	**13.57**
Standard error		**0.19**	**1.34**	**0.20**	**4.44**	**0.02**	**0.06**	**0.39**	**0.01**	**0.06**	**0.20**	**1.30**	**0.01**	**0.49**

=significant at 0.05 probability level; **=significant at 0.01 probability level; *; GY=Grain yield; AD=Anthesis date; ASI=Anthesis-silking interval; EH=Ear height; EPO=Ear position; EPP=Ears per plant; MOI=Grain moisture; SP=Shelling percentage*

Table 4.13: Estimates of general combining ability (GCA) effects of lines and testers for grain yield and selected agronomic traits under drought and low N stress environments in 2015/16

LINE	Line	GY	AD	SD	ASI	EPO	MOI	SEN	GY	AD	SD	ASI	PH	EPO	EPP	MOI
		Drought environments							**low N environments**							
1	E30Y	-1.10	-0.51	-1.15	-0.64	0.015	-0.32	0.24	-0.48	0.24	-0.37	-0.61	-8.97	0.02	0.04	0.08
2	FO215W	0.58	3.12*	3.35*	0.24	-0.017	1.19*	0.18	0.55	1.91	1.96	0.06	-7.79	-0.02	-0.02	1.05***
3	I-16	0.01	-0.44	-0.21	0.24	0.021	-0.03	0.30	0.02	0.62	1.21	0.60	-11.06	0.01	0.04	0.05
4	I-42	0.92	-0.69	-1.40	-0.70	-0.05*	-0.35	-0.45	0.68	-0.80	-0.58	0.22	7.87	-0.05*	0.06	-0.37
5	J80W	0.71	0.68	0.98	0.30	0.001	0.02	0.05	0.07	0.49	1.46	0.97	4.05	0.01	0.02	-0.02
6	K64	0.87	-1.51	-1.58	-0.08	0.021	0.77	-0.20	0.03	-1.05	-1.29	-0.24	-5.76	0.02	0.04	-0.16
7	M162W	-0.61	1.37	1.79	0.42	0.012	0.01	0.30	-0.57	0.20	0.80	0.60	3.07	0.03	-0.07	0.15
8	MO17HtHtN	-0.24	0.12	-0.21	-0.33	-0.023	-0.04	0.18	0.004	-0.38	-0.62	-0.24	13.52*	-0.01	-0.01	-0.11
9	P614MSV	-0.06	-2.26	-2.52	-0.26	0.012	-0.43	-0.20	0.02	-1.80	-2.70	-0.90	1.09	0.01	0.01	-0.26
10	RO421W	0.31	1.12	1.54	0.42	-0.026	-0.05	-0.26	0.19	1.20	1.88	0.68	-0.13	-0.01	-0.02	-0.03
11	RO452W	0.79	-0.82	-1.4	-0.58	0.030	0.02	-0.20	0.38	-0.55	-1.08	-0.53	4.36	0.02	0.03	0.29
12	RO544W	-0.14	-0.38	0.29	0.67	0.001	0.56	-0.14	0.38	-0.38	-0.62	-0.24	-7.44	-0.01	0.05	0.3
13	S198Y	-0.56	3.06*	3.29*	0.24	-0.010	-0.14	-0.45	-0.43	2.41*	2.80*	0.39	-5.31	0.01	-0.09*	0.17
14	SO181Y	0.11	0.12	-0.21	-0.33	0.001	0.59	-0.01	0.23	0.45	0.38	-0.07	-2.81	-0.02	0.02	0.29
15	U127Y	-0.84	-0.07	-0.27	-0.20	-0.013	-1.02*	-0.20	-0.41	-0.51	-0.91	-0.40	2.99	-0.02	-0.04	-0.62
16	U2540W	-0.30	-0.32	-0.15	0.17	0.012	-0.28	-0.14	-0.51	-0.38	-0.16	0.22	-0.86	0.01	-0.04	-0.45
17	U71Y	-0.17	-0.82	-0.58	0.24	-0.003	-0.40	0.68*	0.22	-0.72	-1.25	-0.53	11.76	-0.01	-0.03	-0.42
18	VO495Y	-0.30	-1.76	-1.58	0.17	0.019	-0.11	0.30	-0.38	-0.93	-0.91	0.01	1.44	0.01	-0.01	-0.05
Grand mean		**4.52**	**67.69**	**68.58**	**0.89**	**0.513**	**13.23**	**2.70**	**3.45**	**72.88**	**74.87**	**1.99**	**171.88**	**0.53**	**0.87**	**12.01**
Standard error		**0.58**	**1.41**	**1.60**	**0.39**	**0.012**	**0.49**	**0.29**	**0.37**	**1.03**	**1.38**	**0.50**	**6.79**	**0.02**	**0.04**	**0.37**
Tester																
1	MO17	-0.54	-1.40	-1.62	-0.22	-0.024	-0.10	0.18	-0.34	-1.25	-1.33	-0.09	-6.48	-0.02	-0.028	-0.282
2	B73	-0.41	-1.00	-0.49	0.51	-0.018	-0.34	-0.09	-0.25	-1.26	-1.13	0.13	-4.16	-0.02	-0.059	-0.567
3	CML312	0.54	1.47	1.47	4E-16	-0.002	0.29	-0.16	0.22	1.74	2.06	0.33	8.84	0.003	-0.036	0.283
4	CML444	0.42	0.93	0.64	-0.29	0.044	0.16	0.07	0.38	0.77	0.40	-0.38	1.81	0.037	0.123	0.54
Tester mean		**4.52**	**67.69**	**68.58**	**0.89**	**0.513**	**13.23**	**2.70**	**3.45**	**72.88**	**74.87**	**1.99**	**171.88**	**0.53**	**0.87**	**12.01**
Standard error		**0.48**	**1.22**	**1.17**	**0.32**	**0.027**	**0.24**	**0.13**	**0.31**	**1.30**	**1.37**	**0.26**	**5.93**	**0.02**	**0.07**	**0.44**

=significant at 0.05 probability level; *=significant at 0.001 probability level GY=Grain yield; AD=Anthesis date; SD=Silking date; ASI=Anthesis-silking interval; EH=Ear height; EPO=Ear position; EPP=Ears per plant; MOI=Grain moisture; SEN=Leaf senescence*

4.3.4 Specific combining ability effects

Estimates of SCA effects for grain yield are presented in Table 4.14. The highest (1.05) and significant SCA effects for grain yield across environments were shown by crosses L2 x T4, L4 x T4, L9 x T3; poor specific combiners with low negative and significant SCA effects were L2 x T2 and L7 x T3. The crosses L2 x T4 (3.01; P<0.01) and L12 x T3 (2.05; P<0.05) were the most superior combiners under non-stress environments, the worst combiners were L7 x T3, L2 x T2 and L10 x T3. The cross L4 x T4 was the best combiner for grain yield under drought environments, with positive and significant SCA effect (1.84; P<0.01); poor specific combiners with statically low negative and significant SCA effects were L10 x T4 and L18 x T4. Under low N stress environments, L4 x T4, L14 x T1 and L17 x T4 were the best specific combiners for grain yield, with high positive and significant SCA effects, the poorest was L16 x T4.

Table 4.14: Estimates of specific combining ability (SCA) effects for grain yield across environments, two non-stress, two drought and three low N stress environments in 2015/16

Cross	Name	Across	Non-stress	Drought stress	Low N stress
L1 x T1	E30Y x MO17	0.10	0.80	-0.15	0.03
L1 x T2	E30Y x B73	0.01	-0.88	0.72	0.49
L1 x T3	E30Y x CML312	-0.03	0.90	-0.88	-0.48
L1 x T4	E30Y x CML444	-0.07	-0.82	0.32	-0.05
L2 x T1	FO215W x MO17	-0.09	-0.25	0.16	0.35
L2 x T2	FO215W x B73	-1.05**	-1.98*	-1.21	-0.58
L2 x T3	FO215W x CML312	0.08	-0.78	0.76	0.30
L2 x T4	FO215W x CML444	1.05**	3.01**	0.30	-0.07
L3 x T1	I-16 x MO17	-0.25	-0.59	-0.30	0.22
L3 x T2	I-16 x B73	0.03	-0.90	1.04	0.20
L3 x T3	I-16 x CML312	-0.35	-0.10	-0.97	-0.38
L3 x T4	I-16 x CML444	0.58	1.58	0.24	-0.04
L4 x T1	I-42 x MO17	-0.20	-0.22	-0.07	-0.50
L4 x T2	I-42 x B73	-0.38	-0.33	-0.61	-0.56
L4 x T3	I-42 x CML312	-0.37	-0.20	-1.16	-0.42
L4 x T4	I-42 x CML444	0.95*	0.75	1.84**	1.48**
L5 x T1	J80W x MO17	0.02	0.05	-0.48	0.39
L5 x T2	J80W x B73	0.13	-0.45	0.10	0.77
L5 x T3	J80W x CML312	-0.25	-0.55	0.46	-0.58
L5 x T4	J80W x CML444	0.10	0.96	-0.09	-0.59
L6 x T1	K64 x MO17	0.25	0.93	-0.39	0.20
L6 x T2	K64 x B73	-0.01	0.83	-0.32	-0.44

Cross	Name	Across	Non-stress	Drought stress	Low N stress
L6 x T3	K64 x CML312	-0.17	-1.32	0.61	0.41
L6 x T4	K64 x CML444	-0.07	-0.44	0.11	-0.18
L7 x T1	M162W x MO17	0.30	0.89	0.32	-0.51
L7 x T2	M162W x B73	0.71	0.72	1.18	1.01
L7 x T3	M162W x CML312	-0.82*	-2.32*	-0.40	-0.13
L7 x T4	M162W x CML444	-0.20	0.72	-1.11	-0.37
L8 x T1	MO17HtHtN x MO17	0.35	1.05	-0.05	-0.40
L8 x T2	MO17HtHtN x B73	-0.11	-0.57	0.00	0.50
L8 x T3	MO17HtHtN x CML312	0.36	0.69	0.71	0.35
L8 x T4	MO17HtHtN x CML444	-0.60	-1.16	-0.66	-0.45
L9 x T1	P614MSV x MO17	-0.56	-0.88	-0.63	-0.23
L9 x T2	P614MSV x B73	-0.16	0.31	-0.91	-0.46
L9 x T3	P614MSV x CML312	0.83*	1.40	0.69	0.40
L9 x T4	P614MSV x CML444	-0.10	-0.83	0.85	0.29
L10 x T1	RO421W x MO17	0.34	0.86	0.15	-0.29
L10 x T2	RO421W x B73	0.74	1.79	0.25	0.55
L10 x T3	RO421W x CML312	-0.29	-1.90*	1.26	0.56
L10 x T4	RO421W x CML444	-0.79	-0.76	-1.67*	-0.82
L11 x T1	RO452W x MO17	0.15	-0.13	0.32	0.14
L11 x T2	RO452W x B73	-0.07	0.08	-0.32	-0.38
L11 x T3	RO452W x CML312	0.05	0.22	0.42	0.17
L11 x T4	RO452W x CML444	-0.13	-0.17	-0.42	0.07
L12 x T1	RO544W x MO17	-0.71	-1.41	-0.38	-0.75
L12 x T2	RO544W x B73	0.34	0.66	0.27	0.30
L12 x T3	RO544W x CML312	0.36	2.05*	-0.82	-0.17
L12 x T4	RO544W x CML444	0.01	-1.30	0.92	0.62
L13 x T1	S198Y x MO17	0.28	0.84	0.33	-0.20
L13 x T2	S198Y x B73	0.05	-0.09	0.00	-0.23
L13 x T3	S198Y x CML312	-0.28	-0.29	-0.72	-0.03
L13 x T4	S198Y x CML444	-0.05	-0.47	0.39	0.46
L14 x T1	SO181Y x MO17	0.41	-0.38	0.97	1.27*
L14 x T2	SO181Y x B73	-0.43	0.36	-0.86	-1.07*
L14 x T3	SO181Y x CML312	-0.01	0.80	-0.89	-0.23
L14 x T4	SO181Y x CML444	0.04	-0.77	0.78	0.03
L15 x T1	U127Y x MO17	-0.47	-0.70	-0.73	-0.04
L15 x T2	U127Y x B73	0.40	1.20	0.31	-0.16
L15 x T3	U127Y x CML312	0.24	0.18	0.64	-0.33
L15 x T4	U127Y x CML444	-0.17	-0.68	-0.22	0.53
L16 x T1	U2540W x MO17	0.02	-0.99	-0.04	0.97

Cross	Name	Across	Non-stress	Drought stress	Low N stress
L16 x T2	U2540W x B73	0.48	1.13	0.75	0.12
L16 x T3	U2540W x CML312	0.15	0.02	0.11	0.10
L16 x T4	U2540W x CML444	-0.65	-0.15	-0.81	-1.18*
L17 x T1	U71Y x MO17	0.27	0.55	0.80	-0.34
L17 x T2	U71Y x B73	-0.80	-1.19	-0.77	-0.63
L17 x T3	U71Y x CML312	-0.09	-0.20	-0.64	-0.05
L17 x T4	U71Y x CML444	0.61	0.83	0.62	1.03*
L18 x T1	VO495Y x MO17	-0.21	-0.41	0.16	-0.31
L18 x T2	VO495Y x B73	0.12	-0.70	0.38	0.57
L18 x T3	VO495Y x CML312	0.60	1.40	0.84	0.51
L18 x T4	VO495Y x CML444	-0.51	-0.29	-1.39*	-0.76
Grand mean		**4.43**	**5.98**	**4.52**	**3.45**
Standard error		**0.42**	**0.98**	**0.71**	**0.53**

**=significant at 0.05 probability level; **=significant at 0.01 probability level; L=Line; T=Tester*

4.3.5 Variance components and contribution of GCA and SCA sums of squares

The relative contribution of SCA and GCA effects sums of squares for grain yield and related secondary traits across all environments, non-stress, drought and low N stress environments are presented in Table 4.15 to 4.18. The contribution of lines (females), testers (males) and SCA varied with environments and traits. The GCA sums of squares contributed the greatest proportion to GY across non-stress, stress and across environments. For most traits, the GCA due to lines (GCAl) was more important than GCA due to testers (GCAt) under both stress and non-stress environments.

Table 4.15: Percentage sum of squares of general and specific combining ability effects for grain yield and related agronomic traits across seven environments in 2015/16

Contribution (%)	GY	AD	DS	ASI	EH	PH	EPO	EPP	MOI	PA	SP
SSGCAl	36.13	33.44	40.44	43.32	35.48	51.86	34.39	17.39	32.10	37.82	29.387
SSGCAt	10.42	51.77	42.26	11.68	42.45	23.12	44.40	47.77	43.10	16.26	11.900
SSSCA	53.46	14.79	17.35	45.04	22.06	25.02	21.21	34.84	24.83	45.92	58.713
SCA	0.29	1.46	1.65	-0.11	24.97	20.82	0.0005	0.01	0.20	-0.01	0.0002
GCA	0.23	12.61	13.42	0.31	140.49	147.74	0.0026	0.02	1.04	0.05	0.0001

SS=Sums of squares; GCA=General combining ability; l=line; t=tester; SCA=Specific combining ability; GY=Grain yield; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; EH=Ear height; PH=Plant height; EPO=Ear position; EPP=Ears per plant; MOI=Grain moisture; PA=Plant aspect; SP=Shelling percentage

Table 4.16: Percentage sum of squares of general and specific combining ability effects for grain yield and related agronomic traits across two non-stress environments in 2015/16

Contribution (%)	GY	AD	DS	ASI	EH	PH	EPO	EPP	MOI	PA	SP
SSGCAl	31.88	30.15	32.46	40.39	31.54	25.53	34.487	17.30	27.25	34.28	28.022
SSGCAt	0.24	41.12	40.89	7.43	3.41	5.12	11.188	12.51	36.40	7.69	3.301
SSSCA	67.88	28.73	26.89	52.19	65.05	69.35	54.326	70.19	36.35	58.03	68.677
SCA	1.16	-0.28	-0.99	-0.24	28.87	67.11	6.3E-04	0.01	0.21	-0.05	5.0E-04
GCA	-0.07	4.16	5.07	0.09	1.43	3.51	2.6E-04	0.00	0.54	0.01	2.1E-06

SS=Sums of squares; GCA=General combining ability; l=line; t=tester; SCA=Specific combining ability; GY=Grain yield; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; EH=Ear height; PH=Plant height; EPO=Ear position; EPP=Ears per plant; MOI=Grain moisture; PA=Plant aspect; SP=Shelling percentage

Table 4.17: Percentage sum of squares of general and specific combining ability effects for grain yield and related agronomic traits across two drought environments in 2015/16

Contribution (%)	GY	AD	DS	ASI	EH	PH	EPO	EPP	MOI	PA	SP	SEN
SSGCAl	31.49	43.15	48.26	24.63	27.94	43.97	30.163	29.64	43.10	37.94	28.646	33.51
SSGCAt	21.83	32.68	25.82	16.08	46.88	28.22	52.069	36.10	10.34	19.72	16.004	6.72
SSSCA	46.68	24.17	25.92	59.29	25.18	27.81	17.768	34.25	46.56	42.33	55.350	59.77
SCA	-0.17	0.38	-0.87	-0.34	6.14	-7.31	0.00	0.00	-0.09	-0.06	0.00	0.08
GCA	0.51	3.75	3.60	0.18	100.97	134.75	0.00	0.03	0.15	0.07	0.00	0.03

SS=Sums of squares; GCA=General combining ability; l=line; t=tester; SCA=Specific combining ability; GY=Grain yield; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; EH=Ear height; PH=Plant height; EPO=Ear position; EPP=Ears per plant; MOI=Grain moisture; PA=Plant aspect; SP=Shelling percentage; SEN=Leaf senescence

Table 4.18: Percentage sum of squares of general and specific combining ability effects for grain yield and related agronomic traits across three low N environments in 2015/16

Contribution (%)	GY	AD	SD	ASI	EH	PH	EPO	EPP	MOI	PA	SP	SEN
SSGCAl	27.18	30.19	37.34	35.37	23.78	40.34	30.072	16.16	29.38	23.70	37.708	31.50
SSGCAt	18.39	47.93	36.72	9.81	55.81	30.81	47.647	51.29	42.80	29.39	4.824	6.42
SSSCA	54.43	21.88	25.94	54.82	20.40	28.85	22.281	32.55	28.02	46.91	57.468	62.08
SCA	0.26	1.06	1.10	-0.15	3.15	15.62	0.00	0.00	0.10	0.00	0.00	0.25
GCA	0.28	5.85	6.71	0.22	136.04	129.05	0.00	0.02	0.67	0.07	0.00	0.03

SS=Sums of squares; GCA=General combining ability; l=line; t=tester; SCA=Specific combining ability GY=Grain yield; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; EH=Ear height; PH=Plant height; EPO=Ear position; EPP=Ears per plant; MOI=Grain moisture; PA=Plant aspect; SP=Shelling percentage; SEN=Leaf senescence

4.4 **Discussion**

4.4.1 Analysis of variance and hybrid performance for grain yield and agronomic traits

Analysis of variance revealed significant differences for GY and most agronomic traits among genotypes across sites, non-stress, drought and low N conditions, indicating the presence of variation among genotypes. These results have implications for the breeding and selection of the best performing hybrids under both stress and non-stress conditions. The effect of drought and low N stress observed falls within the range of yield reductions reported in several studies. Ertiro *et al.* (2017) observed 50% and 69% yield reductions in maize hybrids due to drought and low N, respectively. Ndlela (2012) observed 61% yield losses under drought stress and 15% reductions under low N stress. Other investigators (Betran *et al.,* 2003b) reported high reductions of up to 65% under low N, 13% and 50% under intermediate and drought stress, respectively. Meseka *et al.* (2013) reported 40% and 63% yield losses due to mild and severe water stress, and 52% reductions were caused by low N stress. Banziger *et al.* (2000) proposed that yield reductions of 15 - 20% could be observed under moderate drought and 25 - 35% under low N stress conditions. These variations in yield reductions under low N and drought stress environments suggest that yield losses due to drought and low N stress mostly depend on the hybrids evaluated, environments used, duration and intensity of stress.

The majority of the experimental hybrids among the ten superior hybrids across sites performed above the trial mean (Relative yield above 100%), mean of checks and some were superior to the best check. Five hybrids were common under both drought and low N environment. The single cross hybrid L2 x T4 (FO215W x CML444) was generally the best hybrid across environments, with 41% yield advantage over mean, 29% advantage over checks and 9% yield improvement over the best check. This cross consistently appeared in the top ten hybrids across different environments, thus it could be useful for improving maize production under both stress and non-stress environments. The above-mentioned hybrid can therefore perform well under stress environments and without yield penalties under non-stress conditions. The cross L2 x T4 was a late maturing hybrid characterised by the maximum AD, SD, MOI and reduced ASI score across environments. In the past studies, superior hybrids identified by Ndhlela (2012) and Hosana *et al.* (2015) were also late maturing. Late maturing varieties generally have an extended time for accumulating and utilising photosynthetic products during grain filling, which subsequently contribute to high grain yield (Banziger *et al.*, 2000).

On average, drought stress reduced days to flowering by ±7 days, for example the average of 68 and 69 days for AD and SD, respectively, under drought relative to 75 and 76 days under non-stress was recorded. Derera *et al.* (2008) reported a ±10 day reduction in flowering under stress than non-stress conditions. As a drought adaptive response, hybrids grew rapidly to reach physiological maturity. Shorter growing seasons generally reduce leaf area development, which limits the amount of captured radiation and subsequently accumulation of photosynthetic products required for full grain filling (Banziger *et al.*, 2000). The observed earliness under drought stress thus carried overall yield penalties. On average, the top ten superior varieties across all sites consistently had a high average plant height under non-stress, drought and low N conditions compared to the inferior ten. Low N environments had the lowest mean grain yield and significant reductions in plant and ear height. This confirms that reductions in plant and ear height are associated with yield reductions especially under stress environments. Hosana *et al.* (2015) reported high GY in taller plants than shorter plants; this was attributed to high accumulation of synthetic products during the grain filling period. The effect of drought and low N stress on plant height is in agreement with previous studies where 1% and 40% reductions due to drought and low N, respectively were reported (Ertiro *et al.*, 2017).

The hybrid L4 x T4 was the second best hybrid across sites, the yield advantage of this hybrid was 32% over the trial mean, 20% over checks and 2% over the best check. L4 x T4 was also the most superior hybrid under both drought and low N conditions. This hybrid can therefore be used to improve tolerance to both drought and low N stress. The potential hybrids L2 x T4 and L4 x T4 had one common tester (CML444), indicating that CML444 contributed greatly to higher yields and to stress tolerance as indicated by the largest proportion of superior hybrids containing CML444. Other studies have also shown that the top performing hybrids across sites generally have one common parent; for example, Ndlela (2012) similarly observed that most hybrids that were amongst the top ten across sites also had one common male parent, RS61P.

About 50% among the best ten hybrids that were drought tolerant were also superior under low N conditions, with generally greater than 15% yield improvement over the trial mean and over checks, indicating the potential of new varieties, which may be commercialised in South Africa. According to Makumbi *et al.* (2011), these hybrids have favourable genes particularly with additive effects for both parents; a combination resulting from such parents thus results in a combined potential for water and nitrogen use efficiency. This is also associated with the similar adaptive mechanism for tolerance to both stresses (Banziger *et al*., 2002). Ertiro *et al.* (2017) also identified superior experimental hybrids across stress environments, indicating that they were superior for both drought and low N tolerance. In the above-mentioned study, it was reported that the parental lines of superior hybrids were fixed for favourable alleles through selection for favourable traits including ASI and good GCA effects across non-stress and random stress environments in early generation testing, this therefore contributed largely to higher yields across environments. In this study, both tropical and temperate testers were used to develop hybrids; nonetheless, the majority of the top 10 under different environments were derived from tropical CIMMYT testers. Hybrids containing the temperate Corn Belt testers, MO17 (T1) and B73 (T2) were also identified amongst the top 10 superior hybrids, under both stress and non-stress environments. This indicated the potential of temperate material in the South African growing conditions, and hence the likelihood of obtaining tropical x temperate hybrids with broader genetic base and good adaptation.

4.4.2 General combining ability effects

Knowledge of combining ability of inbred lines is important for devising good breeding strategies; from this study, inbred lines and testers with desirable general combing ability effects for yield and other agronomic traits were identified. Some lines combined well across sites while others showed good general combining ability for specific environments. The inconsistencies of inbred lines for grain yield across different environments are also reported in the literature (Ertiro *et al.*, 2017; Makumbi *et al.*, 2011). Overall, the study showed that selection of desired parental lines based on GCA values may be effective in improving yield under both stress and non-stress environments. Line 2 (FO215W) and line 10 (RO421W) were identified as best general combiners across sites, line 2 (FO215W), line 4 (I-42), line 6 (K64) and line 11 (RO452W) showed good levels of GCA effects for yield under stress environments. Therefore, indicating the possibility of obtaining superior stress tolerant hybrids, with high grain yield, when these lines are included as parental lines in hybrid make-ups. Most lines that showed good GCA effects under drought stress were also superior under low N environments. Therefore, selection for superior performance under drought stress will equally improve low N tolerance and the opposite holds, this was also observed by Meseka *et al.* (2013). To achieve high grain yield, good GCA effects for both grain yield and number of ears per plant are desirable. Furthermore, line 2 (FO215W), 4 (I-42), Line 3 (I-16) and line 6 (K64) showed desirable GCA effects for EPP under both stress and non-stress environments and will be ideal for improving maize yields in different environments. The tropical

CIMMYT tester, T4 (CML444) was the most superior tester across all environments, with desirable combining ability for grain yield and EPP, whereas tester 3 (CML312) was the best combiner primarily under low N environments. The contribution of CML444 and CML312 in hybrid yield was also observed from the percentage of superior crosses containing these testers across sites. CIMMYT testers are broad base testers that are widely utilised in many breeding programs in Southern Africa and are well known for high levels of adaptation and tolerance to several abiotic stresses (Ertiro *et al*., 2017); these testers are thus very significant in hybrid breeding programs.

A negative anthesis silking interval is desired to enhance stress tolerance and grain yield. The temperate Corn Belt tester T1 (MO17) was the best general combiner for early maturity, with desirable negative anthesis-silking interval. Therefore, it would be worthwhile to use MO17 in hybrid combinations to enhance maturity. Line 4 (I-42) was the best amongst all inbred lines, displaying negative ASI effects under different environments, and should be considered to improve stress tolerance and grain yield. Selection for reduced ASI to improve hybrid performance under stress conditions has been evinced in several previous investigations (Bolanos and Edmeades, 1996; Magorokosho *et al*., 2003; Ertiro *et al.*, 2017).

4.4.3 Specific combining ability effects

The usefulness of a particular cross in expressing high heterosis is pre-determined by its SCA effect (Meseka *et al*., 2012). The analysis evidenced significant differences between genotypes under different environments. The best cross-combinations with high and significant SCA effects for grain yield across sites were L2 x T4, L4 x T4, and L9 x T3. The cross L2 x T4 was also the most superior under non-stress conditions. Cross combinations that combined well only under drought environments were L10 x T3 and L7 x T2; those that only exhibited good SCA effects under low N were L4 x T4, L14 x T1 and L17 x T4. The cross between L4 and T4 was the most superior under both drought and low N conditions. These cross combinations were all ranked in the top ten superior hybrids in selected environments, indicating a relationship between GY and SCA effects; the same was also observed by Hosana *et al.* (2015). Furthermore, some lines such as L2, L4 and T10 and testers (T3 and T4) used in these crosses showed high GCA effects for GY across stress and non-stress environments. These findings are in agreement with findings by Meseka *et al.* (2013) where most hybrids with at least one drought tolerant inbred line exhibited significant SCA effects. It has been reported that genotypes that generally show improved performance under drought conditions also perform well under low N conditions as a result of the favourable alleles for stress-tolerance (Ertiro *et al.*, 2017); the same was also observed in this study.

However, some of the superior combinations contained inbred lines that showed poor GCA effects for GY in different environments; for example, line 7 had negative GCA effect under drought, whereas line 9, 17 and 14 had positive but small GCA effects across sites and under low N conditions, respectively. This can be correlated with findings by Meseka *et al.* (2013), where drought tolerant inbred lines combined well with drought susceptible inbred lines. It was concluded that even though some lines may not display good performance under stress conditions, but when used in combination with a superior stress tolerant line, a good SCA effect for GY may be expressed in crosses. This has been verified in some studies (Bao *et al.*, 2009; Ndhlela, 2012; Tyagi and Lal, 2005), indicating that poor general combiners may possibly produce hybrids with high SCA effects for grain yield if the other parent in a cross is properly selected. Ejigu *et al.* (2017) also observed that in some cases, high SCA effects could be derived from cross combinations between (i) good × good GCA effects, (ii) poor × poor, and (iii) good × poor GCA effects, suggesting that good SCA effects do not necessarily result from a combination of two superior general combiners. These three scenarios were respectively attributed to (i) additive × additive gene action, (ii) dominance by dominance type of non-allelic gene action, which produces overdominance in crosses, and (iii) favourable additive effects of a good general combining parent and epistatic effects of a poor combiner (Fasahat *et al.*, 2016).

4.4.4 Identification of testers

In this study, line 2 (FO215W), 4 (I-42), 3 (I-16) and line 6 (K64) showed desirable GCA effects for most traits. Lines displaying good GCA effects for more than one trait should be considered for utilisation as (i) parental lines for developing hybrids, (ii) lines for use in recurrent selection programs, (iii) in recycling of inbred lines, and (iv) as testers for screening newly developed inbred lines (Makumbi *et al.*, 2011). The feasibility of identifying new inbred candidate testers based on good GCA effects has been demonstrated (Ertiro *et al.*, 2017). The South African maize breeding program currently utilises single cross and inbred testers from CIMMYT, the testers used are mainly intermediate to late maturing, and they have been useful in developing single-cross and three-way hybrids targeted for the region. From the current study, the lines identified that showed potential for utilisation as new suitable tester lines could also be beneficial in developing early to medium maturing hybrids for use in different environmental conditions.

The definition and choice of a suitable tester has been based on the objective of a breeding program. Several definitions of a good tester thus exist in relation to inbred line evaluation (Matzinger, 1953; Rawlings and Thompson, 1962; Vasal, 1995) and population improvement (Allison and Curnow, 1966). Hallauer (1975) generalised a good tester as one which include simplicity in use, provide information that correctly classifies the relative merit of lines and maximise genetic gains. Pswarayi and Vivek (2008) identified inbred and single cross testers for use in developing early maturing single-cross and double -cross hybrids. The choice of potential lines as testers was based on display of good GCA effects for GY and other traits, classification of lines into heterotic groups and per se grain yield under different environments. Whereas, for potential single cross testers, selection was based on good GCA effects of the inbred lines constituting the single cross, grouping of the inbred lines constituting the single-cross to the same heterotic group, desirable per se performance of the inbred lines constituting the single-cross and good yield potential of the single cross, for use as female parent in three-way and double-cross hybrids. With the given characteristics of an ideal single-cross tester; from this study, some crosses such as L2 x T4 (FO215W x CML444) and L4 x T4 (I-42 x CML444) could qualify as potential single cross testers. However, further assessment of the identified potential testers is required to verify the general attributes of a good tester and therefore to recommend as potential testers for use in developing different hybrids.

4.4.5 Gene action controlling yield and secondary traits

The relative importance of GCA and SCA in crosses is useful in any breeding program in making informed decisions. Significant differences for GY were observed for lines, testers and their interaction under non-stress conditions, with lines being more important than males, indicating that the inheritance of GY under non-stress environments is controlled by both additive and non-additive gene action, but the non-additive gene effects were more important. The importance of additive gene effects in controlling GY was observed under drought environments, while non-additive effects were predominant under low N environments. Betran *et al*. (2003a) also reported an increasing importance of additive effects under drought conditions, indicating that when breeding for drought tolerance; both inbred lines for use in hybrid combinations should have alleles for drought tolerance. This was also confirmed by Derera *et al*. (2008) where combinations between drought tolerant × conventional lines were more tolerant to drought than drought susceptible × conventional lines. These findings suggested that to improve yield for stress environments; selection of potential parents can be used to predict hybrid performance and this will greatly improve the level of stress tolerance.

In some investigations, non-additive genetic effects were also more important under low N (Maseka *et al.*, 2006; Ndlela (2012). However, contradicting results on the mode of gene action conditioning grain yield and other agronomic traits under stress and non-stress environments are available in the literature. Makumbi *et al.* (2011) and Ertiro *et al.* (2017) reported a greater role of additive variance across non-stress, drought and low N environments. Gissa *et al*. (2007) reported the high importance of additive effects for GY under low N conditions; Ndlela (2012) reported the importance of non-additive effects for GY under drought environments. Miti (2007) reported a larger role of additive effects in the expression of grain yield under both low N and drought environments. The differences observed by several investigators could be attributable to the type of germplasm used and the test environments in which the germplasm was evaluated. The type of gene action involved on the inheritance of most secondary traits also varied under different environments. The predominance of SCA (non-additive genetic effects) is a consequence of fluctuations in dominance relationships among parents (Wassimi *et al*., 1986) as cited by Ndlela, (2012). Therefore, for traits indicating the predominance of non-additive effects, selection of parents for hybrid development may not be judged by high GCA effects due to dominance gene effects that may also contribute to the improvement and expression of a particular trait.

4.4.6 Relative contribution of lines and testers

The line by tester mating scheme allows for estimation of relative contribution of lines and testers to hybrid performance using a ratio of their sums of squares (Singh and Chaudhary, 1985). The study revealed that line GCA (GCAl) was more important in controlling grain yield than tester GCA (GCAt) across all environments (36:10%), non-stress (32:0.24%), drought (31:22%) and low N conditions (27:18%), indicating the value of superior lines in grain yield. This suggests that when selecting lines to improve grain yield, parents to use as female parents should be carefully selected. For most secondary traits, the contribution of either GCAl or GCAt varied across environments, implying that the contribution of GCAl and GCAt will always vary depending on the trait and the conditions in which the trait was measured. This has also been observed in several studies, for example, Ndlela (2012) also reported a larger contribution of GCAf than GCAm for grain yield across sites, non-stress conditions and low N conditions and equal contribution under drought conditions. Previous findings by Derera *et al.* (2008) reported greater contributions of

GCAm than GCAf for grain yield under drought conditions, and equal contribution of females and males to hybrid grain yield under stress environments. Modifications of grain yield and most traits by maternal effects particularly under drought stress could explain why a fewer number of hybrids for stress tolerance have been developed (Derera *et al.*, 2008). The presence of significant maternal effects might lead to bias estimates of additive variance (Hallauer and Miranda, 1988). Therefore, examining the role of male or female effect in the expression of GY and secondary traits is important in hybrid breeding to avoid unreliable or overestimation of heritabilities, resulting from biased estimates of GCA variances inflated by the presence of maternal effects.

Conclusion

The findings of this study confirmed progress made in developing drought and low N tolerant hybrids. The result also showed that it is possible to develop higher yielding early to medium maturing hybrids; The potential of temperate material in producing superior hybrids was also demonstrated. The overall best hybrid across all sites was L2 x T4; several hybrids also showed high performance under both drought and low N conditions. The identified potential hybrids should be considered for further testing and later for registration and release. These varieties will maximise productivity gains in SA farmer's fields, primarily smallholder farmers regardless of accessibility and affordability of the full range of resources needed to optimise yields. The observed mode of gene action conditioning GY under different environments indicated that potential parents could be used to predict hybrid performance and improve yield levels under stress and non-stress environments. The study further revealed that selection for parents to use as either male or female in breeding for stress tolerance is necessary. Favourable inbred lines including FO215W, K64, I-16 and I-42 that displayed good overall general combining ability for grain yield and several agronomic traits under different environments were identified for consideration as new inbred testers, in breeding for tolerance to drought and low N stress. Single-crosses FO215W x CML444 and I-42 x CML444 could also qualify as potential single-cross testers for use in three-way-hybrids. Further assessment of the identified potential testers is necessary to verify their suitability for use as new testers in the SA maize breeding program.

References

Agri SA. 2016. “A raindrop in the drought”. Report to the Multi-Stakeholder Task Team on the drought, Agri SA’s status report on the current drought crisis. Agri South Africa, Pretoria, South Africa. http://www.nstf.org.za/wp-content/uploads/2016/06/Agri-SA-Drought-Report_CS4.pdf (Accessed 15/07/2016).

Allison, J.C.S., and R.W. Curnow. 1966. On the choice of tester parent for the breeding of synthetic varieties of maize (*Zea mays* L.). *Crop Science* 6: 541-544.

Annor, B., and B. Badu-Apraku. 2016. Gene action controlling grain yield and other agronomic traits of extra-early quality protein maize under stress and non-stress conditions. *Euphytica* 212: 213–228.

Banziger, M., G.O. Edmeades, and H.R. Lafitte. 2002. The increased N stress tolerance of tropical maize selected for drought tolerance. *Field Crops Research* 75: 223-233.

Banziger, M., G.O. Edmeades, D. Beck, and M. Bellon. 2000. Breeding for drought and Nitrogen stress tolerance in maize: From theory to practice. CIMMYT, Mexico, D.F.

Bao, Y., S. Wang, X. Wang, Y. Wang, X. Li, L. Wang, and H. Wang. 2009. Heterosis and combining ability for major yield traits of a new wheat germplasm shannong 0095 derived from thinopyrum intermedium. *Agricultural Sciences in China* 8: 753-760.

Betran, F.J., D. Beck, M. Banziger, and G.O. Edmeades. 2003a. Genetic analysis of inbred and hybrid grain yield under stress and non-stress environments in tropical maize. *Crop Science* 43: 807-817.

Betran, F.J., J.M. Ribaut, D. Beck, and D.G. de Leon. 2003b. Genetic diversity specific combining ability and heterosis in tropical maize under stress and non-stress environments. *Crop Science* 43: 797-806.

Bolanos, J., and G.O. Edmeades. 1996. Eight cycles of selection for drought tolerance in lowland tropical maize. III. Responses in grain yield, biomass, and radiation utilization. *Field Crops Research* 31: 233-252.

CIMMYT. 1997. Book of abstracts. the genetics and exploitation of heterosis in crops; An international symposium. CIMMYT, Mexico, D.F.

CIMMYT. 2001. Maize inbred lines released by CIMMYT: A Compilation of 454 CIMMYT Maize Lines (CMLs), CML1-CML454. CIMMYT, Mexico, D.F.

Comstock, R.E., H.F. Robinson, and P.H. Harvey. 1949. A breeding procedure designed to make maximum use of both general and specific combining ability. *Agronomy Journal* 41: 360-367.

Derera, J., P. Tongoona, B.S. Vivek, and M.D. Laing. 2008. Gene action controlling grain yield and secondary traits in Southern African maize hybrids under drought and non-drought environments. *Euphytica* 162: 411-422.

Ejigu, Y.G., P.B. Tongoona, and B.E. Ifie. 2017. General and specific combining ability studies of selected tropical white maize inbred lines for yield and yield related traits. *International Journal of Agricultural Science and Research* 7: 381-396.

Ertiro, B.T., Y. Beyene, B. Das, S. Mugo, M. Olsen, S. Oikeh, C. Juma, M. Labuschagne, and B.M. Prasanna. 2017. Combining ability and testcross performance of drought-tolerant maize inbred lines under stress and non-stress environments in Kenya. *Plant Breeding* 136: 197–205.

Fasahat, P., A. Rajabi, J.M. Rad, and J. Derera. 2016. Principles and utilization of combining ability in plant breeding. *Biometrics & Biostatistics International Journal* 4: 1-24.

Fellahi, Z.E.A., A. Hannachi, H. Bouzerzour, and A. Boutekrabt. 2013. Line by tester mating design analysis for grain yield and yield related traits in bread wheat (*Triticum aestivum* L.). *International Journal of Agronomy* 2013: 1-9.

Gevers, H.O. and I.V. Whythe. 1987. Patterns of heterosis in South African maize breeding material. pp 21-26. In: Fourie, AP., and J.G. Duplessis (eds). Proceedings of the seventh South African maize breeding symposium 1986. Summer Grain Centre, Grain Crops Research Institute, Potchefstroom.

Gevers, H.O., and J.K. Lake. 1998. The Natal maize breeding programme: 1948 -1993. pp 16 – 22. In: Gevers, H.O (ed.). Proceedings of eleventh South African maize breeding symposium, 1994. Cedara Agricultural development Institute, Summer grain Sub-Centre, Pietermaritzburg, Kwa-Zulu Natal.

Gissa, D.W., H. Zelleke, M.T. Labuschagne, T. Hussien, and H. Singh. 2007. Heterosis and combining ability for grain yield and its components in selected maize inbred lines. *South African Journal of Plant and Soil* 24: 133-137.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing system. *Australian Journal of Biological Sciences* 9: 463-493.

Haley, S.D. 1996. Quantitative genetics: Orientation and Introduction. Brookings, USA.

Hallauer, A.R. 1975. Relation of gene action and type of tester in maize breeding procedures. *Proceeding of Annual Corn and Sorghum Research Conference* 30: 50-165.

Hallauer, A.R., and J.B. Miranda. 1988. Testers and combining ability, in Quantitative genetics in maize breeding. In: Hallauer, A.R. and J. B. Miranda. (ed). Iowa State University Press, Ames, USA.

Hosana, G.H., S. Alamerew, B. Tadesse, and T. Menamo. 2015. Test cross performance and combining ability of maize (*Zea Mays* L.) inbred lines at Bako, Western Ethiopia. *Global Journals Inc. (USA)* 15: 1-25.

Kempthorne, O.1957. An introduction to genetic statistics. John Wiley and Sons. Inc., New York. U.S.A

Magorokosho, C., K.V. Pixley, and P. Tongoona. 2003. Selection for drought tolerance in two tropical maize populations. *African Crop Science Journal* 11: 151-161.

Makumbi, D., J.F. Betran, M. Banziger, and J.M. Ribaut. 2011. Combining ability, heterosis and genetic diversity in tropical maize (*Zea mays* L.) under stress and non-stress conditions. *Euphytica* 180: 143–162.

Maseka, S.K., A. Menkir, A.E.S. Ibrahim, and S.O. Ajala. 2006. Genetic analysis of performance of maize inbred lines selected for tolerance to drought under low Nitrogen. *Maydica* 51: 487-495.

Matzinger, D.F. 1953. Comparison of three types of testers for the evaluation of inbred lines of corn *Agronomy Journal* 45: 493-495.

Medici, L.O., M.B. Pereira, P.J. Lea, and R.A. Azevedo. 2004. Diallel analysis of maize lines with contrasting responses to applied Nitrogen. *Agricultural Sciences* 142: 535-541.

Meseka, S., and J. Ishaaq. 2012. Combining ability analysis among Sudanese and IITA maize germplasm at Gezira Research Station. *Journal of Applied Biosciences* 57: 4198– 4207.

Meseka, S.K., A. Menkir, A.E.S. Ibrahim, and S.O. Ajala. 2013. Genetic analysis of maize inbred lines for tolerance to drought and low Nitrogen. *Jonares* 1: 29-36.

Miti, F. 2007. Breeding investigations of maize (*Zea mays* L.) genotypes for tolerance to low Nitrogen and drought in Zambia. PhD thesis. University of KwaZulu-Natal, Republic of South Africa.

Ndhlela, T. 2012. Improvement strategies for yield potential, disease resistance and drought tolerance of Zimbabwean maize inbred lines. PhD Thesis. University of the Free State, South Africa.

Olver, R.C. 1998. Maize breeding in Zimbabwe. pp 22-27. In: Gevers, H.O (ed.). Proceedings of eleventh South African maize breeding symposium, 1994. Cedara Agricultural development Institute, Summer grain Sub-Centre, Pietermaritzburg, KZN.

Pswarayi, A., and B.S. Vivek. 2008. Combining ability amongst CIMMYT's early maturing maize (*Zea mays* L.) germplasm under stress and non-stress conditions and identification of testers. *Euphytica* 162: 353-362.

Rawlings, J.O., and D.L. Thompson. 1962. Performance level as criterion for the choice of maize testers. *Crop Science* 2: 217-220.

Rodriguez, F., G. Alvarado, A. Pacheco, J. Crossa, and J. Burgue. 2015. "AGD-R (Analysis of Genetic Designs with R for Windows) Version 3.0", http://hdl.handle.net/11529/10202 International Maize and Wheat Improvement Center [Distributor] V11 [Version].

Singh, R.K., and B.D. Chaudhary. 1977. Biometrical methods in Quantitative genetic Analysis. Kalyani Publishers, New Delhi.

Singh, R.K., and B.D. Chaudhary. 1985. Biometrical Methods in Quantitative Genetics Analysis. Kalyani Publishers, New Delhi.

Sprague, G.F., and L.A. Tatum. 1942. General versus specific combining ability in single crosses of corn. *American Society of Agronomy* 34: 923-932.

Tyagi, A.P., and P. Lal. 2005. Line x tester analysis in sugar cane (*Saccharum officinarum*). *South Pacific Journal of Natural Science* 23: 30-36.

Vasal, S.K. 1995. Achievement, challenges and future directions of hybrid maize research and development in CIMMYT, Paper presented at Workshop -Cotonou, Africa; May 1995.

VSN International. 2016. GenStat for Windows 18*th* Edition. VSN International, Hemel Hempstead, UK. Web page: GenStat.co.uk.

WWW-SA. 2011. The World Wide Fund for Nature, Cape Town, South Africa.

http://awsassets.wwf.org.za/downloads/wwf_ar_2011_final_web.pdf (Accessed 14/09/2015).

CHAPTER 5

Genotype by environment interaction and grain yield stability of newly developed maize (*Zea mays* L.) hybrids across stress and non-stress environments

Abstract

Maize yields in South Africa vary from environment to environment due to genotype by environment interaction (G × E); identifying higher yielding and stable genotypes for grain yield across environments in crucial for improving maize productivity. The objectives of the study were to identify higher yielding and stable hybrids across stress and non-stress environments and to identify representative test environments for testing and selection of superior genotypes. Forty-two and 72 newly developed single-cross hybrids were evaluated separately for grain yield performance, along with commercial checks across non-stress, low nitrogen (N) and drought environments in the 2014/15 (season 1) and 2015/16 (season 2) seasons, respectively. The hybrids were evaluated at seven environments in season 1 and six environments in season 2, in a (0, 1) α-lattice design with two replicates. Additive main effects and multiplicative interaction (AMMI) and genotype plus genotype by environment (GGE) analyses were employed. Based on GGE analyses, higher yielding and stable varieties were detected. In season 1, hybrid L7 x T3 (MO17HtHtN x CML444) and L2 x T3 (I-39 x CML444) were the most stable and high yielding genotypes after the ideal commercial check. Hybrids L2 x T4 (FO215W x CML444), L4 x T4 (I-42 x CML444) and L17 x T4 (U71Y x CML444) were stable and high yielding in season 2 with, L2 x T4 being the most ideal. These stable hybrids are best suited for wide adaptation across non-stress and stress environments. Hybrids containing tropical CIMMYT testers were more stable than those derived from temperate Corn Belt material. The ideal test environments for testing were Potchefstroom and Vaalharts. However, the observed G x E pattern was not repeatable, indicating a need for verifying the performance of genotypes or using different test locations to evaluate genotypes.

Keywords: Additive main effects and multiplicative interaction (AMMI); genotype plus genotype by environment (GGE); ideal test environments; maize grain yield; stability

5.1 Introduction

Maize (*Zea mays* L.) is the most widely grown food crop in South Africa. A large portion of the white maize produced in South Africa serves as a staple food commodity for the majority of the South African population, whereas a significant amount of the yellow maize produced is mainly used for animal feed purposes (BFAP, 2014). Maize is grown in various and diverse environments, the majority of the growing regions are characterised by frequent occurrence of drought (Agri SA, 2016), low soil fertility (WWF-SA, 2011) and limited use of improved and adapted varieties, particularly by smallholder farmers. Developing maize varieties that are tolerant to drought and low soil fertility, particularly Nitrogen (N) will mitigate the challenges posed by climate change. The international Maize and Wheat Improvement Centre (CIMMYT) and partners have made substantial progress in the breeding and identification of drought and low N tolerant maize varieties. However, varieties developed have been specific to certain regions.

Maize is a widely cultivated and adapted cereal crop; when superior varieties are developed, they are distributed to farmers in different regions with a wide range of environmental conditions. Some varieties may fail to adapt in certain regions due to geographical differences such that superior varieties in one environment may not be consistently superior in other environments (Beyene *et al.*, 2011; Makumbi *et al.*, 2015; Russell *et al.*, 2002; Setimela *et al.*, 2007). The varying conditions may cause varieties to rank differently from one environment to another due to the presence of genotype by environment interaction (G × E). The G × E complicates breeding and selection of superior and adapted varieties under stress and non-stress environments (Beyene *et al.*, 2011). Cultivars that show minimal G × E are phenotypically more stable and their yield potential is always guaranteed than those showing high G × E (Duarte and Zimmermann, 1992).

Varieties with high mean performance and stability (consistency in ranking across varying environmental conditions) are more ideal (Yan and Tinker, 2005), and may be recommended for a wide range of environments (Duarte and Zimmermann, 1992). Therefore, in addition to high mean performance, breeders also account for yield stability across the range of environments in order to exploit the G × E effects. The G × E effect can be minimised by grouping similar locations into two or more groups (mega-environments), where maize varieties will perform consistently with minimum crossover interaction (Russell *et al.*, 2002). Mega environments allow breeders to easily identify areas with similar biotic and abiotic stresses for hybrid development and germplasm exchange (Setimela *et al.*, 2005).

Multi-environment trial (MET) data are important in the evaluation of environments and genotypes and their interaction, to effectively identify superior genotypes and mega-environments (Yan *et al*., 2007; Cooper *et al*., 1997). The additive main effects and multiplicative interaction (AMMI) (Crossa, 1990; Gauch and Zobel, 1988) and the genotype plus genotype by environment (GGE) biplots analysis (Yan *et al.*, 2000) are the commonly used statistical tools for evaluating the response of genotypes to different environments. The difference from AMMI is that GGE biplot analysis is based on environment-centred principal component analysis (PCA), whereas AMMI analysis refers to double-centred PCA (Yan *et al.*, 2007). The GGE approach is useful for (i) visualising the G x E relationships which facilitates mega-environment delineation, the "which won where" view is useful for this visualization (Gauch and Zobel, 1997), (ii) evaluating the interrelationship among environments, to identify ideal environments based on their discriminative ability of genotypes and representativeness power of the test locations (Cooper *et al.*, 1997; Yan and Rajcan, 2002), and (ii) evaluating the interrelationship among genotypes and comparison for mean yield performance and stability (Yan, 2001). Evaluating genotypes for G × E and yield stability is important for developing and selecting higher yielding and broadly adapted maize varieties (Rad *et al.*, 2013). Newly developed cultivars must therefore be evaluated to determine the magnitude of G × E and to identify stable genotypes. The objectives of the study were to identify higher yielding and stable genotypes across stress and non-stress environments and to identify suitable environments for testing genotypes.

5.2 Materials and Methods

5.2.1 Germplasm and study sites

Forty-two and 72 F_1 hybrids derived from a line by tester mating design in the 2014/15 (season 1) and 2015/16 (season 2) winter nurseries were evaluated along with four commercial checks. Similar material was used for generating crosses in season 1 and 2, but due to missing crosses in season 1, combined analysis across seasons could not be performed. Inbred lines and testers used are presented in Table 5.1 and 5.2.

Table 5.1: Pedigree and descriptions of parental and check materials used in the study during 2014/15 season

Code	Name	Pedigree	Description
L1	B1138T	TEKOYELLOW	Line
L2	I-39	I-39	Line
L3	U2540W	M162W1.DO940Y-J34	Line
L4	M162W	K64R2.B1138T	Line
L5	K64	Pride off Saline	Line
L6	K64R-22	K64R-22	Line
L7	MO17HtHtN	MO17HtHtN	Line
L8	P594MSV	MLSxVHMO17	Line
L9	SO181Y	KO326Y2.NPPES1	Line
L10	SO713W	POWS1(S4)	Line
L11	VO500Y	POWS12.Y	Line
L12	SO1224Y	M28Y1.KO288Y	Line
L13	U71Y	M28Y2.NP	Line
L14	P612MSV	B73xVHKG/C1	Line
T1	B73	BSSS C5 (Iowa Stiff Stalk Synthetic)	Tester
T2	CML312	S89500-F2-2-2-1-1-B	Tester
T3	CML444	P43-C9-1-1-1-1-1-B	Tester
Check1	CAP9004	Capstone	Check
Check2	PAN6479	Pannar	Check
Check3	WE3127	WEMA	Check

Table 5.2: Pedigree and descriptions of parental and check materials used in the study during 2015/16 season

Code	Name	Pedigree	Description
L1	E30Y	B390YxM136Y	Line
L2	FO215W	NPPES14.O2S14	Line
L3	I-16	I-16	Line
L4	I-42	I-42	Line
L5	J80W	D800W2.HtN	Line
L6	K64	Pride off Saline	Line
L7	M162W	K64R2.B1138T	Line
L8	MO17HtHtN	MO17HtHtN	Line
L9	P614MSV	B73xVHKG/C1	Line
L10	RO421W	DO940Y-11.O2(W)	Line
L11	RO452W	DO940Y-13.NHK	Line
L12	RO544W	BO160W.3J400W	Line
L13	S198Y	M28Y1.DO620Y	Line

Code	Name	Pedigree	Description
L14	SO181Y	KO326Y2.NPPES1	Line
L15	U127Y	M162W.1KO326Y	Line
L16	U2540W	M162W1.DO940Y-J34	Line
L17	U71Y	M28Y2.NP	Line
L18	VO495Y	POWS12.Y	Line
T1	MO17	(CL.187-2 x C103)	Tester
T2	B73	BSSS C5 (Iowa Stiff Stalk Synthetic)	Tester
T3	CML312	S89500-F2-2-2-1-1-B	Tester
T4	CML444	P43-C9-1-1-1-1-1-B	Tester
Check1	CAP9004	Capstone	Check
Check2	PAN6479	Pannar	Check
Check3	SNK2147	Sensako	Check
Check4	WE3127	WEMA	Check

The study was conducted in seven and six different environments during 2014/15 and 2015/16 growing seasons, respectively. These environments were characterised by variable weather conditions and soil properties. Test environments used are designated E1 to E7. All seven environments were used for evaluations during 2014/15 and six (E1 to E4 and E6 to E7) were used in 2015/16, respectively (Table 5.4). The planting season for a majority of the trials was October/November (summer) except in Makhathini (E5) where the planting season was March/April (winter). The environments used were composed of non-stress, low nitrogen (low N) and drought stress environments which were partitioned into managed (MD) and random (RD) drought stress. The agro-climatic and geographical information of the study sites is presented in Table 5.3 and the codes of environments are shown in Table 5.4.

Table 5.3: Weather data and geographic information for the study locations used during 2014/15 and 2015/16 growing seasons

Environment	**Site**	**Season**	**Geographical position**			**Annual Rainfall (mm)**			***Temperature (^0C)***					
									Min			Max		
			Latitude	Longitude	Altitude (m.a.s.l)	Long-term	2014/15	2015/16	Long-term	2014/15	2015/16	Long-term	2014/15	2015/16
NS and DT	Potchefstroom	Summer	26.74^0 S	27.08^0 E	1349	541	519	364	15	14	16	29	29	31
NS and LN	Cedara	Summer	29.54^0 S	30.26^0 E	1068	662	619	521	14	13	14	25	25	27
Combined LN+DT	Vaalharts/Taung	Summer	27.95^0 S	24.84^0 E	1180	356	214	239	15	15	16	32	34	35
DT	Makhathini	Winter	27.39^0 S	32.18^0 E	77	153	127		14	9		28	29	

masl- metres above sea level (m); NS-non-stress; DT-drought stress; LN- Low nitrogen stress

Under non-stress conditions, trials received optimal fertilisation and supplementary irrigation. A compound fertiliser NPK (2:3:1) was applied as a basal fertiliser at a rate of 25 kg N ha^{-1}, 17 kg P ha^{-1} and 8 kg K ha^{-1} prior to sowing. Trials were top-dressed with Lime ammonium nitrate (33% N), at a rate of 150 kg ha^{-1}, at four weeks after seedling emergence. Phenotyping for low N tolerance was achieved in fields that had been previously depleted of nitrogen by growing unfertilised, non-leguminous crops (oats, wheat and sorghum) at high density for several seasons and removing the crop biomass after each season. Only Vaalharts was not depleted because of the sandy soil texture and therefore, natural depletion. For managed low N blocks, triple super phosphate (46% P_2O_5) and potassium chloride (61% K_2O) were applied at planting at a rate of 25 kg P_2O_5 ha^{-1} and 25 kg KCL ha^{-1} with no further top dressing. Screening for drought tolerance was achieved under random and managed drought stress conditions. Random drought received adequate fertilisation as applied in non-stress blocks, but no supplementary irrigation during the growing season, irrigation was only applied at the beginning of the season to establish a good plant stand. Under managed drought stress, trials were grown during a rain-free period in winter, with irrigation applied adequately until two weeks before flowering to simulate drought stress during flowering through to grain filling (Banziger *et al.*, 2000). In the 2015/16 season, there were irrigation problems that coincided with flowering and grain filling periods at Vaalharts (E3), the site was therefore treated as combined low N and drought stress. In all environments, a pre-emergence herbicide, Bateleur Gold (1.3 L ha^{-1}) and the post-emergence (Basagran, 2.5 L ha^{-1}) herbicide were used to control weeds and later followed by subsequent manual weeding. Insecticides were controlled using Karate, at 70 ml h^{-1}.

Table 5.4: Locations and codes of environments used in 2014/15 and 2015/16 seasons

Location	**Code**	**2014/15**	**2015/16**
		Management	**Management**
Potchefstroom	E1	low N	Low N
Taung[1]/Cedara[2]	E2	low N	Low N
Vaalharts	E3	Low N	Low + drought
Potchefstroom	E4	Random drought	Random drought
Makhathini	E5	Managed drought	-
Potchefstroom	E6	Non-stress	Non-stress
Cedara	E7	Non-stress	Non-stress

[1]2014/15; [2]2015/16

5.2.2 Experimental design and data collection

All trials were laid out in an (0,1) α-lattice design. At each location, each entry was hand-planted in a two-row plot of 4 m, with a spacing of 0.75 m between rows and 0.25 m between plants, with two replications. Two seeds were planted per station, at four weeks after emergence; all trials were thinned to a population density of about 53 333 plants ha^{-1}. Data on grain yield and secondary traits was recorded in all trials. Grain yield was estimated on plot basis and adjusted to 12.5% grain moisture content.

5.2.3 Data analysis

To test for G × E, combined analysis of variance were done from grain yield data using GenStat 18th edition (VSN International, 2016). For G x E and yield stability analyses, a three-way (genotype, environment and grain yield) MET raw data from seven (season 1) and six (season 2) environments was used to perform the analyses using both GGE and AMMI procedures. Adjusted GY from ANOVA was subjected to GGE biplot analysis to decompose the GGE of each experiment (Yan *et al*., 2000; Yan, 2001), to investigate the stability of genotypes. The GGE biplot shows the first two principal components (PC1 and PC2) derived from subjecting environment-centered yield data (the yield variation due to GGE) to singular value decomposition (Yan *et al.,* 2000). Means were compared at $P \leq 0.05$. The model used was:

$$Y_{ijk} = \mu + H_i + E_j + r_k (E_j) + (HE)_{ij} + \varepsilon_{ijk}$$

Where: Y_{ijk} is the phenotypic value of the hybrid i, when tested within the kth replication, nested within the jth environment, μ is the grand mean, H_i = is the effect of the hybrid i, E_j is the effect of the j^{th} environment, r_k is the effect of the k^{th} replication nested in the jth environment, $(HE)_{ij}$ is the hybrid and environment interaction effect associated with hybrid *i* and environment *j and* ε_{ijk} is the random error term. The replications, genotypes and sites were treated as fixed effects.

The AMMI and GGE models were expressed by (Yang *et al.*, 2009) as:

AMMI model:

$$\mathrm{Y}_{ij}= \mu+\mathrm{T}_{i}+ \delta_{j}+\sum_{k=1}^{t} \lambda_k \alpha_{ik} \gamma_{jk}+\varepsilon_{ij}$$

GGE model:

$$\mathrm{Y}_{ij}= \mu+\delta_{j}+\sum_{k=1}^{t} \lambda_k \alpha_{ik} \gamma_{jk}+\varepsilon_{ij}$$

Where Y_{ij} is the yield of the ith genotype in jth environment; μ is the overall mean; T_i is the effect of the ith genotype mean deviation, δ_j is the effect of the jth environment; λ_k is the eigen value of the principal component (PCA) axis; α_{ik} and γ_{jk} are the genotype and environment PCA scores (singular values) for the PCA axis; t is the maximum number of multiplicative terms retained in a model. The α_{ik} and γ_{jk} for k=1 are primary effects of the genotypes and environments, respectively and ε_{ij} is the residual of a model associated with the genotype i in environment j. The GGE model first fits additive effects for the main effects of genotypes and environments followed by multiplicative effects for GE interaction by Principal Component (PC) analysis (Zobel *et al*. (1988), in the AMMI model only the GE interaction is fitted.

The AMMI stability value (ASV) was used to compare the stability of the genotype (Purchase, 1997) as:

$$\mathrm{ASV} = \sqrt{\left[\frac{IPCA1SS}{IPCA2SS}(IPCA1\ score)\right]^2 + (IPCA2\ score)^2}$$

Where ASV is the AMMI stability value, SS is the sum of squares, IPCA1 is the first principal component axis; IPCA2 is the second principal component axis.

5.3 Results

5.3.1 Analysis of variance

The combined analysis for the 2014/15 and 2015/16 season, respectively, are presented in Table 5.5 and Table 5.6. The combined analysis of variance showed highly significant genotypes (P<0.001), environments (P<0.001) and genotype × environment interaction (P<0.001) in both seasons. Among all sources of variation, the environments accounted for large sum of squares, followed by the genotype × environment interaction in both season 1 and season 2.

Table 5.5: Combined analysis of variance of grain yield of 45 genotypes tested across seven environments in South Africa during 2014/15 season

Source of variation	df	Sum of squares	Mean squares
Replications (R)	1	16.55	16.55
Environments (E)	6	2154.18	359.03***
Genotype (G)	44	220.87	5.02***
Genotype × environment (G × E)	264	592.25	2.24***
Error	314	469.06	1.49
Total	629	3452.90	

****significant at P<0.001; df=degrees of freedom*

Table 5.6: Combined analysis of variance of grain yield of 76 genotypes tested across seven environments in South Africa during 2015/16 season

Source of variation	df	Sum of squares	Mean squares
Replications (R)	1	24.422	24.422
Genotype (G)	75	313.31	4.18***
Environment (E)	5	1819.00	363.80***
Genotype × environment (G × E)	375	1279.55	3.41***
Residual	455	1101.84	2.42
Total	911	4538.12	

****significant at P<0.001; df=degrees of freedom*

5.3.2 AMMI Analysis

The AMMI analysis of variance showed highly significant (P<0.001) main effects of genotypes, environments and their interaction (G × E) in both seasons with varying magnitude of sources of variation (Table 5.7 and Table 5.8). Of total variation, the environments contributed 62.4% and 40.2% in season 1 and 2, respectively. The G × E contributed about 23.5% of which 6.4% and 17.1% were due to the genotype and interaction main effects, respectively. In season 2, of 35%

G × E variation, 7.0 was due to genotypes and 28.1% was due to the interaction. The G × E interaction was further partitioned into interaction principal component axes (IPC1 and IPC2) and the residual effects; the IPC axes were all significant at P<0.001. Of total variation, 42.3% was due to IPC1 and 20.1% was due to IPC2 and 37.6% due to residual effects in season1. In season 2, the IPC1, IPC2 and G × E residuals accounted for 44.6%, 25.6% and 29.8%, respectively.

Table 5.7: AMMI Analysis of variance for grain yield of 45 single cross hybrid across seven environments in 2014/15

Source	df	Sum of square	Mean square	Total Variation (%)	G × E explained (%)
Genotypes (G)	44	221	5.02***	6.40	
Environments (E)	6	2154	359.03***	62.38	
Block	7	83	11.79***	2.40	
Interactions (G×E)	264	592	2.24***	17.14	
IPCA 1	49	251	5.12***		42.33
IPCA 2	47	119	2.53***		20.07
Residuals	168	223	1.32		37.61
Error	308	403	1.31	11.67	

****significant at P<0.001; DF=degrees of freedom*

Table 5.8: AMMI Analysis of variance for grain yield of 76 single cross hybrid across seven environments in 2015/16

Source	df	Sum of squares	Mean square	Total Variation (%)	G × E explained (%)
Genotypes (G)	75	318	4.24***	6.98	
Environments (E)	5	1831	366.29***	40.18	
Block	6	74	12.31***	1.62	
Interactions (G×E)	375	1279	3.41***	28.07	
IPCA 1	79	571	7.23***		44.64
IPCA 2	77	327	4.25***		25.57
Residuals	219	381	1.74		29.79
Error	450	1054	2.34	23.13	

****significant at P<0.001; DF=degrees of freedom*

5.3.3 GGE biplots analysis

Different GGE biplot views for grain yield of 45 single cross hybrids evaluated across seven environments in 2014/15 are shown in Figure 5.1, 5.3, 5.5, and 5.7. The biplots of 76 hybrids evaluated across six environments in season 2 are presented in Figure 5.2, 5.4, 5.6 and 5.8. The first principal components (PC1) representing the X-axis explained 42.86% of the total variation, while PC2, the Y-axis explained 20.19% of the total variation, both axes explained 63.06% of total variation for grain yield in 2014/15 season. During 2015/16, the first principal component (PC1) explained 38.79% of the total variation, PC2 explained 28.63%; the total variation for grain yield relative to genotype (G) and G x E was 67.41%.

5.3.4 Test environments evaluation

The discriminative vs representative view of the GGE biplots of the test environments in 2014/15 season 1 is shown in Figure 5.1. The length of a genotype vector approximate the standard deviation of cultivar means evaluated in a particular environment. Cedara non-stress (E7) environment was the most discriminative environment among genotypes followed by E1 (Potch Low N), while E5 (Makhathini, MD) and E2 (Taung Low N) were the least discriminating. Other environments E3 (Vaalharts LN), E4 (Potch RD) and E6 (Potch non-stress) had more or less similar discriminating power of the hybrids evaluated, and the most representative environment and the ideal environment was E6 (Potch Non-stress). In season 2, the most discriminative environments were E6 (Potch Opt) and E3 Vaalharts (LN+RD). Environment, E1 (Potch LN), E2 (Cedara LN), E4 (Potch RD) and E7 (Cedara non-stress) were equally not discriminating among hybrids (Figure 5.2). Vaalharts (LN+RD) environment (E3) was the most ideal and representative environment of all test environments (Figure 5.4).

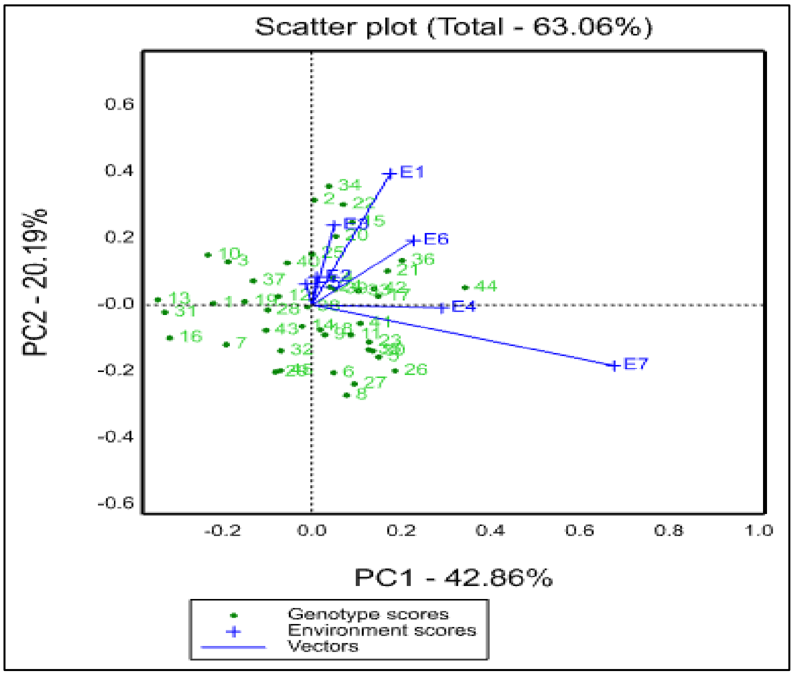

Figure 5.1: Representativeness of the test environments in 2014/15; Constructed based on: Transform=0; Scaling=0; Centering=2; SVP=2

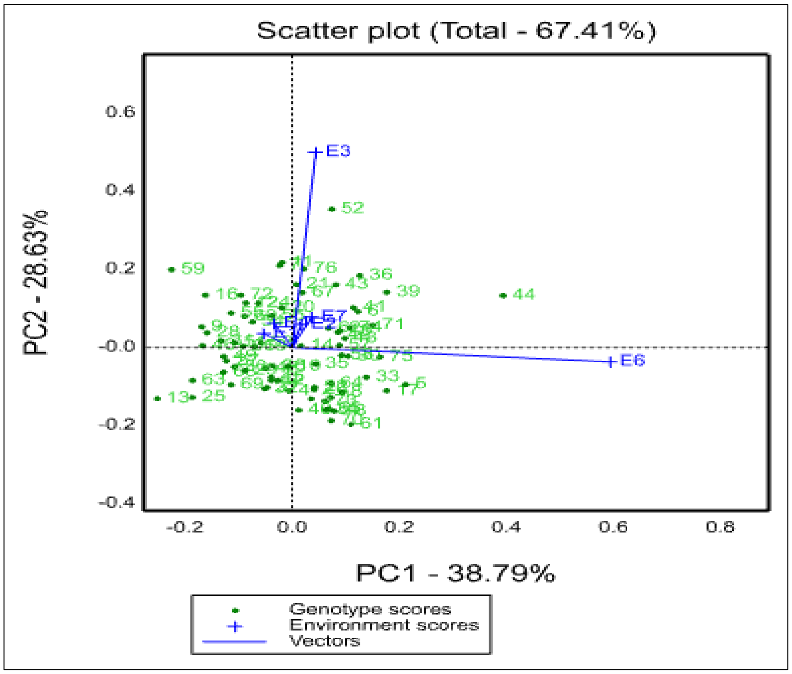

Figure 5.2: The vector view of the GGE biplot showing discriminative power and representativeness of the test environments in 2015/16; Constructed based on: Transform=0; Scaling=0; Centering=2; SVP=2

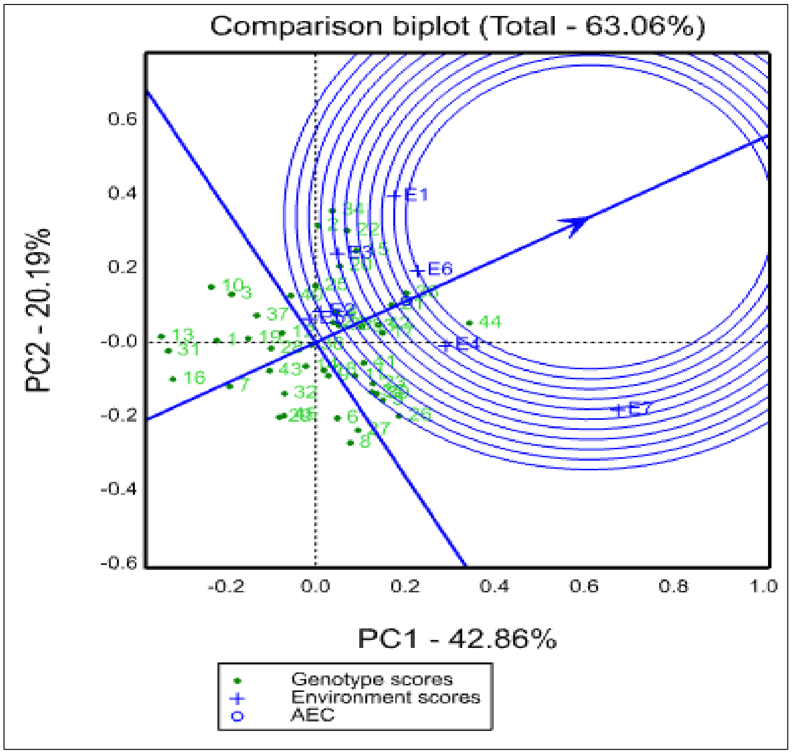

Figure 5.3: The biplot view for comparison of all environments with the ideal environment in 2014/15 season; Constructed based on: Transform=0; Scaling=0; Centering=2; SVP=2

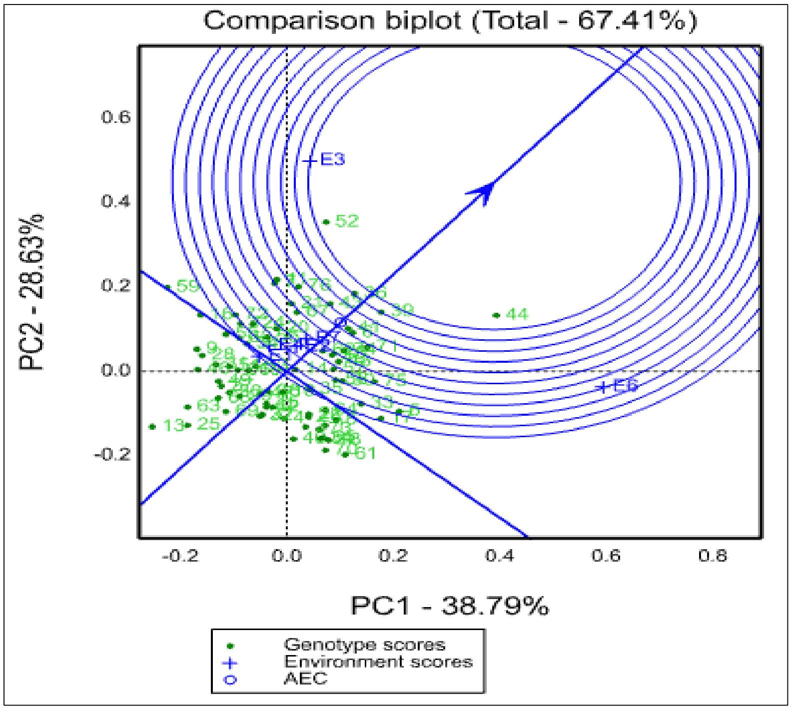

Figure 5.4: The biplot view for comparison of all environments with the ideal environment in 2015/16 season; Constructed based on: Transform=0; Scaling=0; Centering=2; SVP=2

5.3.5 Relationship among environments and mega environments analysis

In the 2014/15 season, the interrelationship between environments was observed between E7, E4 and E6 (Figure 5.1). The environments E1, E3, E2 and E5 were also positively correlated. In the 2015/16 (Figure 5.2) season, a positive correlation was found between E1 and E4; Environment 3 was strongly correlated with E6 and E2. Environment 5 and E1, with an angle greater than 90, were negatively correlated. The “which won where” view of GGE biplots for season 1 and season 2 are shown in Figure 5.5 and 5.6, respectively. The polygon divided the season 1 biplot into seven sectors with different winning genotypes; only two sectors were containing the environments. The first sector was comprised of four stress environments, which were E1 (Potch LN), E2 (Tang LN), E3 (Vaalharts low N) and E5 (Makhathini MD); and the winning genotype in this sector entry 44 (WE3127). The second sectors had three environments which included E4 (Potch RD), E6 (Potch non-stress) and E7 (Cedara non-stress) with entry 34 (L7/T1= MO17HtHtN/B73) as the winning genotype, however only slightly higher than several other hybrids with markers in close proximity to it. Other winner (vertex) genotypes were not associated with any environment those were; entry 8 (L11/T2; VO500Y/CML312), 26 (L4/T2; M162W/CML312) and 16 (L4/T1; P612/B73).

The polygon in season 2 divided the biplot into six sectors (Figure 5.6), with only three sectors containing environments. The first sector had only E5 (Potch non-stress) with entry 44 (L2/T4; FO215W/CML444) as a winner genotype. The second sector was formed by three environments, those were E2 (Cedara LN), E3 (Vaalharts LN+RD) and E6 (Cedara non-stress), entry 52 (L4/T4; I-42/CML444) was the superior genotype in this sector, the third sector was formed by E1 (Potch low N) and E4 (Potch RD); the winning genotype in this sector was 59 (L6/T3; K64/CML312). Other genotypes including 13 (L12/T1; RO544W/MO17) and 61 (L7/T1; M162W/MO17) were not associated with any environment.

Table 5.9: AMMI adjusted mean grain yield (t ha^{-1}), IPCA scores of top 20 genotypes in season 2, ranked by AMMI stability value (ASV)

#Genotype	Genotype	Mean	IPCAg1	IPCAg2	ASV	Rank
44	L2/T4	6.31	-1.23	-0.66	0.84	1
17	L13/T1	4.27	-0.89	-0.07	0.86	2
5	L10/T1	5.32	-0.87	0.45	0.98	3
75	SNK2147	4.81	-0.66	-0.14	1.09	4
33	L17/T1	4.73	-0.63	0.15	1.13	5
61	L7/T1	4.14	-0.73	0.51	1.14	6
34	L17/T2	3.73	-0.55	0.06	1.19	7
18	L13/T2	4.11	-0.56	0.36	1.24	8
1	L1/T1	4.02	-0.51	0.16	1.25	9
70	L9/T2	4.12	-0.57	0.47	1.26	10
64	L7/T4	4.09	-0.46	0.00	1.29	11
30	L16/T2	4.54	-0.44	-0.09	1.31	12
8	L10/T4	4.64	-0.50	0.40	1.31	13
71	L9/T3	5.38	-0.43	-0.18	1.32	14
58	L6/T2	4.22	-0.43	-0.30	1.35	15
73	CAP9004	4.25	-0.44	0.40	1.37	16
65	L8/T1	4.35	-0.48	0.53	1.37	17
22	L14/T2	3.87	-0.37	0.06	1.38	18
26	L15/T2	3.92	-0.37	0.08	1.38	19
57	L6/T1	4.41	-0.36	-0.34	1.42	20

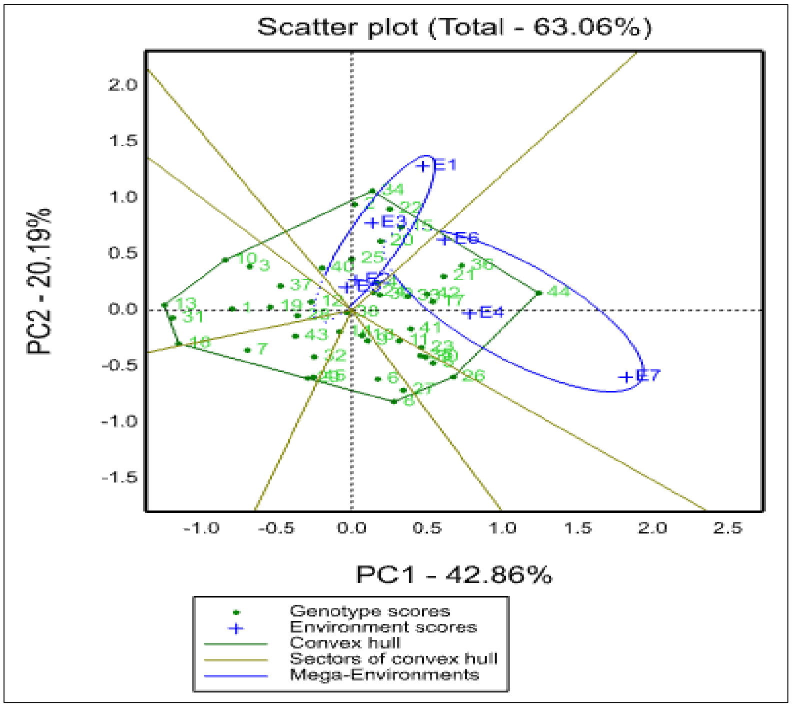

Figure 5.5: The “which-won-where” view of the GGE biplot under each mega environment in 2014/15; Constructed based on: Transform=0; Scaling=0; Centering=2; SVP=Symmetrical

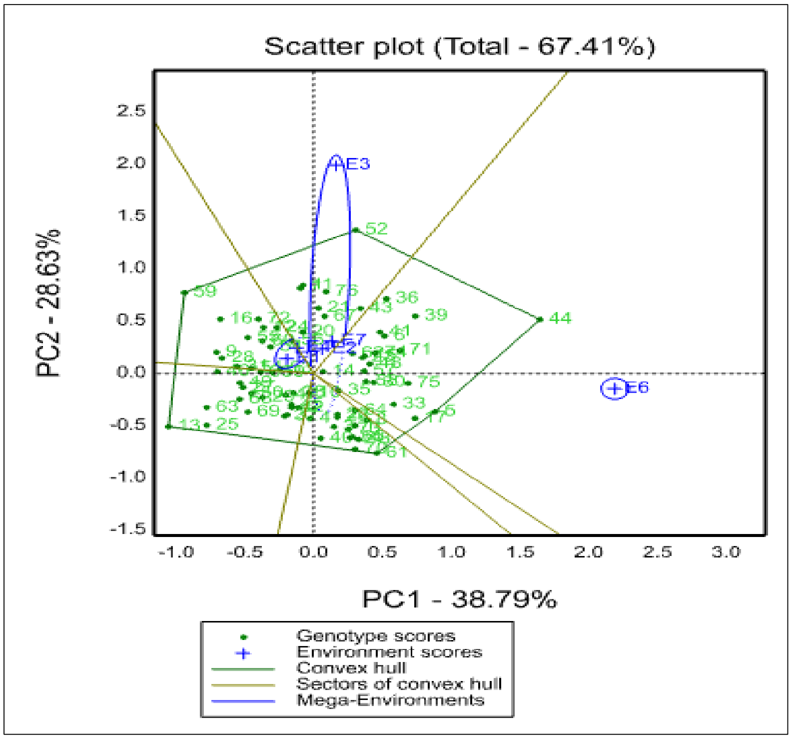

Figure 5.6: The “which-won-where” view of the GGE biplot under each mega environment in 2015/16; Constructed based on: Transform=0; Scaling=0; Centering=2; SVP=Symmetrical

5.3.6 Performance of genotypes vs ideal genotype

The biplots comparing all genotypes with ideal genotype for grain yield mean performance and stability are shown in Figure 5.7 and Figure 5.8, for 2014/15 and 2015/16, respectively. The experimental genotypes is season 1 were all outperformed by the commercial check, entry 44 (WE3127) which was the most ideal genotype in the 2014/15 season. Following the check were hybrids 36 (L7 x T3; MO17HtHtN x CML444), 21 (L2 x T3; I-39 x CML444), 22 (L3 x T1; U2540W x B73) and 17 (L14 x T2; P612MSV x CML312). In the 2015/16, the most ideal genotypes were entry 44 (L2 x T4; FO215W x CML444), other higher yielding and stable genotypes included entry 52 (L4 x T4; I-42 x CML444), 36 (L17 x T4; U71Y x CML444), 39 (L18 x T3, VO495Y x CML312) and 43 (L2 x T3; FO215W x CML312). Based on the AMMI stability value (Table 5.9), entry 44 (L2 x T4; FO215W x CML444) in season 2 was also the most superior genotype with the lowest ASV of 0.84. High yielding but unstable genotypes included entry 15 (L13 x T3; U71Y x CML444) in season 1 and entry 11 (L11 x T3; RO452W x CML312).

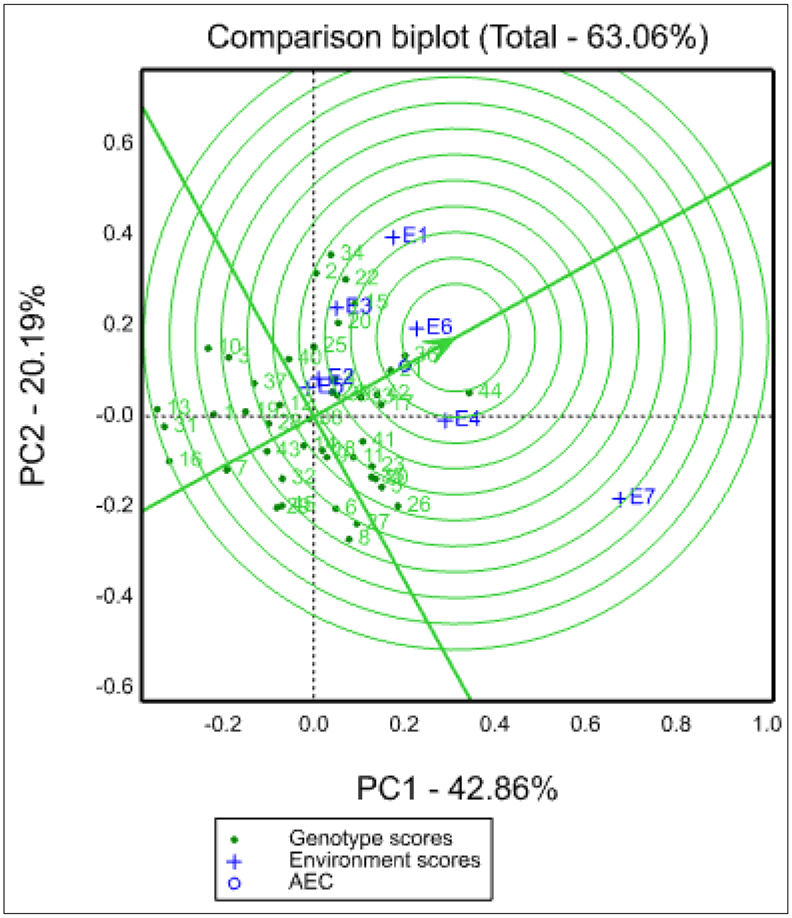

Figure 5.7: Biplot view comparing all genotypes with the ideal genotype across environments in 2014/15; Constructed based on: Transform=0; Scaling=0; Centering=2; SVP=1

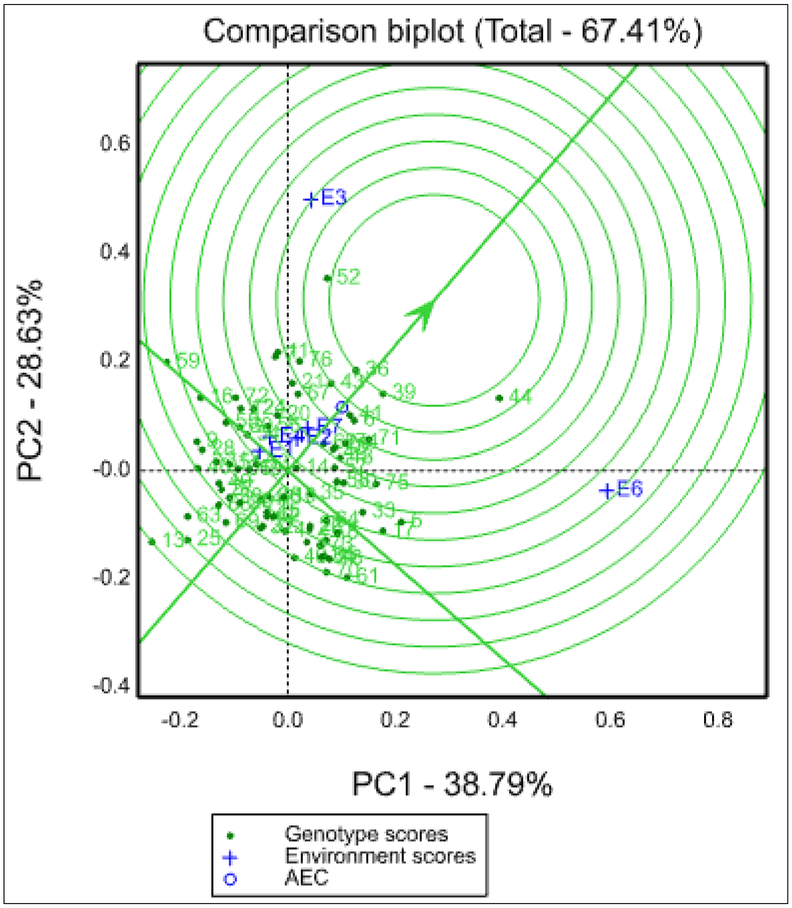

Figure 5.8: Biplot view comparing all genotypes with the ideal genotype across environments in 2015/16; Constructed based on: Transform=0; Scaling=0; Centering=2; SVP=1: See codes of environments in Table 5.4.

5.4 **Discussion**

5.4.1 Analysis of variance

The significant mean squares observed for genotype by environment interaction indicated differential response of the genotypes at different environments due to the presence of G × E. The greater magnitude of sum of squares due to environments indicated the presence of large variability among sites used in evaluating genotypes, and therefore potential sites for identifying superior and adapted genotypes. The large contribution of environments in influencing a genotype's performance and stability across environments has been reported in several studies (Abakemal *et al.*, 2016; Makumbi *et al.*, 2015; Ndhlela *et al.*, 2014; Setimela *et al.*, 2007; Shaibu *et al.*, 2016; Sserumaga *et al.*, 2016). Gauch and Zobel (1997) stated that in standard METs, environmental effects generally account for the greatest total sums of treatments, which is about

80%, whereas, the genotype and interaction effect both contribute almost 10%. Based on the AMMI analysis in both season 1 and 2, the first two IPCAs accounted for more than 50% of the G × E, suggesting that by using only the first two PCs to explain meaningful G × E patterns, the best-fit model for AMMI can be predicted (Gauch and Zobel, 1997).

5.4.2 Discriminative vs representative of test environments

The discriminativeness and representativeness of sites was revealed from the current study. Test environments with longer vectors are more discriminative of the performance of genotypes across environments than environments with short vectors (Yan *et al*., 2007). Based on the vector length, the environment that had the longest vector of all test environments in season 1 was E7 (Cedara non-stress) followed by E1 (Potch Low N), these were the most discriminative environments among genotypes. In season 2, the most discriminative environments were Potchefstroom (E6, low N) and Vaalharts (E3 LN+RD). Testing genotypes in these environments may give sufficient information on genotype's differences compared to the least discriminative environments. In this study, it was observed that the least discriminating environments, which had shorter vectors and located closer to the biplot origin (Yan *et al*., 2007) were mainly stress environments, including low N environments and managed drought stress. It is therefore impossible to obtain adequate information on the differences in the performance of all genotypes within these environments. According to Gauch and Zobel (1997), stress environments with low productivity are prone to large errors and are less discriminating between genotypes. Abakemal *et al.* (2016) also indicated that lack of discriminating power of the environments is generally attributed to unfavourable seasonal conditions and therefore, genotypic differences based on short environmental vectors may not be reliable.

Environments that are more representative are selected based on the cosine of the environmental vector and the average environmental axes (AEA). The AEA passes through the average environment (indicated by a small circle) and the biplot origin relative to the genotype mean performance. According to Yan and Kang (2003), environments with long vectors and small angles with the AEC abscissa are more representative of mega environments and are ideal for testing and selecting superior genotypes. In this study, the most representative environments were Potchefstroom (non-stress) is season 1 and Vaalharts (low N + RD) in season 2. These two environments were also identified as ideal environments for evaluating genotypes. The ideal environment should be both highly discriminative among genotypes and representative of the mega environments (Yan, 2001). The small circle located on the AEC abscissa and with an arrow pointing to it represents the average environments. The ideal environment is located at the centre of a set of concentric lines which measures the distance between each environment and the ideal environment (Abakemal *et al.,* 2016). Potchefstroom non-stress (season 1) and Vaalharts (low N + RD) in season 2 were both in close proximity to the ideal environments and were thus identified as the best environments for evaluating genotypes. These environments showed a greater discriminative and representative power and may be chosen over other sites for use as suitable test environments, especially when resources are limiting. Vaalharts combined both low N and drought stress; this environment could easily differentiate among inbred lines because, in general, it represented the actual farmers' fields where drought and low N stress generally occur concurrently anytime. To test for performance of genotypes under low N conditions, Vaalharts should always be prioritised. Vaalharts is located in a mid-altitude area, characterised by low average annual rainfall and high temperatures during dry seasons, this site may be considered as a potential site for selecting for stress tolerance. In the study by Setimela *et al.* (2007), Potchefstroom was also among the most representative locations, its general representative power of test environments has therefore been proven, this suggests that Potchefstroom should always be considered as a test environment, particularly for non-stress trials. In this study, the identified ideal environments were non-stress in season 1 and stress environment in season 2. Banziger *et al.* (2006) postulated that to achieve breeding progress, test environments should include both low and high yielding areas because selection of genotypes only under high yielding environments is usually associated with poor performance when selection is done under poor environments. The identified environments therefore represent the average performance across all locations and are generally good test environments.

5.4.3 Relationship between environments and mega environment analyses

From the GGE biplot, useful information on the relationship between environments was detected. According to Yan (2002), the cosine of the angle between the vectors of two environments approximate the correlation between them. An acute angle implies a strong positive correlation between two environments; conversely, an obtuse angle is an indication of a strong but negative correlation. An angle formed by two environments at a right angle (90^0) indicates the absence of correlation. The observed relationship indicated the possibility of grouping similar environments.

The "which won where" view of a GGE biplot was used to visualise the higher yielding genotypes in different environments. The polygon was drawn on genotypes that were furthest from the biplot origin so that all genotypes in a biplot were accommodated in a polygon (Yan, 2001). The perpendicular lines to each side of the polygon were drawn to divide the biplot into sectors. The study revealed that the target environments could be delineated into mega-environments, with different winning genotypes. Yan and Rajcan (2002) defined a mega environment as a group of environments that share the same set of superior genotypes. The seven environments in season 1 were divided into two mega environments. The first mega environment was formed mainly by low N stress environments which were E1 (Potch LN), E2 (Tang, LN), E3 (Vaalharts low N) except E5 (Makhathini), which was a managed drought stress environment; the higher yielding hybrid for this mega environment was entry 34 (L7 x T1; MO17HtHtN x B73). The second cluster was mainly formed by non-stress environments (E6 and E7), except one random drought (E4, Potch), with entry 44 (commercial check, WE3127) as a winner genotype. The presence of mega environments suggest that the sets of environments in a mega environment can give similar information regarding the performance of genotypes; one environment can therefore be dropped without losing any useful information on genotype performance (Yan and Tinker, 2005). The observations from this season thus suggested that in mega environment 1, when resources are limiting the number of low N environments may be minimised by dropping some test environments particularly those that provide little or no information (least discriminating) regarding the performance of genotypes. For example, E2 (Taung, LN) was the least discriminating test environment within a mega environment, it can therefore be easily dropped without losing information on the genotype's performance. The observed delineations by stress and non-stress environments are in agreement with previous studies. Ertiro *et al.* (2017) evaluated hybrids across non-stress, low N and drought stress environments; the tested hybrids fell into two mega environments with the first mega environment containing mainly non-stress sites and the second sector mainly encompassed stress environments.

Results from season 2 classified the six environments into three mega environments with E6 (Potchefstroom non-stress) forming one mega environment; E2 (Cedara LN), E3 (Vaalharts LN and drought), and E7 (Cedara non-stress) formed the second mega environment, while E1 (Potch low N) and E4 (Potch RD) formed the third mega environment. In the second mega environment, E3 which was Vaalharts, combining both drought and low N stress may be chosen over Cedara. This environment is the most ideal environment with high discriminative power among genotypes and was also more representative of the test environments; it is therefore suitable for evaluating and selecting superior genotypes under stress. According to Gauch and Zobel (1997), too many or too few mega-environments may reduce average yield; therefore, four mega environments are generally ideal for testing genotypes. However, in this study the fewer mega environments identified may be considered ideal because of the total number of environments used. Some genotypes such as entry 8 (L11 x T2; VO500Y x CML312), 26 (L4 x T2; M162W x CML312) and 16 (L4 x T1; P612 x B73) in season 1 and entry 13 (L12 x T1; RO544W x MO17) and 61 (L7 x T1; M162W x MO17) in season 2 were superior (vertex) genotypes, but were not associated with any mega environment. This suggested that these genotypes might show either poor or good performance for grain yield depending on the environment (Kaya *et al.*, 2006; Setimela *et al.*, 2007). According to Yan and Tinker (2005), dividing test environments into mega-environments and recommending genotypes is more reliable and recommended if crossover interactions are repeatable across the years. For this study, the pattern was not repeatable, particularly due to differences in genotypes evaluated in the two seasons, this indicate a need for an additional experiment to validate the pattern and therefore identify key environments and recommend genotypes based on multi-environment trials. Where the G × E pattern is not repeatable, different test environments are recommended for evaluating genotypes.

5.4.4 Genotypes performance vs ideal genotypes

The comparison biplot was used to compare genotypes with ideal genotype. The biplot comparing all genotypes with ideal genotypes accounted for 63.06% of the variation in GY in season 1 and 67.41% in season 2. The AEC abscissa, also referred to as the average environmental axis ranks genotypes relative to the direction of high mean performance and stability (Yan *et al.*, 2007). The AEC abscissa passes through the biplot origin and the average environment indicated by a small circle defined by the average PC1 and PC2 scores across the environments (Yan, 2002). Genotypes with high mean performance and stable within a mega environment are considered 'ideal' genotypes (Yan, 2001); the term stability relates to the consistent rank of a genotype across environments. The check hybrid entry 44 (WE3127) was the best genotype in season 1, entry 36 (L7 x T3; MO17HtHtN x CML444), 21 (L2 x T3; I-39 x CML444) and 22 (L3 x T1; U2540W x B73) were the most stable hybrids among experimental hybrids after the commercial check.

All experimental hybrids evaluated in season 2 outperformed the commercial checks; this indicated the superiority of the newly developed hybrids over commercial checks. These hybrids were entry 44 (L2 x T4; FO215W x CML444), which was the most stable hybrid; entry 52 (L4 x T4; I-42 x CML444) and 36 (L17 x T4; U71Y x CML444) were also higher yielding and stable. Entry 44 (L2 x T4; FO215W x CML444) was identified as the ideal hybrid with high mean and stable performance across environments. An ideal genotype has the longest vector of all genotypes and has minimal to zero G × E interaction (Yan *et al.*, 2000; Yan and Rajcan, 2002). In a biplot, this type of cultivar is located at the centre of concentric circles, which are drawn to visualise the distance between each genotype and the ideal cultivar (Yan, 2001). A genotype in close proximity to the ideal cultivar is therefore desirable.

Furthermore, the AMMI stability value (ASV) was used to check for stability of hybrids in season 2; a low ASV or a value closer to zero is an indication of stable genotypes (Purchase, 1997). Based on the ASV entry 44 (L2 x T4; FO215W x CML444) was the most stable genotype with the lowest ASV (0.84). This hybrid was identified as a late maturing hybrid (data not shown), with a superior performance across non-stress, drought and low N environments. These findings are in agreement with findings by Setimela *et al.* (2007), who also found that the late maturing, moisture and low soil fertility-tolerant cultivars were adapted in most regions. These results suggest that the identified hybrids with high mean grain yield and stability are broadly adapted and could therefore be used to improve yields across a wide-range of stress and non-stress environments. Broad adaptations are associated mainly with genotype than G × E effect (Gauch, 2013); such genotypes eliminate the need for sub-dividing the test environments into mega environments (Gauch and Zobel, 1997).

Some higher yielding genotypes such as entry 15 (L13 x T3; U71Y x CML444) in season 1 and entry 11 (L11 x T3; RO452W x CML312) were not among the most stable, suggesting that they may have specific adaptation to certain stress or non-stress environments. Higher yielding and unstable genotypes were previously reported by other investigators (Badu-Apraku *et al.*, 2012; Makumbi *et al.*, 2015; Sserumaga *et al.*, 2016), such genotypes have a narrower adaptation which is greatly influenced by G × E (Gauch, 2013). The study comprised of a wide range of non-stress, low N and drought environments, therefore these genotypes may be less responsive particularly under stress environments, which could therefore make them unstable. Moreover, it was observed that the most stable genotypes were mainly those involving the tropical CIMMYT testers than temperate Corn Belt testers, MO17 and B73. However, among the higher yielding hybrids, there were some involving temperate testers but were least stable. Use of temperate material in South Africa is negligible; differences in environmental conditions may thus interfere with photoperiod reaction and therefore overall performance of the temperate lines when grown in different tropical conditions. Hybrids containing temperate material may therefore be recommended for specific environments.

Conclusion

The results indicated that yield performance of maize single-cross hybrids was influenced largely by G × E effect than genotype effects. However, superior genotypes with high mean yield and stable performance could be identified. Hybrids derived from tropical CIMMYT testers were more stable than those containing temperate Corn Belt testers; the most stable was FO215W x CML444. The identified stable hybrids will improve maize production and productivity across various maize growing regions in South Africa. The higher yielding but unstable genotypes may be considered for specific environments in selected regions. Further testing of these genotypes in multi-locations is required for verifying the observed patterns, and recommending potential hybrids for commercialisation.The study further indicated the possibility of delineating the used test locations into mega-environments and identifying the ideal target test locations. Potchefstroom and Vaalharts were the most suitable environments for evaluating the performance of genotypes under non-stress and stress conditions, respectively. However, the observed G x E pattern was not repeatable, indicating a need for verifying the performance of genotypes or using different test locations to evaluate genotypes.

References

Abakemal, D., H. Shimelis, and J. Derera. 2016. Genotype-by-environment interaction and yield stability of quality protein maize hybrids developed from tropical highland adapted inbred lines. *Euphytica* 209: 757-769.

Agri SA. 2016. “A raindrop in the drought”. Report to the Multi-Stakeholder Task Team on the drought, Agri SA’s status report on the current drought crisis. Agri South Africa, Pretoria, South Africa. http://www.nstf.org.za/wp-content/uploads/2016/06/Agri-SA-Drought-Report_CS4.pdf (Accessed 15/07/2016).

Badu-Apraku, B., M. Oyekunle, K. Obeng-Antwi, A.S. Osuman, S.G. Ado, N. Coulibaly, C.G. Yallou, M. Abdulai, G.A. Boakyewaa, and A. Didjeira. 2012. Performance of extra-early maize cultivars based on GGE biplot and AMMI analysis. *Agricultural Sciences* 150: 473–483.

Banziger, M., G.O. Edmeades, D. Beck, and M. Bellon. 2000. Breeding for drought and Nitrogen stress tolerance in maize: From theory to practice. CIMMYT, Mexico, D.F.

Banziger, M., P.S. Setimela, D. Hodson, and B. Vivek. 2006. Breeding for improved drought tolerance in maize adapted to Southern Africa. *Agricultural Water Management* 80: 212–224.

Beyene, Y., S. Mugo, C. Mutinda, T. Tefera, H. Karaya, S. Ajanga, J. Shuma, R. Tende, and V. Kega. 2011. Genotype by environment interactions and yield stability of stem borer resistant maize hybrids in Kenya. *African Journal of Biotechnology* 10: 4752-4758.

BFAP. 2014. Trade flow patterns in South Africa-Adjusting to structural changes. The Bureau for Food and Agricultural Policy, Pretoria, South Africa. http://www.bfap.co.za/documents/research%20reports/BFAP%20MT%20Trade%20flow%20final%20June%202014.pdf (Accessed 10/11/2016)

Cooper, M., R.E. Stucker, I.H. de Lacy, and B.D. Harch. 1997. Wheat breeding nurseries, target environments, and indirect selection for grain yield. *Crop Science* 37: 1168–1176.

Crossa, J. 1990. Statistical analysis of multilocation trials. *Advanced Agronomy* 44: 55-85.

Duarte, J.B., and M.J.O. Zimmermann. 1992. Comparison of three methods used for the study of adaptation and phenotypic stability in the common bean (*Phaseolus Vulgaris* L.). *Brasilian Journal of Genetics* 15: 125-136.

Ertiro, B.T., Y. Beyene, B. Das, S. Mugo, M. Olsen, S. Oikeh, C. Juma, M. Labuschagne, and B.M. Prasanna. 2017. Combining ability and testcross performance of drought-tolerant maize inbred lines under stress and non-stress environments in Kenya. *Plant Breeding* 136: 197–205.

Gauch, H.G. 2013. A Simple Protocol for AMMI Analysis of yield trials. *Crop Science* 53: 1860-1869.

Gauch, H.G., and R.W. Zobel. 1988. Predictive and postdictive success of statistical analyses of yield trials. *Theoretical and Applied Genetics* 76: 1-10.

Gauch, H.G., and R.W. Zobel. 1997. Identifying mega-environments and targeting genotypes. *Crop Science* 37: 311-326.

Kaya, Y., M. Akcura, and S.Taner. 2006. GGE-biplot analysis of multi-environment yield trials in bread wheat. *Turkish Journal of Agriculture* 30: 325-337.

Makumbi, D., A. Diallo, F. Kanampiu, S. Mugo, and H. Karaya. 2015. Agronomic performance and genotype x environment interaction of herbicide-resistant maize varieties in Eastern Africa. *Crop Science* 55: 540–555.

Ndhlela, T., L. Herselman, C. Magorokosho, P. Setimela, C. Mutimaamba, and M. Labuschagne. 2014. Genotype × environment interaction of maize grain yield using AMMI biplots. *Crop Science* 54: 1–8.

Purchase, J.L. 1997. Parametric analysis to describe GxE interaction and yield stability in winter wheat. PhD Thesis. University of the Orange Free State, Bloemfontein, South Africa.

Rad, M.R.N., M.A. Kadir, M.Y. Rafii, H.Z.E. Jaafar, M.R. Naghavia, and F. Ahmadi. 2013. Genotype × environment interaction by AMMI and GGE biplot analysis in three consecutive generations of wheat (*Triticum aestivum*) under normal and drought stress conditions. *Australian Journal of Crop Science* 7: 956-961.

Russell, W.K., K.M. Eskridge, D.A. Travnicek, and F.R. Guillen-Portal. 2002. Clustering environments to minimize change in rank of cultivars. *Crop Science* 43: 858-864.

Setimela, P.S., B. Vivek, B., Banziger, M., Crossa, J. and F. Maideni. 2007. Evaluation of early to medium maturing open pollinated maize varieties in SADC region using GGE biplot based on the SREG model. *Field Crops Research* 103: 161–169.

Setimela, P.S., Z. Chitalu, J. Jonazi, A. Mambo, D. Hodson, and M. Banziger. 2005. Environmental classification of maize testing sites in the SADC region and its implication for collaborative maize breeding strategies in the subcontinent. *Euphytica* 145: 123–132.

Shaibu, A.S., A.A. Adnan, and J.M. Jibrin. 2016. Stability performance of extra early maturing maize (*Zea mays* L.) varieties under high and low Nitrogen environments in Sudan Savanna. *Cogent Food & Agriculture* 2: 1-12.

Sserumaga, J.P., S.O. Oikeh, S. Mugo, G. Asea, M. Otim, Y. Beyene, G. Abalo, and J. Kikafunda. 2016. Genotype by environment interactions and agronomic performance of doubled haploids testcross maize (*Zea mays* L.) hybrids. *Euphytica* 207: 353-365.

WWF-SA. 2011. Agriculture: facts & trends - South Africa. World Wildlife Fund South Africa, Cape Town, South Africa. http://awsassets.wwf.org.za/downloads/facts_brochure_mockup_04_b.pdf (Accessed 08/05/2017).

Yan, W. 2001. GGE Biplot- A windows application for graphical analysis of multi- environment trial data and other types of two-way data. *Agronomy Journal* 93: 1111-1118.

Yan, W. 2002. Singular-value partitioning in biplot analysis of multienvironment trial data. *Agronomy Journal* 94: 990-996.

Yan, W., and I. Rajcan. 2002. Biplot evaluation of test sites and trait relations of soybean in Ontario. *Crop Science* 42: 11-20.

Yan, W., and M.S. Kang. 2003. GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists. CRC Press, Boca, Raton, FL.

Yan, W., and N.A. Tinker. 2005. An integrated biplot analysis system for displaying, interpreting, and exploring genotype x environment interaction. *Crop Science* 45: 1004–1016.

Yan, W., L.A. Hunt, Q.Sheng, and Z. Szlavnics. 2000. Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Science* 40: 597–605.

Yan, W., M.S. Kang, B. Ma, S. Woods, S. and P.L. Cornelius. 2007. GGE Biplot vs. AMMI Analysis of Genotype-by-Environment data. *Crop Science* 47: 641-653.

Yang, R.C., J. Crossa, P.L. Cornelius, and J.Burgueno. 2009. Biplot analysis of genotype × environment interaction: Proceed with caution. *Crop Science* 49: 1564–1576.

CHAPTER 6

Heterosis in maize (*Zea Mays* L.) grain yield under non-stress and stress environments

---

Abstract

The superiority of new cultivars under stress and non-stress conditions determines the success of a breeding program and subsequently improvements in maize performance. Quantifying the level of heterosis for grain yield is important for measuring breeding progress and to devise appropriate breeding strategies. The aims of the study were to (i) evaluate the performance of new maize hybrids under stress and non-stress conditions and to (ii) assess the level of heterosis in grain yield under non-stress, drought and low N stress conditions. One hundred and forty single-cross hybrids alongside with five commercial checks were evaluated for grain yield and secondary traits under non-stress, drought and low N stress environments in South Africa, during the 2015/16 season. Adequate levels of variability were observed among hybrids studied indicating breeding progress through selection. Reduction of up to 22% and 42% were observed in grain yield due to drought and low N stress, respectively. Among hybrids studied, five hybrids showed competitive yields and positive heterosis relative to the standard check varieties. The most superior hybrid under both drought and low N environments was L9 x T3 (I-42 x CML444); this hybrid have potential for commercialisation and boosting farmers yields under stress environments. The hybrids L3 x T3 (FO215W x CML444), L21 x T2 (RO421W x B73), L21 x T1 (RO421W x MO17) and L19 x T4 (P612MSV x CML312) performed well under non-stress environments. The potential of temperate Corn Belt testers, MO17 and B73 was also revealed in grain yield heterosis, indicating the possibility of incorporating temperate × tropical hybrids in the South African maize breeding programs. These results indicate the possibility of maize genetic improvements for stress tolerance in South Africa.

Keywords: Heterosis; grain yield; non-stress; stress conditions; temperate material

6.1 Introduction

South Africa remains the main maize producer in the African continent because of technology adoption. However, the country is till characterised by lack of infertile land, irregular rainfall distribution and a larger proportion of maize grown under rain-fed conditions (ARC, 2014). Several factors are also responsible for the fluctuations in maize grain yields obtained. Drought and low soil nitrogen (N) are among the primary abiotic constraints to maize productivity. Drought combined with low N stress can cause severe yield losses of greater than 50% (Banziger *et al.*, 2000). The combined effect of drought and low N stress have serious implications for food security in South Africa and most parts of the continent, and the effect is predicted to continue with climate change, especially in areas relying on uneven and unpredictable rainfall. The gap between small-scale and commercial farmer’s maize grain yields is also often too large mainly due to lack of adapted varieties. Developing maize varieties with acceptable tolerance to drought and low N stress is therefore part of a long-term solution to increased maize productivity and alleviating poverty.

The success of any breeding program is determined by the superiority of new varieties. Improvement of grain yield and associated secondary traits in new varieties requires knowledge of heterosis (Masuka *et al.*, 2017). Heterosis (hybrid vigor) is expressed as (i) the superiority of the F_1 hybrid relative to the mean of the parents, (ii) better parent (Heterobeltiosis) and (iii) standard commercial check (Narayanam and Phundan, 1993). Exploitation of heterosis in new varieties is fundamental for maximising the effectiveness of new varieties, with potential for improving yields (Shull, 1952). The expression of maximum herosis in hybrids is influenced by the level of genetic diversity of the parental lines (Hallauer and Miranda, 1988). Maize breeding programs are thererefore focused on exploiting maximum heterosis in hybrids.

Heterosis estimates based on mid and better-parent heterosis may have more limitations than standard heterosis. These estimates generally require information on the perfomance of inbred lines. Including inbred lines in experimental trials is not always possible, especially when there are many crosses evaluated (Chigeza, 2013). New varieties that compete favourably with the commercial checks and give high yield advantage are always the primary target in all breeding programs. Standard heterosis provides estimates based on direct comparisons of new hybrids with commercial checks. This type of estimate is therefore the most practical and economic approach that provides adequate information about the merit of new varieties. The study was therefore undertaken to quantify the level of standard heterosis in maize grain yield under non-stress, drought and low Nitrogen stress environments.

6.2 Materials and Methods

6.2.1 Germplasm and trial design

Forty-two maize inbred lines (Table 6.1) obtained from the Agricultural Research Council maize genebank of South Africa were used to generate single cross hybrids in a line by tester mating scheme. Each line was selected from a pool of the South African maize germplasm; lines were selected focusing on different heterotic groups, and therefore all lines used represents the seven South African maize heterotic groups. Single cross hybrid were developed by crossing all inbred lines with four testers which included two inbred line testers representing the tropical heterotic Group A of CIMMYT, and the temperate group A of the USA Corn Belt (CML312 and B73), and their heterotic Group B counterparts (CML444 and MO17), respectively. CML312 is an intermediate maturing line, drought tolerant, subtropical, and resistant to grey leaf (GLS), *Turcicum leaf blight* (TLB) and *Exerohilum turcicum* and is *Maize streak virus* (MSV) susceptible, whereas CML444 is a late maturing inbred line, drought and low N tolerant, mid-altitude or subtropical tester, MSV and TLB susceptible (CIMMYT, 2001). The temperate inbred lines B73 is the Iowa stiff stalk synthetic representing Reid heterotic group while MO17 is a Lancaster Sure Crop (Bidhendi *et al.*, 2012). The successful 140 single cross hybrids were evaluated along with five commercial checks in a 29 × 5 (0, 1) α-lattice design, with two replications, 2 – row plot and 4 m row in all environments. Two seeds were sown per hill and later thinned to 53 333 plants ha^{-1}, the intra and inter-row spacing of 25 and 75 cm were used, respectively.

Table 6.1: Description of maize lines and testers used and their respective heterotic groups

Genotype code	Inbred line name	Source	Pedigree	SA Heterotic group
1	B1138T	*ARC, South Africa	TEKOYELLOW	F
2	BO394Y	ARC, South Africa	F2834T.4O2	F
3	E30Y	ARC, South Africa	B390YxM136Y	F
4	RO544W	ARC, South Africa	BO160W.3J400W	F
5	UO705Y	ARC, South Africa	YOFE1(S4)	F
6	VO430Y	ARC, South Africa	(HO466Y.1BO310Y)	F
7	I-16	ARC, South Africa	I-16	I
8	I-20	ARC, South Africa	I-20	I
9	I-34	ARC, South Africa	I-34	I
10	I-39	ARC, South Africa	I-39	I
11	I-41	ARC, South Africa	I-41	I
12	I-42	ARC, South Africa	I-42	I
13	K64	ARC, South Africa	Pride off Saline	K
14	K64R-22	ARC, South Africa	K64R-22	K
15	M162W	ARC, South Africa	K64R2.B1138T	K
16	R2565Y	ARC, South Africa	K64R2(I137TN1.K64R)	K
17	U127Y	ARC, South Africa	M162W.1KO326Y	K
18	U2540W	ARC, South Africa	M162W1.DO940Y-J34	K
19	MO17HtHtN	ARC, South Africa	MO17HtHtN	L
20	NC258	ARC, South Africa	NC258	L
21	P588MSV	ARC, South Africa	MRSxVHMO17	L
22	P590MSV	ARC, South Africa	MRSxVHMO17	L
23	P594MSV	ARC, South Africa	MLSxVHMO17	L
24	P598MSV	ARC, South Africa	21A-6xVHMO17	L
25	J80W	ARC, South Africa	D800W2.HtN	M
26	RO421W	ARC, South Africa	DO940Y-11.O2(W)	M
27	RO452W	ARC, South Africa	DO940Y-13.NHK	M
28	SO181Y	ARC, South Africa	KO326Y2.NPPES1	M
29	SO503W	ARC, South Africa	KO315Y2.NPPES1	M
30	VO617Y	ARC, South Africa	(1)"M37W.TE/TEO"	M
31	FO215W	ARC, South Africa	NPPES14.O2S14	P
32	SO607W	ARC, South Africa	POWS1(S4)	P
33	SO713W	ARC, South Africa	POWS1(S4)	P
34	VO495Y	ARC, South Africa	POWS12.Y	P
35	VO500Y	ARC, South Africa	POWS12.Y	P
36	VO501Y	ARC, South Africa	POWS12.Y	P
37	P28	ARC, South Africa	P28	R
38	P612MSV	ARC, South Africa	B73xVHKG/C1	R
39	P614MSV	ARC, South Africa	B73xVHKG/C1	R

Genotype code	Inbred line name	Source	Pedigree	SA Heterotic group
40	S198Y	ARC, South Africa	M28Y1.DO620Y	R
41	SO1224Y	ARC, South Africa	M28Y1.KO288Y	R
42	U71Y	ARC, South Africa	M28Y2.NP	R
	Inbred line testers			
1	B73	Iowa State University-USA	BSSS C5 (Iowa Stiff Stalk Synthetic)	Temperate A
2	MO17	University of Missouri-USA	(CL.187-2 x C103)	Temperate B
3	CML312	CIMMYT-Zimbabwe	S89500-F2-2-2-1-1-B	Tropical A
4	CML444	CIMMYT-Zimbabwe	P43-C9-1-1-1-1-1-B	Tropical B

**ARC – Agricultural Research Council of South Africa; SA – South Africa; USA – United States of America*

6.2.2 Trial management and data collection

The sites Potchefstroom (26.736S, 27.076E, 1349 m.a.s.l) and Cedara (29.542S, 30.265E, 1068m.a.s.l) were used for non-stress experiments. Non-stress trials received adequate irrigation and fertilisation based on soil recommendations, except Cedara which is a rainfed site and therefore no supplementary irrigation was applied. Low nitrogen experiments were conducted in Potchefstroom, Cedara and Vaalharts (27.958S, 24.840E, 1180 m.a.s.l). Nitrogen stress was managed following the low N stress management protocol defined by CIMMTY (Banziger *et al.*, 2000). Low N environments were previously depleted of N by growing unfertilised non-leguminous crops; oats, wheat and sorghum at high plant density for several seasons and removing the stover from the field each season after harvest. Vaalharts is naturally depleted of N because of sandy soil texture. Drought trials were conducted at Potchefstroom under random drought stress with irrigation applied at planting and emergence, while managed drought stress was conducted at Vaalharts. Vaalharts was initially treated as a low N stress block, however irrigation failure during the growing season coincided with the drought stress management protocol of two weeks prior to flowering; the site was therefore treated as a combined environment, with both low N and drought stress. The soils at Potchefstroom and Cedara are mostly clay loam. Weeds and insect pests were controlled manually and by using recommended chemicals. Data was recorded for days to anthesis (DA) and silking (AD), anthesis silking interval (ASI), plant height (PH), ear height (EH), field (FW) and grain weight (GW), grain moisture (GM), shelling percentage (SP), leaf senescence (SEN) and plant aspect (PA). Grain yield was determined on plot basis and adjusted to 12.5% grain moisture content.

6.2.3 Statistical analysis

Data for all traits across environments were subjected to the analysis of variance using Genstat 18th edition (VSN International, 2016); significant means were compared at $P \leq 0.05$. The model used was:

$$Y_{ijkl} = \mu + H_i + E_j + r_k (E_j) + B_l (E_j r_k) + (HE)_{ij} + \varepsilon_{ijkl}$$

Where: Y_{ijkl} is the phenotypic value of the hybrid i, when tested in lth block nested within the kth replication and within the jth environment, μ is the grand mean, H_i = is the effect of the hybrid i, E_j is the effect of the *j*th environment, r_k is the effect of the *k*th replication nested in the jth environment, B_l is the effect of the *l*th incomplete block nested in kth replication and jth environment, $(HE)_{ij}$ is the hybrid and environment interaction effect associated with hybrid *i* and environment *j and* ε_{ijkl} is the random error term. The blocks within replications were treated as random effects and replications, genotypes and environments were treated as fixed effects.

Variance components, phenotypic and genotypic variances were computed as:

Genotypic variance $(\sigma_g^2) = \frac{\text{MSg} - \text{MSE}}{\text{r}}$

Environmental Variance $(\sigma_e^2) = \frac{\text{MSe}}{\text{rl}}$

Genotype x environmental variance $(\sigma_{ge}^2) = \frac{\text{MSge} - \text{MSE}}{\text{l}}$

Phenotypic variance $(\sigma_P^2) = \sigma_g^2 + \sigma_e^2 + \sigma_{ge}^2$

Where MSg, MSe and MSE are mean squares of genotype, environments and error, respectively, r and l are the number of replicates and locations. The mean values were used to determine phenotypic, genotypic and environmental coefficient of variation (Singh and Chaudhary, 1985) as follows:

Genotypic coefficient of variation (GVC%) = $\frac{\sqrt{\sigma_g^2}}{\bar{X}} \times 100$

Phenotypic coefficient of variation (PCV %) = $\frac{\sqrt{\sigma_p^2}}{\bar{X}} \times 100$

Environmental coefficient of variation (ECV %) = $\frac{\sqrt{\sigma_{\mathrm{E}}^{2}}}{\bar{\mathrm{X}}} \times 100$

Broad sense heritability was estimated across environments (Hallauer and Miranda, 1988) as:

$$\mathrm{H} = \frac{\sigma_{\mathrm{g}}^{2}}{\sigma_{\mathrm{g}}^{2}+\frac{\sigma_{\mathrm{ge}}^{2}}{\mathrm{e}}+\frac{\sigma_{\mathrm{e}}^{2}}{\mathrm{re}}}$$

Where: σ_{g}^{2}, σ_{p}^{2} and σ_{e}^{2} is the genotypic, phenotypic and environmental variance components, respectively, and $\bar{\mathrm{X}}$ is the grand mean of a trait, r and l is the number of replications and locations.

The magnitude of standard heterosis was estimated relative to the standard checks (Narayanam and Phundan, 1993) for traits that showed significant differences as:

Standard heterosis (SH %) = $\frac{\mathrm{F1-SC}}{\mathrm{SC}} X100$

Where F_1 is the mean value of the F_1 hybrid and SC is the mean of the best standard check. The significance of heterosis was determined using t-test. Standard error of the differences and the t-value were calculated as follows (Wynne *et al.*, 1970):

SE (d) = $\sqrt{2\mathrm{MSE}/\mathrm{r}}$

$$\mathrm{t} = \frac{\mathrm{F1-SC}}{\mathrm{SE(d)}}$$

Where, SE (d) is standard error, MSE is error mean square, r is the number of replications and SC is the mean value of a standard check. The t-value obtained was tested against the tabular t-value at error degree of freedom.

6.3 Results

6.3.1 Analysis of variance

Mean squares for grain yield and secondary traits under non-stress, low N and drought stress environments are presented in Table 6.2 to 6.4. Highly significant differences (P<0.01) among genotypes were shown by grain yield and most secondary traits under non-stress and all stress environments, except shelling percentage (SP) and plant aspect under non-stress environments. The G x E was also significant (P<0.05) for most traits under stress and non-stress environments.

Table 6.2: Analysis of variance for grain yield and secondary traits under non-stress environments in 2015/16

Source	**df**	**GY**	**EPP**	**AD**	**SD**	**ASI**	**PH**	**EH**	**GM**	**PA**	**SP**
Environment	1	9.9*	0.29***	8876.5***	9711.7***	14.60*	79344.7***	123936.5***	0.001	7.1***	0.056***
Environment.Rep	2	20.2***	0.15***	737.0***	768.1***	0.93	24788.7***	5201.4***	12.9***	3.8**	0.001
Environment.Rep.Block	112	6.8***	0.05***	14.8***	20.9***	2.67***	931.0***	328.1***	1.6***	0.49***	0.002**
Genotype	144	5.4***	0.04***	21.5***	26.9***	2.18*	310.3***	156.3***	2.2***	0.27	0.002
Genotype.Environment	144	3.0***	0.04***	7.1*	10.3*	2.29**	288.3**	126.1***	1.7***	0.32	0.002*
CrossvsCheck	1	37.0**	0.08	143.2***	140.4**	0.05	236.2	176.10	0.3	0.48	0.002
Environment.CrossvsCheck	1	12.7	0.22**	13.7	13.0	0.04	210.20	484.30	4.60	0.38	0.014**
Residual	176	2.1	0.02	5.48	7.49	1.59	180.30	76.80	0.75	0.23	0.001

****significant at P<0.001; **P<0.01; *P<0.05; df=Degree of freedom; GY=Grain yield; EPP=Ears per plant; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; PH=Plant height; EH=Ear height; GM=Grain moisture; PA=Plant aspect; SP= Shelling percentage*

Table 6.3: Analysis of variance for grain yield and secondary traits under low N stress conditions in 2015/16

Source	df	GY	EPP	AD	SD	ASI	PH	EH	GM	SP	PA	SEN
Environment	2	535.2***	0.03	34570.5***	35199.0***	237.8***	231493.2***	22847.5***	423.8***	0.025***	41.61***	260.90***
Environment.Rep	3	52.0***	0.72***	43.8***	68.2***	4.6	5436.20***	809.8***	18.9***	0.024***	0.97**	7.53***
Environment.Rep.Block	168	3.2***	0.05***	10.4***	19.2***	5.4***	560.80***	310.9***	1.3***	0.002	0.53***	1.69***
Genotype	144	2.7***	0.06**	22.7***	32.0***	4.9**	497.80***	370.6***	2.4***	0.003**	0.45***	0.99***
Genotype.Environment	288	1.7*	0.03**	4.4***	7.4	3.3	195.70*	92.4	0.9	0.002	0.29	0.60
CrossvsCheck	1	0.6	0.13	89.2***	253.9***	42.1***	389.90	78.0	0.1	0.0001	0.001	0.52
Environment.CrossvsCheck	2	0.4	0.12*	19.2	61.3**	12.7*	400.50	41.6	1.3	0.003	0.09	0.13
Residual	263	1.7	0.02	3.1	6.7	3.4	160.80	87.7	0.7	0.002	0.27	0.54

****significant at P<0.001; **P<0.01; *P<0.05; df=Degree of freedom; GY=Grain yield; EPP=Ears per plant; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; PH=Plant height; EH=Ear height; GM=Grain moisture; PA=Plant aspect; SP= Shelling percentage; SEN = Leaf senescence*

Table 6.4: Analysis of variance for grain yield and secondary traits under drought conditions in 2015/16

Source	df	GY	EPP	AD	SD	ASI	PH	EH	GM	SP	PA	SEN
Environment	1	7.5	6.1***	8126.3***	6848.4***	54.6***	14024.8***	36373.1***	635.3***	0.16**	47.18***	80.44***
Environment.Rep	2	77.4***	1.3***	110.7***	158.7***	6.5	5721.6***	1468.6***	7.3**	0.03**	1.31*	10.55***
Environment.Rep.Block	112	5.3***	0.1***	13.0***	18.0***	2.9	658.0***	309.7***	2.5**	0.002	0.72***	1.31***
Genotype	144	3.1**	0.1***	18.2***	21.1***	2.5	535.2***	297.1***	2.4**	0.003*	0.60**	0.93**
Genotype.Environment	144	3.0**	0.1**	4.9	6.5	2.0	256.4*	90.3	2.1*	0.002	0.43	0.65
CrossvsCheck	1	0.4	0.3*	114.9***	238.7***	22.4**	101.5	36.5	0.0	0.001	0.11	0.20
Environment.CrossvsCheck	1	0.3	0.0	0.01	1.2	1.2	416.2	87.6	1.6	0.002	0.71	0.65
Residual	176	2.1	0.0	4.0	6.0	2.0	185.5	72.7	1.6	0.002	0.40	0.64

****significant at P<0.001; **P<0.01; *P<0.05; df=Degree of freedom; GY=Grain yield; EPP=Ears per plant; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; PH=Plant height; EH=Ear height; GM=Grain moisture; PA=Plant aspect; SP= Shelling percentage; SEN = Leaf senescence*

6.3.2 Mean performance

Grain yield under non-stress environments ranged from 3.33 to 10.13 t ha^{-1}, with the mean of 5.87 t ha^{-1}. The highest yield was exhibited by L3 x T3 (FO215W x CML444), with 73% yield advantage relative to the trial mean, 41% and 24% relative to the mean of all checks and best checks, respectively (Table 6.5). Under low conditions, the average yield was 3.42 t ha^{-1}, which was 42% lower that of non-stress environments and the range was 1.97 to 5.99 t ha^{-1} (Table 6.6). Drought stress reduced GY by 22% of the yield under non-stress conditions, the yield under drought conditions ranged from 2.38 to 7.70 t ha^{-1}, with the average of 4.57 t ha^{-1} (Table 6.7). Maximum yield under low N and drought stress environments was shown by the crosses L9 x T3 (I-42 x CML444), which also exhibited the highest yield advantage of 76%, 69% and 13% relative to the grand mean, mean of checks and the best check, respectively under low N stress and 68%, 64% and 15% under drought stress conditions. When both stress and non-stress environments were combined, the highest yield (6.76 t ha^{-1}) was recorded from the cross L3 x T3 (FO215W x CML444), with 46, 31 and 11% yield advantage relative to the trial mean, check hybrids and the best check (Table 6.8). Grain yield advantage of the top 10 experimental hybrids relative to the trial mean was 41% under non-stress and 43% under both drought and low N stress environments. Relative to the checks, yields were 115%, 139% and 137% under non-stress, drought and low N stress, respectively. The means of the top 10 hybrids were 8.30, 6.54, 4.87 and 6.03 t ha^{-1} under non-stress, drought, low N stress and across all management conditions.

Table 6.5: Mean performance of top and bottom 10 testcrosses and commercial checks for grain yield (t ha^{-1}) and other agronomic traits under two non-stress environments

Cross	**Absolute GY**	**Relative yield**	**%Checks**	**%Best Check**	**EPP**	**AD**	**SD**	**ASI**	**PH**	**EH**	**GM**	**SP**	**PA**
L3 x T3	10.13	173	141	124	1.2	79.5	80.3	0.8	192.29	109.96	15.23	0.87	4.63
L21 x T2	9.59	163	133	117	0.9	76.5	78.8	2.3	184.63	103.37	12.45	0.89	4.00
L21 x T1	8.63	147	120	105	1.1	74.5	74.5	0.0	200.63	104.71	14.10	0.87	4.13
L19 x T4	8.52	145	118	104	1.0	74.0	75.5	1.5	183.00	107.67	13.58	0.85	4.38
L4 x T3	7.82	133	109	95	1.2	76.0	77.8	1.8	183.09	115.05	14.58	0.85	4.13
L39 x T4	7.74	132	107	95	1.0	75.8	77.0	1.3	208.67	121.12	13.83	0.85	4.63
L24 x T4	7.72	132	107	94	1.1	75.5	76.5	1.0	197.34	98.62	14.20	0.86	4.25
L16 x T4	7.69	131	107	94	1.0	76.0	77.8	1.8	192.50	108.83	14.28	0.87	4.00
L36 x T4	7.58	129	105	93	1.0	75.0	76.8	1.8	199.59	117.33	13.95	0.86	4.38
L26 x T4	7.54	129	105	92	1.0	73.8	75.3	1.5	219.75	119.92	12.83	0.83	4.00
L31 x T3	4.14	71	58	51	0.8	77.0	78.0	1.0	193.71	106.96	12.63	0.81	5.00
L31 x T1	4.08	70	57	50	0.9	73.8	75.0	1.3	169.63	90.33	13.65	0.81	4.50
L11 x T4	4.04	69	56	49	0.7	77.8	78.8	1.0	171.04	99.75	13.25	0.79	4.88
L44 x T1	4.01	68	56	49	0.9	73.8	75.8	2.0	181.84	105.42	12.43	0.83	4.88
L18 x T1	3.77	64	52	46	0.8	72.3	73.0	0.8	191.50	110.04	12.55	0.80	4.75
L40 x T1	3.75	64	52	46	0.7	73.3	74.0	0.8	187.17	95.13	12.68	0.80	4.75

Cross	Absolute GY	Relative yield	%Checks	%Best Check	EPP	AD	SD	ASI	PH	EH	GM	SP	PA
L38 x T4	3.72	63	52	45	0.8	80.3	84.0	3.8	193.96	119.34	14.28	0.85	5.00
L37 x T2	3.41	58	47	42	0.7	76.3	79.0	2.8	179.88	110.88	12.63	0.78	4.50
L13 x T4	3.38	58	47	41	0.7	79.3	80.8	1.5	183.67	110.17	14.53	0.81	4.88
L29 x T1	3.33	57	46	41	0.7	75.3	76.0	0.8	183.42	107.04	13.35	0.83	4.25
Check1	6.28	107	87	77	0.9	78.8	80.8	2.0	193.17	112.21	13.50	0.82	4.63
Check2	7.37	126	102	90	0.9	78.5	79.3	0.8	178.21	111.15	13.35	0.86	4.50
Check3	6.61	113	92	81	1.1	73.8	74.0	0.3	195.46	119.21	13.10	0.84	4.75
Check4	8.19	140	114	100	1.2	80.8	81.5	0.8	181.05	108.71	15.15	0.87	4.63
Check5	7.56	129	105	92	1.0	74.8	76.0	1.3	186.30	106.46	12.80	0.82	4.63
Mean	***5.87***				***0.93***	***74.67***	***75.69***	***0.81***	***190.21***	***108.63***	***13.47***	***0.83***	***4.47***
Checks	***7.20***												
Best Check	***8.19***												
#Sites	***2.00***												
SE	***1.74***				***0.17***	***3.49***	***3.99***	***1.41***	***22.58***	***11.15***	***0.98***	***0.03***	***0.56***
LSD	***3.43***				***0.33***	***6.86***	***7.86***	***2.78***	***44.44***	***21.95***	***1.93***	***0.07***	***1.09***
CV	***29.70***				***18.00***	***4.70***	***5.30***	***134.90***	***11.90***	***10.30***	***7.30***	***4.10***	***12.40***
Min	***3.33***				***0.68***	***69.75***	***70.50***	***-0.50***	***164.17***	***87.38***	***11.98***	***0.78***	***3.63***
Max	***10.13***				***1.18***	***80.25***	***84.00***	***3.75***	***219.75***	***128.00***	***16.55***	***0.89***	***5.00***

SE=Standard error; LSD=Least significant difference; CV=Coefficient of variation; GY=Grain yield; EPP=Ears per plant; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; PH=Plant height; EH=Ear height; GM=Grain moisture; SP= Shelling percentage; PA=Plant aspect

Table 6.6: Mean performance of top and bottom 10 testcrosses and commercial checks for grain yield (t ha^{-1}) and other agronomic traits under three low N stress environments

Cross	Absolute GY	Relative yield	%Checks	%Best Check	EPP	AD	SD	ASI	PH	EH	GM	SP	PA	SEN
L9 x T3	5.99	176	169	113	0.99	74.83	77.17	2.33	182.6	95.6	12.58	0.83	4.08	3.00
L33 x T3	5.07	149	143	96	0.98	72.50	73.50	1.00	189.0	104.5	12.55	0.80	4.17	4.25
L8 x T4	5.01	147	142	95	0.99	71.33	72.17	0.83	201.0	94.9	11.68	0.83	3.42	2.75
L24 x T3	4.83	142	136	91	1.11	72.17	73.67	1.50	164.9	87.7	12.05	0.80	4.50	3.50
L38 x T3	4.82	142	136	91	1.03	75.50	78.83	3.33	179.5	106.0	12.95	0.82	4.25	4.00
L27 x T1	4.60	135	130	87	0.86	71.17	72.67	1.50	164.4	79.1	12.43	0.84	4.33	3.75
L8 x T3	4.60	135	130	87	0.93	75.00	75.83	0.83	187.3	100.6	12.75	0.76	4.42	3.75
L16 x T2	4.59	135	130	86	0.97	71.83	73.17	1.33	170.1	86.1	11.25	0.80	4.08	3.75
L6 x T3	4.58	135	129	86	1.05	72.83	73.83	1.00	184.2	100.2	13.35	0.82	3.92	4.00
L3 x T4	4.52	133	128	85	0.87	75.67	76.83	1.17	175.7	90.5	13.90	0.83	4.25	4.25
L25 x T1	2.48	73	70	47	0.82	75.00	76.67	1.67	155.8	75.6	11.50	0.76	4.92	3.00

Cross	Absolute GY	Relative yield	%Checks	%Best Check	EPP	AD	SD	ASI	PH	EH	GM	SP	PA	SEN
L46 x T2	2.44	72	69	46	0.63	73.33	74.00	0.67	183.8	92.3	11.12	0.82	4.17	3.75
L36 x T1	2.41	71	68	45	0.75	71.00	74.83	3.83	169.0	86.6	11.55	0.83	4.58	3.75
L27 x T2	2.35	69	66	44	0.75	73.17	75.83	2.67	160.2	78.3	12.45	0.80	4.83	3.50
L35 x T1	2.29	67	65	43	0.76	74.00	77.33	3.33	168.2	92.2	11.55	0.79	5.00	3.75
L18 x T1	2.28	67	64	43	0.87	70.17	71.83	1.67	143.3	66.0	11.45	0.76	4.92	4.25
L40 x T1	2.19	64	62	41	0.73	68.67	69.83	1.17	159.4	70.8	11.25	0.76	4.75	3.75
L32 x T3	2.14	63	61	40	0.96	75.00	77.17	2.17	163.9	94.9	12.53	0.76	4.75	4.25
L13 x T1	2.02	59	57	38	0.70	73.00	74.83	1.83	163.5	81.1	11.77	0.78	4.83	4.00
L5 x T1	1.97	58	56	37	0.78	72.17	73.83	1.67	151.8	68.7	11.17	0.79	4.67	3.25
Check1	5.30	156	150	100	0.90	74.17	76.33	2.17	182.8	100.0	12.07	0.85	3.83	3.75
Check2	3.52	104	100	66	0.85	73.33	76.50	3.17	170.0	91.3	12.02	0.79	4.42	4.25
Check3	2.30	68	65	43	0.65	74.50	79.50	5.00	161.8	87.4	11.38	0.79	4.83	4.00
Check4	3.31	97	94	62	0.83	78.00	80.17	2.17	155.9	87.7	12.25	0.77	4.83	4.00
Check5	3.25	96	92	61	0.82	72.50	75.33	2.83	169.4	86.3	11.75	0.78	4.50	3.25
Mean	***3.40***				***0.87***	***72.81***	***74.71***	***1.90***	***171.5***	***89.0***	***11.95***	***0.79***	***4.49***	***3.69***
Checks	***3.54***													
Best Check	***5.30***													
#Sites	***3***													
SE	***1.39***				***0.18***	***1.96***	***2.82***	***1.88***	***15.71***	***10.37***	***0.93***	***0.04***	***0.56***	***0.86***
LSD	***2.74***				***0.35***	***3.85***	***5.55***	***3.69***	***30.87***	***20.38***	***1.83***	***0.09***	***1.09***	***1.69***
CV	***40.9***				***20.50***	***2.70***	***3.80***	***98.70***	***9.20***	***11.70***	***7.80***	***5.60***	***12.40***	***23.30***
Min	***1.97***				***0.63***	***68.67***	***69.50***	***-0.17***	***143.25***	***66.00***	***10.77***	***0.74***	***3.42***	***2.50***
Max	***5.99***				***1.25***	***78.67***	***80.83***	***4.67***	***205.29***	***117.58***	***14.07***	***0.84***	***5.00***	***5.75***

SE=Standard error; LSD=Least significant difference; CV=Coefficient of variation; GY=Grain yield; EPP=Ears per plant; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; PH=Plant height; EH=Ear height; GM=Grain moisture; PA=Plant aspect; SP= Shelling percentage; SEN= Leaf senescence

Table 6.7: Mean performance of top and bottom 10 testcrosses and commercial checks for grain yield (t ha^{-1}) and other agronomic traits under two drought environments

Cross	**Absolute GY**	**Relative yield**	**%Checks**	**%Best Check**	**EPP**	**AD**	**SD**	**ASI**	**PH**	**EH**	**GM**	**SP**	**PA**	**SEN**
L9 x T3	7.70	168	164	115	1.0	69.8	69.5	-0.3	214.7	107.7	13.58	0.86	3.38	1.75
L38 x T3	6.69	146	142	100	1.2	69.5	71.0	1.5	195.7	117.0	13.65	0.83	3.75	2.50
L21 x T4	6.63	145	141	99	1.0	68.5	69.8	1.3	215.9	103.8	14.08	0.85	3.25	2.25
L5 x T4	6.54	143	139	97	0.9	69.8	70.3	0.5	190.0	90.3	14.13	0.84	2.88	2.25
L11 x T4	6.54	143	139	97	1.0	66.3	67.3	1.0	196.5	108.0	13.85	0.87	3.00	3.00
L3 x T4	6.40	140	136	95	1.0	72.5	72.3	-0.3	186.5	94.6	16.00	0.85	3.25	2.75
L26 x T4	6.29	137	134	94	1.1	69.8	70.0	0.3	199.7	100.7	13.40	0.85	3.25	1.75
L23 x T4	6.28	137	133	94	0.9	68.8	68.8	0.0	205.4	107.6	13.90	0.83	4.13	2.25
L10 x T4	6.24	136	133	93	1.0	70.5	71.5	1.0	205.7	106.0	14.20	0.81	3.63	2.75
L45 x T1	6.15	135	131	92	1.4	65.8	66.0	0.3	177.0	94.1	13.28	0.84	4.38	3.00
L13 x T3	3.23	71	69	48	1.0	69.3	70.5	1.3	187.5	104.7	13.70	0.76	4.50	3.75
L12 x T2	3.19	70	68	48	0.7	63.0	64.5	1.5	166.2	82.3	13.43	0.81	4.50	2.75
L33 x T2	3.16	69	67	47	0.7	67.3	68.0	0.8	185.7	88.6	12.33	0.80	4.25	3.25
L20 x T2	3.14	69	67	47	0.9	65.0	66.0	1.0	169.3	82.5	13.10	0.80	4.25	2.75
L5 x T1	3.10	68	66	46	0.9	67.0	67.5	0.5	168.9	78.3	13.25	0.84	4.00	2.25
L42 x T4	3.08	67	65	46	1.0	68.3	69.0	0.8	188.5	96.6	13.15	0.81	3.75	2.50
L42 x T1	2.73	60	58	41	0.7	66.0	66.0	0.0	168.1	87.5	12.68	0.77	4.50	4.25
L18 x T1	2.56	56	54	38	0.7	67.0	68.0	1.0	161.5	76.1	13.48	0.78	4.75	3.25
L31 x T1	2.40	53	51	36	0.7	67.0	68.0	1.0	169.2	79.8	11.15	0.81	4.63	3.00
L41 x T2	2.38	52	50	35	0.9	64.0	64.3	0.3	185.2	82.3	12.60	0.83	4.38	2.75
Check1	6.71	147	143	100	0.9	68.0	69.3	1.3	198.2	102.4	13.40	0.88	3.50	3.00
Check2	4.96	108	105	74	0.9	67.5	69.5	2.0	175.5	86.9	14.10	0.82	4.00	2.75
Check3	3.62	79	77	54	0.8	71.8	74.8	3.0	175.5	94.8	12.18	0.81	4.75	2.00
Check4	3.92	86	83	58	0.8	73.3	74.8	1.5	171.8	101.9	13.45	0.79	4.38	2.75
Check5	4.33	95	92	65	0.8	69.3	70.8	1.5	189.0	91.8	13.10	0.80	3.88	2.25
Mean	***4.57***				***0.97***	***67.59***	***68.41***	***1.05***	***184.24***	***94.28***	***13.21***	***0.81***	***4.03***	***2.65***
Checks	***4.71***													
Best Check	***6.71***													
#Environments	***2***													
SE	***1.79***				***0.24***	***2.17***	***2.70***	***1.50***	***17.06***	***10.46***	***1.34***	***0.04***	***0.68***	***0.87***
LSD	***3.51***				***0.48***	***4.27***	***5.32***	***2.95***	***33.58***	***20.58***	***2.64***	***0.08***	***1.34***	***1.72***
CV	***39***				***25.20***	***3.20***	***4.00***	***184.70***	***9.30***	***11.10***	***10.10***	***5.10***	***16.90***	***33.00***
Min	***2.38***				***0.58***	***62.75***	***62.75***	***-1.50***	***158.95***	***72.83***	***11.15***	***0.74***	***2.63***	***1.00***
Max	***7.7***				***1.57***	***74.25***	***75.00***	***4.00***	***215.88***	***126.25***	***16.00***	***0.87***	***4.75***	***4.25***

SE=Standard error; LSD=Least significant difference; CV=Coefficient of variation; GY=Grain yield; EPP=Ears per plant; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; PH=Plant height; EH=Ear height; GM=Grain moisture; PA=Plant aspect; SP= Shelling percentage; SEN= Leaf senescence

Table 6.8: Mean performance of top and bottom 10 testcrosses and commercial checks for grain yield and other agronomic traits across environments

Cross	Absolute GY	Relative yield	%Checks	%Best Check	EPP	AD	SD	ASI	PH	EH	GM	PA	SP	SEN
L3 x T3	6.76	146	131	111	1.0	77.3	77.8	0.5	174.9	97.7	14.43	4.08	0.83	3.25
L9 x T3	6.58	143	128	108	1.0	73.3	74.4	1.1	196.7	105.7	13.38	3.94	0.85	2.38
L26 x T4	6.10	132	118	100	1.0	72.6	73.6	1.0	200.2	104.3	12.75	3.78	0.83	2.38
L21 x T2	6.06	131	118	99	0.9	72.4	74.4	2.0	176.9	91.4	12.17	4.18	0.83	2.38
L16 x T4	6.03	131	117	99	0.9	74.3	75.8	1.5	187.9	100.4	12.94	3.71	0.83	3.00
L33 x T3	5.95	129	115	97	0.9	71.5	72.3	0.8	194.0	111.5	12.99	4.10	0.81	4.13
L39 x T4	5.73	124	111	94	0.9	73.4	74.5	1.1	196.8	110.7	13.07	4.31	0.82	2.63
L38 x T3	5.71	124	111	94	1.0	74.4	76.6	2.2	189.9	111.3	13.78	4.25	0.82	3.25
L3 x T4	5.71	124	111	94	0.9	74.5	75.0	0.6	184.0	97.1	15.16	3.96	0.85	3.50
L36 x T4	5.66	123	110	93	0.9	72.9	74.1	1.2	197.3	112.1	13.63	3.72	0.83	3.13
L20 x T1	3.72	81	72	61	0.9	68.7	69.4	0.8	183.1	98.0	12.53	4.46	0.81	4.50
L5 x T1	3.68	80	71	60	0.9	70.6	72.1	1.6	166.2	82.9	12.61	4.39	0.82	2.75
L44 x T1	3.65	79	71	60	0.8	69.9	71.6	1.7	178.6	91.7	12.11	4.32	0.81	3.75
L41 x T2	3.55	77	69	58	1.0	67.8	68.2	0.3	185.9	85.8	11.92	4.56	0.82	3.38
L24 x T1	3.52	76	68	58	0.7	71.9	73.3	1.4	162.2	80.2	12.95	4.61	0.79	2.50
L29 x T1	3.48	75	68	57	0.8	72.2	73.1	0.9	177.8	98.3	13.39	4.49	0.81	3.88
L13 x T4	3.47	75	67	57	0.7	74.8	77.3	2.6	180.9	103.0	13.34	4.60	0.79	3.50
L40 x T1	3.26	71	63	54	0.8	68.4	69.1	0.7	172.4	81.9	12.33	4.54	0.78	2.88
L31 x T1	3.05	66	59	50	0.8	70.7	72.1	1.4	168.2	83.1	11.88	4.57	0.80	3.00
L18 x T1	2.87	62	56	47	0.8	69.8	70.9	1.1	165.4	84.0	12.49	4.81	0.78	3.75
Check1	6.10	132	118	100	0.9	73.6	75.4	1.8	191.4	104.9	12.99	3.99	0.85	3.38
Check2	5.28	115	103	87	0.9	73.1	75.1	2.0	174.6	96.5	13.16	4.31	0.82	3.50
Check3	4.18	91	81	68	0.8	73.3	76.1	2.8	177.6	100.5	12.22	4.78	0.81	3.00
Check4	5.14	111	100	84	1.0	77.3	78.8	1.5	169.6	99.4	13.62	4.61	0.81	3.38
Check5	5.05	109	98	83	0.9	72.2	74.0	1.9	181.6	94.8	12.55	4.33	0.80	2.75
Mean	***4.61***				***0.92***	***71.69***	***72.94***	***1.25***	***182.00***	***97.29***	***12.88***	***4.33***	***0.81***	***3.17***
Checks	***5.15***													
Best Check	***6.10***													
#Environments	***6.00***													
SD	***1.17***				***0.14***	***1.84***	***2.29***	***1.17***	***13.13***	***7.87***	***0.78***	***0.42***	***0.03***	***0.64***
SE	***1.65***				***0.20***	***2.60***	***3.24***	***1.65***	***18.57***	***11.13***	***1.10***	***0.60***	***0.04***	***0.90***
LSD	***3.24***				***0.39***	***5.11***	***6.36***	***3.24***	***36.43***	***21.84***	***2.16***	***1.18***	***0.08***	***1.77***
CV	***37.20***				***21.70***	***3.60***	***4.40***	***122.7***	***10.30***	***11.60***	***8.60***	***13.80***	***5.10***	***28.40***
Min	***2.87***				***0.74***	***67.83***	***68.17***	***-0.31***	***159.70***	***80.22***	***11.71***	***3.55***	***0.76***	***1.88***
Max	6.76				1.24	77.31	79.36	3.14	202.90	121.10	15.16	4.82	0.85	4.75

SE=Standard error; LSD=Least significant difference; CV=Coefficient of variation; GY=Grain yield; EPP=Ears per plant; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; PH=Plant height; EH=Ear height; GM=Grain moisture; PA=Plant aspect; SP= Shelling percentage; SEN= Leaf senescence

6.3.3 Standard Heterosis

Estimates of standard heterosis for grain yield under different environments are presented in Table 6.9. The data shown is for the top and bottom 10 varieties. Heterosis for grain yield ranged from -58.7 to 23.7% under non-stress environments, with the average of -29.0; four hybrids (L3 x T3, L21 x T2, L21 x T1 and L19 x T4) showed positive heterosis, the highest heterosis was manifested by L3 x T3 (FO215W x CML444). The average across environments was -24.7, and the range was -52.9 – 10.8; the cross L3 x T3 also showed maximum heterosis across environments. Under low N and drought conditions, the average heterosis was -35.9 and -31.9, respectively. The range was -62.9 – 12.9 under low N and -64.6 to 14.8 under drought environments. The cross L9 x T3 (I-42 x CML444) showed positive and maximum heterosis under both low N and drought stress environments.

Table 6.9: Standard heterosis of top and bottom 10 testcrosses for grain yield and other agronomic traits across environments, non-stress, low N and drought stress

Across environments		**Non-stress**		**Low N Stress**		**Drought stress**	
Cross	Heterosis	Cross	Heterosis	Cross	Heterosis	Cross	Heterosis
L3 x T3	10.81	L3 x T3	23.73	L9 x T3	12.87	L9 x T3	14.82
L9 x T3	7.92	L21 x T2	17.05	L33 x T3	-4.35	L38 x T3	-0.27
L26 x T4	0.03	L21 x T1	5.34	L8 x T4	-5.47	L21 x T4	-1.13
L21 x T2	-0.61	L19 x T4	4.07	L24 x T3	-8.95	L5 x T4	-2.51
L16 x T4	-1.1	L4 x T3	-4.54	L38 x T3	-9.2	L11 x T4	-2.54
L33 x T3	-2.51	L39 x T4	-5.45	L8 x T3	-13.32	L3 x T4	-4.59
L39 x T4	-6.05	L24 x T4	-5.73	L27 x T1	-13.34	L26 x T4	-6.25
L38 x T3	-6.38	L16 x T4	-6.02	L16 x T2	-13.53	L23 x T4	-6.43
L3 x T4	-6.44	L36 x T4	-7.4	L6 x T3	-13.64	L10 x T4	-6.96
L36 x T4	-7.18	L26 x T4	-7.89	L3 x T4	-14.72	L45 x T1	-8.29
L20 x T1	-38.93	L31 x T3	-49.40**	L41 x T2	-52.58*	L13 x T3	-51.88*
L5 x T1	-39.69	L31 x T1	-50.16**	L25 x T1	-53.24**	L12 x T2	-52.45*
L44 x T1	-40.21	L11 x T4	-50.64**	L36 x T1	-54.49**	L33 x T2	-52.88*
L41 x T2	-41.77	L44 x T1	-51.06**	L27 x T2	-55.69**	L20 x T2	-53.21*
L24 x T1	-42.34*	L18 x T1	-53.91**	L35 x T1	-56.82**	L5 x T1	-53.86**
L29 x T1	-42.92*	L40 x T1	-54.20**	L18 x T1	-57.05**	L42 x T4	-54.15**
L13 x T4	-43.16*	L38 x T4	-54.54**	L40 x T1	-58.80**	L42 x T1	-59.33**
L40 x T1	-46.47*	L37 x T2	-58.34***	L32 x T3	-59.61**	L18 x T1	-61.86**
L31 x T1	-50.07*	L13 x T4	-58.66***	L13 x T1	-61.91**	L31 x T1	-64.15**
L18 x T1	-52.92*	L29 x T1	-59.33**	L5 x T1	-62.87**	L41 x T2	-64.58**
Mean	***-24.66***		***-28.95***		***-35.9***		***-31.88***
Min	***-52.92***		***-58.66***		***-62.87***		***-64.58***
Max	***10.81***		***23.73***		***12.87***		***14.82***

****significant at P<0.001; **P<0.01; *P<0.05*

6.3.4 Variance components

Estimates of heritability and variance components for grain yield and agronomic traits under stress and non-stress environments are presented in Table 6.10. Broad sense heritability estimates for grain yield were 0.63 under non-stress, 0.52 under low N and 0.33 under drought stress conditions and 0.49 across environments. Heritability for most traits including EPP, AD, SD, PH, EH, GM and SP were above 50% across all environments. Under non-stress environments, the genotypic variance accounted for the largest proportion of the phenotypic variance in most traits than under stress conditions where environmental variation influenced most traits.

Table 6.10: Variance components for grain yield and secondary traits under two drought, three low N stress and two non-stress environments in 2015/16

Components	**Environment**	**GY**	**EPP**	**AD**	**SD**	**ASI**	**PH**	**EH**	**GM**	**SP**	**PA**	**SEN**
Grand mean	Non-stress	5.87	0.93	74.67	75.69	1.05	190.21	108.63	13.47	0.83	4.47	
	Low Nitrogen stress	3.40	0.87	72.81	74.71	1.90	171.55	88.95	11.95	0.79	4.49	3.69
	Drought stress	4.57	0.97	67.59	68.41	0.81	184.24	94.28	13.21	0.81	4.03	2.65
	Across Environments	4.61	0.92	71.69	72.94	1.25	182.00	97.29	12.88	0.81	4.33	3.17
Genetic variance	Non-stress	1.61	0.01	8.00	9.70	0.29	65.00	39.73	0.74	0.00	0.02	
	Low Nitrogen stress	0.49	0.02	9.84	12.64	0.74	168.50	141.46	0.82	0.00	0.09	0.23
	Drought stress	0.49	0.04	7.07	7.60	0.27	174.85	112.17	0.39	0.00	0.10	0.15
	Across Environments	0.86	0.02	8.30	9.98	0.43	136.12	97.79	0.65	0.00	0.07	0.19
Phenotypic variance	Non-stress	2.57	0.02	10.19	12.99	1.04	164.08	84.02	1.23	0.05	0.08	
	Low N stress	0.94	0.03	11.05	14.55	1.56	220.33	164.94	1.05	0.00	0.16	0.38
	Drought stress	1.45	0.04	8.08	9.08	0.77	221.23	130.35	0.79	0.00	0.20	0.30
	Across Environments	1.65	0.03	9.77	12.21	1.12	201.88	126.44	1.02	0.02	0.15	0.34
Phenotypic coefficient of variation (%)	Non-stress	27.31	15.78	4.28	4.76	97.30	6.73	8.44	8.23	25.59	6.27	
	Low N stress	28.50	18.55	4.57	5.11	65.56	8.65	14.44	8.58	3.89	8.94	16.73
	Drought stress	26.37	21.86	4.20	4.41	108.01	8.07	12.11	6.72	3.54	11.06	20.84
	Across Environments	27.40	18.73	4.35	4.76	90.29	7.82	11.66	7.84	11.01	8.76	18.79
Genotypic coefficient of variation (%)	Non-stress	21.62	10.09	3.79	4.11	51.68	4.24	5.80	6.40	1.52	3.25	
	Low N stress	20.56	15.62	4.31	4.76	45.13	7.57	13.37	7.58	2.72	6.60	12.90
	Drought stress	15.24	19.46	3.93	4.03	63.79	7.18	11.23	4.76	2.33	7.74	14.39

Components	Environment	GY	EPP	AD	SD	ASI	PH	EH	GM	SP	PA	SEN
	Across Environments	19.14	15.06	4.01	4.30	53.53	6.33	10.14	6.25	2.19	5.87	13.64
Environmental coefficient of variation (%)	Non-stress	12.48	7.79	1.57	1.81	60.13	3.53	4.03	3.23	2.07	5.35	
	Low N stress	19.16	8.27	1.20	1.73	48.30	3.70	5.26	3.56	2.72	5.81	9.94
	Drought stress	15.91	9.96	1.49	1.78	87.16	3.70	4.52	4.75	2.66	7.90	15.08
	Across Environments	15.85	8.67	1.42	1.77	65.20	3.64	4.61	3.85	2.48	6.35	12.51
Heritability (H %)	Non-stress	0.63	0.41	0.79	0.75	0.28	0.40	0.47	0.60	0.00	0.27	
	Low N stress	0.52	0.71	0.89	0.87	0.47	0.76	0.86	0.78	0.49	0.55	0.59
	Drought stress	0.33	0.79	0.87	0.84	0.35	0.79	0.86	0.50	0.43	0.49	0.48
	Across Environments	0.49	0.64	0.85	0.82	0.37	0.65	0.73	0.63	0.31	0.44	0.54

GY=Grain yield; EPP=Ears per plant; AD=Anthesis days; SD=silking days, ASI=Anthesis-silking interval; PH=Plant height; EH=Ear height; GM=Grain moisture; SP= Shelling percentage; PA=Plant aspect; SEN=Leaf senescence

6.4 Discussion

6.4.1 Mean performance of hybrids

Compared to yield levels of the non-stress environments, drought stress reduced grain yield by 22% while yield under low N stress dropped by 42% of the yield under non-stress environments. Varying levels of yield reductions under low N and drought stress have been reported in the literature. Some investigations have reported up to 50% or greater yield reductions under both drought stress and low N stress (Betran *et al.*, 2003; Cairns *et al.*, 2013; Ertiro *et al.*, 2017; Meseka *et al.*, 2013), while others observed below 20% reductions due to drought and low N stress (Ndhlela, 2012). The effect of drought and low N on yield levels thus depends on the material used, test environments, duration and intensity of stress. Stress triggered early maturity and reduced plant height in most hybrids. The effect of stress on flowering was mostly observed under drought stress; drought stress triggered early flowering by up to 10%, while under low N stress, early flowering was triggered by only up to 3%. Earliness is generally triggered under stress conditions as a mechanism of stress escape. Reductions in plant height due to drought and low N stress were 3% and 10%, respectively and up to 13% and 18% for ear height. The results are in agreement with authors who also observed shorter plants due to low N and drought stress (Ertiro *et al.*, 2017; Oyekunle *et al.*, 2015).

Most farmer's fields are generally affected by various abiotic stresses simultaneously in the same block and during the same growing season; therefore, selected varieties should display tolerance to multiple stresses. Among varieties that were superior under stress conditions, three were common and early to intermediate flowering under both drought and low N, those were, L9 x T3 (I-42 x CML444), L38 x T3 (VO501Y x CML444) and L3 x T4 (FO215W x CML312). These varieties may therefore be prioritised as early to intermediate flowering hybrids for improving yields under both low N and drought stress environments. The effect was observed due to the existence of the common adaptive mechanism in the tolerance to drought and low N stress, which is documented in the literature (Banziger *et al.*, 1999). Many reports have therefore shown that selection under drought tolerance generally leads to improved performance under low N stress environments (Cairns *et al.*, 2013; Ertiro *et al.*, 2017; Meseka *et al.*, 2013; Zambezi and Mwambula, 1997). Ideally, tolerance to abiotic stresses has to be achieved without penalties in yield potential under non-stress environments, such that a single variety targeted for stress environments should also be superior when growing conditions are ideal. However, in general there is a very poor to no correlated response between stress and non-stress environments (Cairns *et al.*, 2013; Miti, 2007), which is an indication of independent genetic control. This has been associated with the increase in stress level and therefore subsequent increase in mean grain yield differences between stress than non-stress environments (Banziger *et al.*, 1997), and to the contribution of quantitative trait locus (QTLs) with large effects in stress tolerance than under non-stress conditions (Cairns *et al.*, 2013). Therefore, in the current study, many varieties that were superior under non-stress conditions were not consistently superior under stress.

According to Weber *et al.* (2012) and Ertiro *et al.* (2017), there is a possibility of obtaining higher yielding hybrids under stress environments that carries no yield penalties under non-stress environments; this may be achieved through simultaneous selection for stress and non-stress. Ertiro *et al.* (2017) successfully identified two of fifteen varieties with consistent performance across stress and non-stress environments, the success was driven by use of germplasm that has been previously subjected to several cycles of simultaneous selection for performance under non-stress and multiple stress conditions. In this study, the cross L26 x T4 (SO1224Y x CML312) was ranked among the top 10 under non-stress and drought stress environment, this hybrid may be used to improve tolerance under stress. Though it did not appear under low N conditions, many studies have confirmed that selection under drought is generally translated to spill-over effects under low N stress conditions (Derera *et al.*, 2008, Meseka *et al.*, 2013); this may thus be observed in the advanced stages of selection.

The cross L3 x T3 (FO215W x CML444) was the most superior only under non-stress environments; this single-cross may therefore be useful only in environments with minimal abiotic stresses. Among the top 10 varieties under non-stress and stress environments, it was observed that there were also a few crosses containing temperate material, T1 (MO17) and T2 (B73). For example, crosses L21 x T2 (RO421W x B73) and L21 x T1 (RO421W x MO17) under non-stress, L45 x T1 (CML444 x MO17) under drought and L27 x T1 (SO181Y x MO17), L16 x T2 (P28 x B73) and L21 x T2 (RO421W x B73) across all environments. This suggest that there is a possibility of obtaining superior hybrids adapted to the South African growing conditions by incorporating temperate germplasm into the South African maize hybrid programs. The area planted with temperate material in South Africa is generally very little. However, South Africa falls within subtropical to warm temperate conditions, which are comparable to Argentina in the Southern hemisphere. During the maize-growing season, most parts of the country particularly the main maize growing regions may resemble both tropical and temperate growing conditions. Therefore, both tropical and temperate material have potential for adaptation. The MO17 and B73 lines are good sources of germplasm in the temperate zones (Stojakovic *et al.*, 2010), hence, they could be utilised to generate tropical x temperate maize hybrids and to expand the level of variety adaptation in South Africa. These testers therefore have potential for contributing unique and favorable alleles that may be lacking in the South African maize germplasm.

6.4.2 Standard heterosis

The maize crop is increasingly grown as hybrids in most parts of the continent, newly developed hybrids must therefore be able to compete effectively with the commercial check hybrids for steady yields. Standard heterosis relative to the best check is therefore valued in maize grain yield improvements across different environmental conditions. Among five checks used in the study, WE3127, PAN6479, CAP9004, DKC8073 and SNK2147, the check DKC8073 was the best check under non-stress environments whereas WE3127 was the most superior under drought, low N stress and across environments. The two checks were therefore used to estimate standard heterosis for all experimental hybrids in the respective environmental conditions. Grain yield showed the highest heterosis under non-stress (23.7%) than drought (14.8%) and low N stress (12.9%) conditions, and the averages were -29.0%, - 31.9% and -35.9% under non-stress, drought and low N stress, respectively. High heterosis under non-stress than stress environments is attributed to the fact that, estimate of standard heterosis may be influenced by the level of stress, which may be associated with management practices (Tollenaar and Lee, 2006).

The highest heterosis recorded for hybrids L3 x T3 (FO215W x CML444) under non-stress and across environments, and L9 x T3 (I-42 x CML444) under both low N and drought is an indication of superior performance relative to the existing checks. These varieties were therefore the most superior than the standard checks and could be exploited further for commercial importance in improving maize yields under both stress and non-stress environments. Among hybrids that showed positive heterosis under non-stress environments, crosses L21 x T1 (RO421W x MO17) and L21 x T2 (RO421W x B73) were derived from temperate Corn Belt testes, MO17 and B73, indicating the usefulness of temperate material in improving heterosis in South African maize hybrids.

6.4.3 Heritability and variance components

Sufficient variability offers potential for maize genetic improvement. The observed variation in variance components suggest that the influence of the environmental factors in grain yield and most secondary traits was minimal. Genetic factors had a major role and therefore, improvement of the majority of traits may be possible through selection. The observations confirms the value of the new hybrids for cultivation and improving yields in low inputs and rainfed environments.

Conclusion

The study indicated the presence of substantial heterotic potential among varieties studied, and therefore the value of breeding and deploying new maize varieties with improved performance under low-input and water-limited environments. Hybrids that outperformed the standard checks were identified. The identified superior hybrids with high mean grain yield and positive heterosis for grain yield under non-stress were, L3 x T3 (FO215W x CML444), L21 x T2 (RO421W x B73), L21 x T1 (RO421W x MO17) and L19 x T4 (P612MSV x CML312), while under stress environments, the cross L9 x T3 (I-42 x CML444) was the most superior compared to the best check. These hybrids may be advanced and further exploited for improving yields under stress and non-stress environments. The study also revealed the possibility of generating tropical × temperate maize hybrids with high mean grain yields and good levels of adaptation to the maize growing regions of South Africa.

References

Acquaah, G. 2007. Principles of plant genetics and breeding. Blackwell publishing, USA, UK, Australia.

Banziger, M., G.O. Edmeades, and H.R. Lafitte. 1999. Selection for drought tolerance increases maize yields across a range of nitrogen levels. *Crop Science* 39: 1035-1040.

Banziger, M., G.O. Edmeades, and S. Quarrie. 1997. Drought stress at seedling stage: Are there genetic solutions? pp 348-354. In: Edmeades, G.O., Banziger, M., Mickelson, H.R, and C. B. Pena-Valvidia (eds.). Developing Drought and Low N Tolerant Maize. Proceedings of a Symposium, March 25-29, 1997. CIMMYT, El Batan, Mexico, D.F.

Banziger, M., G.O. Edmeades, D. Beck, and M. Bellon. 2000. Breeding for drought and Nitrogen stress tolerance in maize: From theory to practice. CIMMYT, Mexico, D.F.

Betran, F.J., D. Beck, M. Banziger, and G.O. Edmeades. 2003. Genetic analysis of inbred and hybrid grain yield under stress and nonstress environments in tropical maize. *Crop Science* 43: 807-817.

Bidhendi, M.Z., R. Choukan, F. Darvish, K. Mostafavi, and E. Majidi. 2012. Classifying of maize inbred lines into heterotic groups using diallel analysis. *World Academy of Science, Engineering and Technology* 6: 1161-1164.

Cairns, J.E., J. Crossa, P.H. Zaidi, P. Grudloyma, C. Sanchez, J.L. Araus, S. Thaitad, D. Makumbi, C. Magorokosho, M. Banziger, A. Menkir, S. Hearne, and G.N. Atlin. 2013. Identification of drought, heat, and combined drought and heat tolerant donors in maize (*Zea mays* L.). *Crop Science* 53: 1335-1346.

CIMMYT. 2001. Maize inbred lines released by CIMMYT: A Compilation of 454 CIMMYT Maize Lines (CMLs), CML1-CML454. CIMMYT, Mexico, D.F.

Derera, J., P. Tongoona, B.S. Vivek, and M.D. Laing. 2008. Gene action controlling grain yield and secondary traits in Southern African maize hybrids under drought and non-drought environments. *Euphytica* 162: 411-422.

Ertiro, B.T., Y. Beyene, B. Das, S. Mugo, M. Olsen, S. Oikeh, C. Juma, M. Labuschagne, and B.M. Prasanna. 2017. Combining ability and testcross performance of drought-tolerant maize inbred lines under stress and non-stress environments in Kenya. *Plant Breeding* 136: 197–205.

Hallauer, A.R., and J.B. Miranda. 1988. Quantitative genetics in maize breeding. 2nd ed. Iowa State University Press, Ames, IA.

Masuka, B., G.N. Atlin, M. Olsen, C. Magorokosho, M. Labuschagne, J. Crossa, M. Banziger, K.V. Pixley, B.S. Vivek, A. von Biljon, J. Macrobert, G. Alvarado, B.M. Prasanna, D. Makumbi, A. Tarekegne, B. Das, M. Zaman-Allah, and J.E. Cairns. 2017. Gains in maize genetic improvement in Eastern and Southern Africa: CIMMYT hybrid breeding pipeline. *Crop Science* 57: 168-179.

Meseka, S.K., A. Menkir, A.E.S. Ibrahim, and S.O. Ajala. 2013. Genetic analysis of maize inbred lines for tolerance to drought and low Nitrogen. *Jonares* 1: 29-36.

Miti, F. 2007. Breeding investigations of maize (*Zea mays* L.) genotypes for tolerance to low Nitrogen and drought in Zambia. PhD thesis, University of KwaZulu-Natal, Republic of South Africa.

Narayanam, S.S., and P. Phundan. 1993. Biometrical Techniques in Plant Breeding. Kayani Publishers, Ludhiana.

Ndhlela, T. 2012. Improvement strategies for yield potential, disease resistance and drought tolerance of Zimbabwean maize inbred lines. PhD Thesis, University of the Free State, South Africa.

Ogunniyan, D.J., and S.A. Olakojo. 2014. Genetic variation, heritability, genetic advance and agronomic character association of yellow elite inbred lines of maize (*Zea mays* L.). *Nigerian Journal of Genetics* 28: 24-28.

Oyekunle, M., B. Badu-Apraku, S. Hearne, and J. Franco. 2015. Genetic diversity of tropical early-maturing maize inbreds and their performance in hybrid combinations under drought and optimum growing conditions. *Field Crops Research* 170: 55-65.

Shull, G.F. 1952. Beginnings of the heterosis concept. In: Gowen, J.W (ed.). Iowa State College Press., Ames, IA.

Singh, R.K., and B.D. Chaudhary. 1985. Biometrical Methods in Quantitative Genetics Analysis. Kalyani Publishers, New Delhi.

Stojakovic, M., M. Ivanovic, G. Bekavac, and Z. Stojakovic. 2010. Grain Yield of B73 x Mo17-type maize hybrids from different periods of breeding. *Cereal Research Communications* 38: 440-448.

Tollenaar, M., and E. Lee. 2006. Dissection of physiological processes underlying grain yield in maize by examining genetic improvement and heterosis. *Maydica* 51: 399-408.

VSN International. 2016. GenStat for Windows 18*th* Edition. VSN International, Hemel Hempstead, UK. Web page: GenStat.co.uk.

Weber, V.S., A.E. Melchinger, C. Magorokosho, D. Makumbi, M. Banziger, and G.N. Atlin. 2012. Efficiency of managed-stress screening of elite maize hybrids under drought and low Nitrogen for yield under rainfed conditions in Southern Africa. *Crop Science* 52: 1011–1020.

Wynne, J., D. Emery, and P. Rice. 1970. Combining ability estimates in Arachis hypogaea L. II. Field performance of F1 hybrids. *Crop Science* 10: 713-715.

Zambezi, B.T., and C. Mwambula. 1997. The impact of drought and low soil Nitrogen on maize production in the SADC region. pp 29-33. In: Edmeades, G.O., Banziger, M., H.R. Mickelson, and C.B. Pena-Valvidia (eds.). Developing drought- and Low N-tolerant maize. Proceedings of a Symposium, March 25 – 29, 1996. CIMMYT, El Batan, Mexico, D.F.

CHAPTER 7

General discussion and research overview

7.1 Introduction

This chapter provides a general review of the research findings by highlighting the major objectives, implications of the findings and recommendations. The study focused on the following specific objectives:

I. To determine the alignment of South African public maize inbred lines to the public inbred lines testers from the tropical International Maize and Wheat Improvement Centre (CIMMYT) and the temperate USA Corn Belt sources.
II. To asses yield potential of single-cross experimental hybrids under non-stress, drought and low N stress conditions and determine combining ability and the mode of gene action conditioning the inheritance of grain yield under stress and non-stress environments.
III. To identify higher yielding and stable varieties across stress and non-stress environments and to identify ideal test environments.
IV. To determine the level of heterosis in maize grain yield under stress and non-stress environments.

7.2 General discussion and major findings

Heterotic orientation of South African public maize inbred lines towards tropical CIMMYT and temperate USA-Corn Belt testers

Assigning maize inbred lines into heterotic groups is an important aspect of hybrid breeding programs. The majority of the germplasm used in South Africa and by most of the national research systems (NARS) is obtained from CIMMYT. Following the CIMMYT A and B heterotic grouping scheme, thus simplify the breeding process. A small number of heterotic groups is desirable in breeding programs to optimise heterosis and to improve breeding efficiency (Fan *et al.*, 2014; Tams *et al.*, 2006).

From the results of the study, it was possible to simplify the heterotic grouping of public maize germplasm in South Africa on the basis of their orientation towards tropical CIMMYT and USA temperate Corn Belt testers. The SNP markers genotyping was highly effective in confirming classification of the germplasm collection in South Africa. The data obtained from SNP’s markers could be linked to the SCA effect-based heterotic orientation under non-stress environments. The SNP markers divided the germplasm lines into two principal clusters of USA Corn Belt temperate and the South African maize lines. The South African lines were more inclined towards the tropical CIMMYT than the USA temperate testers. The heterotic orientation of inbred lines demonstrated that the majority of inbred lines containing the Opaque-2 gene (QPM) clustered together into two groups, while the normal endosperm maize lines formed distinct groups based on temperate and tropical alignment. The I heterotic group placed in the closest cluster with both CIMMYT testers, CML444 and CML312, indicating that these lines have never been introgressed with temperate germplasm, and were more pro-tropical germplasm than the rest of the lines studies. Lines with a history of temperate introgression in the pedigree analysis were aligned towards the temperate lines. The groups obtained could therefore be simplified into two groups of quality protein maize and three groups of normal maize. Normal endosperm maize lines groups were formed by (i) a temperate group consisting of B73 and MO17, (ii) the I-group lines of South Africa that were closest to the tropical CIMMYT tester lines, (iii) and a group of pure South African lines showing no alignment with both tropical and temperate testers. The QPM lines formed two groups based on the origin of inbred lines.

The study further showed that inbred lines that originated from the same population did not always cluster together as observed in many previous studies (Barata and Carena, 2006; Fan *et al.*, 2003; Yu *et al.*, 2001). This was observed when lines originating from some of the groups such as the K and L heterotic group were expected to group together with the K and L group but were placed in the I group. These deviations indicate that the classification of South African lines in the whole K and L collection may not be presumed based on the clustering of individual lines, however confirmation of the entire groups with SNP genotyping is required. Moreover, lines including RO421W (M), K64 (K) and U2540W (K), which were expected to cluster with the M and K heterotic groups of South Africa were not associated with any of the South African lines, and they formed singleton clusters. They have also displayed a lack of association with neither the USA temperate and tropical CIMMYT lines. These lines qualified as representatives of distinct germplasm groups within the South African inbred lines collection. Clustering of lines using the SNPs markers was generally consistent with pedigree data, except in only a few cases. The inbred lines used in the study were representatives of the seven South African heterotic groups. Therefore, from the results obtained, the observed patterns may be used to deduce or make inferences of the classification of the South African inbred lines that were not included in the study, expect for a few groups which did not cluster as expected. Furthermore, there was a lack of phenotypic correlation between genetic distance with grain yield and specific combining ability, indicating that genetic distances did not efficiently predict hybrid performance and heterosis. For the South African germplasm collection, utility of genetic distances between inbred lines will only be valued in guiding selection of lines for use in crosses. To assess the performance of hybrids and determine the level of heterosis, conducting multi-environment trials is essential.

Combining ability and gene action for major traits in maize under stress and non-stress environments

Low Nitrogen (N) fertility and drought stress are the primary stresses responsible for low and unstable maize yields in South Africa. Smallholder farmers in marginal areas are more vulnerable because their maize crop is generally produced under low inputs and low soil moisture. Lack of cultivars that are adapted to these abiotic stresses worsen these effects. To attain maximum yield potential, developing drought and low N tolerant varieties is crucial. From the current study, it was revealed that hybrids that are tolerant to drought and efficient in use of soil N, and without yield penalties when conditions are optimum could be identified. The most superior hybrids, with high mean grain yield and favourable agronomic traits were FO215W x CML444 and I-42 x CML444). However, superior performance of FO215W x CML444 was associated with late maturity, implying that in the current study selection for lateness would be effective. The late maturing varieties accumulate and utilise photosynthetic products for a long period of time during the grain filling stage, while the early maturing hybrids grow rapidly to reach physiological maturity as a drought adaptive response associated with reduced yields (Banziger *et al.*, 2000). Contrarily, some studies have reported that high grain yield is linked to early maturity (Bello *et al.*, 2012), because late maturing varieties may carry yield penalties when exposed to prolonged periods of stress. Therefore, in pursuit of genetic improvements of low N and drought tolerance, understanding the physiological and developmental mechanisms of drought tolerance to biotic stresses is crucial for improving breeding strategies and facilitating breeding progress.

The study further showed that even though some varieties consistently performed well throughout non-stress and stress environments, for the majority, higher yielding varieties under non-stress conditions were not necessarily higher yielding under low N and drought conditions. Yet, most of the superior varieties under drought were also common under low N stress conditions because of the common adaptive mechanism. In most farmers' fields, both of these abiotic stresses generally occur simultaneously in the same growing season, thereby increasing correlation between drought and low N tolerance. In general, there is no significant correlation between hybrid ranking under non-stress and stress environments (Derera, 2005); this is due to differences in genes controlling grain yield under stress and non-stress environments (Souza *et al.*, 2009). Therefore, when breeding for drought and low N tolerance, varieties that are indirectly selected under non-stress conditions to improve GY performance under stress conditions might not be efficient under stress conditions. To obtain N efficient and drought tolerant varieties, developed hybrids should be selected directly under low N and as well as under low moisture conditions that are representatives of farmers growing conditions, or selection should be performed simultaneously and in several cycles under both stress and non-stress conditions. Hybrids derived from temperate testers were also identified among the superior hybrids. Including temperate material in hybrid development will thus improve maize yields, mainly under stress environments.

Low N and drought tolerance are complex polygenic traits; to understand the genetic control of these abiotic stresses, the inheritance of grain yield was studied. In the current study, additive gene effects were more important in controlling grain yield under drought and non-additive effects were important under low N and non-stress environments. Previous reports also found that additive gene effects are more important than non-additive genetic effects in conditioning the inheritance of GY particularly under drought conditions (Annor and Badu-Apraku, 2016; Ertiro *et al.*, 2017; Miti, 2007). Additive gene action is linked to high heritability indicating that GY can be easily improved through selection; therefore, in the current study, tolerance to abiotic stress can be rapidly improved through selection of superior inbred lines showing good performance under these stresses. In some studies, contradicting findings on the mode of gene action were reported, for example, Ndhlela (2012) reported the role of non-additive gene action in the inheritance of GY under both drought and low N tolerance. Other investigators reported the importance of non-additive effects only under low N (Betran *et al.*, 2003; Makumbi, 2005; Medici *et al.*, 2004; Meseka *et al.*, 2013) than drought environments, indicating varying response of genotypes to low N and drought stress. Thus, conclusions regarding the predominance of either additive or non-additive gene action in the expression of GY and major traits stress environments are based on the type of germplasm and test environments used.

In addition to genetic effects, the study also revealed that the contribution of lines (maternal effects) and testers (paternal effects) in the inheritance and expression of traits varied in magnitudes depending on the trait and conditions in which the traits were measured. These observations reaffirm the conclusion made by Derera *et al.* (2008), where the importance of females and males varied with traits and environments. The study showed that maternal effects were more significant in controlling grain yield across stress and non-stress environments, indicating that when selecting parents for yield improvement under different conditions, the choice of parents for use as female parents must be carefully considered.

Inbred lines including line 2 (FO215W), 4 (I-42), 3 (I-16) and line 6 (K64) showed desirable GCA effects for most traits; these lines thus showed potential for utilisation as new suitable tester lines for developing early to medium maturing hybrids for use in different environmental conditions. Some single crosses such as L2 x T4 (FO215W x CML444) and L4 x T4 (I-42 x CML444) could also qualify as potential single cross testers. The choice of potential lines as inbred and single cross testers has been made based on various characteristics including display of good GCA effects for GY and other traits and good yield (Ertiro *et al.*, 2017; Pswarayi and Vivek, 2008; Rajendran *et al.,* 2014). However, in this study, further assessment of the identified potential testers is essential to verify the general attributes of a good tester and to best recommend for use in a breeding program. The study further showed that some inbred lines may not show good general combining ability when compared to a series of lines, but when used in a cross with a superior inbred line, a good SCA in a cross particularly for GY might be possibly expressed if the superior parent is properly selected. These findings suggested that the performance of a particular inbred line might or might not be always judged by its general combining ability. For example, high SCA effects could be derived from cross combinations including (i) good × good GCA effects, (ii) poor × poor and (iii) good × poor GCA effects. These three combinations were respectively attributed to (i) additive × additive gene action, (ii) dominance by dominance type of non-allelic gene action, which produces overdominance in crosses and (iii) favourable additive effects of a good general combining parent and epistatic effects of a poor combiner (Fasahat *et al.*, 2016). Thus, suggesting that good SCA of parents does not necessarily result from a combination of two superior general combiners, as different gene actions may also contribute to the improvement and expression of a particular trait. Inbred lines with poor GCA effects should therefore not always be quickly eliminated from the gene pool before extensive screening.

Genotype by environment interaction and yield stability of newly developed maize hybrids across stress and non-stress environments

Maize yields generally vary from region to region due to genotype × environment interaction, identifying higher yielding and stable genotypes for grain yield across environments in fundamental for improving maize productivity across many production areas. From the current study, it was shown that the test environments could be delineated into two mega-environments in season 1 and three mega-environments in season 2. This indicated the possibility of targeting fewer environments for evaluating and selecting superior genotypes. The best environments that were discriminating among genotypes and representative of all test environments were Potchefstroom (non-stress) and Vaalharts (low N and drought) in season 1 and 2, respectively. In season 1, stress environments mainly formed the first mega environment and the second cluster was formed by non-stress environments. Similar observations on delineations by stress and non-stress environments were made by Ertiro *et al.* (2017). These findings suggested that some environments that were least discriminating within a mega environment could be dropped without losing useful information regarding the performance of genotypes. In season 2, a combination of stress and non-stress environments within one mega environment indicated the need for confirming mega-environments. However, it was clearly revealed that among all test environments, the environment Vaalharts is suitable for evaluating and selecting superior genotypes for stress tolerance, while Potchefstroom is suited for non-stress trials. These environments must therefore be always included when evaluating genotypes. However, tests environments and subsequently mega environments are best and accurately recommended when the genotype by location interaction pattern for the trait in repeatable (Yan *et al*., 2014). Therefore, in this study, the observed G × E interaction pattern was not repeatable and thus requires confirmation, for meaningful recommendations of test environments and genotypes.

Potential hybrids with stable performance were identified; two hybrids were identified in season 1, those were L7 x T3 (MO17HtHtN x CML444) and L2 x T3 (I-39 x CML444). Three were identified in season 2; L2 x T4 (FO215W x CML444), L4 x T4 (I-42 x CML444) and L17 x T4 (U71Y x CML444) were stable genotypes. The hybrid I-42 x CML444 was the most superior in close proximity to the ideal cultivar in GGE biplot analysis, and the most stable. Hybrids L13 x T3 (U71Y x CML444) in season 1 and L11 x T3 (RO452W x CML312) in season 2 were higher yielding but unstable, these varieties may be best suited as potential hybrids for cultivation in specific regions. Further testing of these hybrids in multi-locations and seasons is required to verify their suitability for wide or narrower adaptation, and for release in different SA maize growing regions. Hybrid containing temperate USA Corn Belt testers, MO17 and B73 were least stable than those with tropical CIMMYT testers. Differences in environmental conditions, mainly the presence of stress could have influenced the performance of these genotypes, however they still showed potential yield in their crosses mostly under non-stress environments.

Heterosis in maize grain yield under stress and non-stress environments

Heterosis (hybrid vigor) is the key phenomenon in the expression of grain yield and favorable agronomic traits in new varieties. The performance of new varieties is generally evaluated relative to the existing commercial check hybrids; the estimate of standard heterosis thus determines the value of new varieties in commercialisation and whether they should be retained in a breeding program for further testing in multi-environmental trials. Standard heterosis therefore has practical importance and economic value in the breeding program (Hundera *et al.*, 2017). The findings of the study revealed that among hybrids studied, five hybrids had competitive yields relative to the standard checks and therefore have potential for further evaluation and commercialisation. Hybrid I-42 x CML444 was the most superior with high heterosis and high mean yield under stress environments while FO215W x CML444, RO421W x B73, RO421W x MO17 and P612MSV x CML312 performed well under non-stress environments. Given that South Africa falls within subtropical to warm temperate conditions, which are comparable to Argentina in the Southern hemisphere, the study also demonstrated that temperate testers MO17 and B73 have potential for improving mean yield and heterosis in the South African maize hybrids. To consider these testers in hybrid development will introduce new favourable alleles. Moreover, exploiting temperate germplasm with broad genetic base will expand the level of adaptation in new varieties. Overall, results indicated that there are possible genetic improvements of maize hybrids for stress tolerance and further increase in maize yields under climate change. The identified varieties require further testing in multi-environments and seasons to confirm their suitability for registration and release.

7.3 General conclusions

- The heterotic grouping of public maize germplasm in South Africa could be simplified into two broader groups based on biochemical composition of the grain, the identified groups were quality protein maize and normal endosperm maize, which were further divided into two and three groups, respectively.

- Additive gene effects were more important in controlling grain yield under drought, while non-additive were predominant under low N and non-stress environments.
- Hybrid I-42 x CML444 was the most superior with high heterosis and high mean yield under stress environments while FO215W x CML444, RO421W x B73, RO421W x MO17 and P612MSV x CML312 performed well under non-stress environments.
- Hybrids, I-42 x CML444 and FO215W x CML444 were higher yielding and stable, with I-42 x CML444 being the most ideal.
- The best environments for testing and discriminating genotypes under non-stress and stress conditions were Potchefstroom and Vaalharts, respectively.
- Inbred lines FO215W, I-16, I-42, K64 and single crosses FO215W x CML444 and I-42 x CML444 displayed desirable characteristics of potential inbred line and single cross testers for use in hybrid development.
- The temperate Corn Belt testers, MO17 and B73 showed potential for improving mean grain yield and heterosis in hybrids, mainly under non-stress environments, however, they were least stable in crosses.

7.4 General recommendations

- Some deviations were observed in the classification of a few lines from heterotic groups L and K of South Africa; classification of South African lines in the whole collection (L and K) requires confirmation with SNP genotyping.
- Further testing of the new varieties is recommended to validate and sustain future genetic gains in maize yields across different maize growing conditions of South Africa.
- Further assessment of the identified potential testers is necessary to verify their suitability for use as new testers in the South African maize breeding program.
- The observed G × E pattern requires confirmation, for meaningful recommendations of test environments and genotypes.
- Incorporating temperate germplasm into the South African maize breeding programs should be considered for introducing favourable alleles and improving variety performance.

References

Annor, B., and B. Badu-Apraku. 2016. Gene action controlling grain yield and other agronomic traits of extra-early quality protein maize under stress and non-stress conditions. *Euphytica* 212: 213–228.

Banziger, M., G.O. Edmeades, D. Beck, and M. Bellon. 2000. Breeding for drought and Nitrogen stress tolerance in maize: From theory to practice. CIMMYT, Mexico, D.F.

Barata, C., and M. Carena. 2006. Classification of North Dakota maize inbred lines into heterotic groups based on molecular and testcross data. *Euphytica* 51: 339-349.

Bello, O.B., S.Y. Abdulmaliq, S.A. Ige, J. Mahamood, F. Oluleye, M.A. Azeez, and M.S. Afolabi. 2012. Evaluation of early and late/intermediate maize varieties for grain yield potential and adaptation to a Southern Guinea savanna agro-ecology of Nigeria. *Scholarly Journal of Agricultural Science* 2: 42-51.

Betran, F.J., D. Beck, M. Banziger, and G.O. Edmeades. 2003. Genetic analysis of inbred and hybrid grain yield under stress and nonstress environments in tropical maize. *Crop Science* 43: 807-817.

Derera, J., P. Tongoona, B.S. Vivek, and M.D. Laing. 2008. Gene action controlling grain yield and secondary traits in Southern African maize hybrids under drought and non-drought environments. *Euphytica* 162: 411-422.

Ertiro, B.T., Y. Beyene, B. Das, S. Mugo, M. Olsen, S. Oikeh, C. Juma, M. Labuschagne, and B.M. Prasanna. 2017. Combining ability and testcross performance of drought-tolerant maize inbred lines under stress and non-stress environments in Kenya. *Plant Breeding* 136: 197–205.

Fan, X.M., J. Tan, H.M. Chen, and J.Y. Yang. 2003. Heterotic grouping for tropical and temperate maize inbreds by analysing combining ability and SSR markers. *Maydica* 48: 251-257.

Fan, X.M., Y.D. Zhang, W.H. Yao, Y.Q. Bi, L. Liu, H.M. Chen, and M.S. Kang. 2014. Reciprocal diallel crosses impact combining ability, variance estimation, and heterotic group classification. *Crop Science* 54: 89–97.

Fasahat, P., A. Rajabi, J.M. Rad, and J. Derera. 2016. Principles and utilization of combining ability in plant breeding. *Biometrics & Biostatistics International Journal* 4: 1-24.

Hundera, N.B., B. Abate, and M. Nigussie. 2017. Standard Heterosis of Maize (*Zea mays* L.) Inbred Lines for Grain Yield and Yield Related Traits at Southern Ethiopia, Hawassa. *American-Eurasian Journal of Agriculture & Environtal Science* 17: 257-264.

Makumbi, D. 2005. Phenotypic and genotypic characterization of white maize inbreds, hybrids and synthetics under stress and non-stress environments. PhD thesis. Texas A&M University.

Medici, L.O., M.B. Pereira, P.J. Lea, and R.A. Azevedo. 2004. Diallel analysis of maize lines with contrasting responses to applied Nitrogen. *Agricultural Sciences* 142: 535-541.

Meseka, S.K., A. Menkir, A.E.S. Ibrahim, and S.O. Ajala. 2013. Genetic analysis of maize inbred lines for tolerance to drought and low Nitrogen. *Jonares* 1: 29-36.

Miti, F. 2007. Breeding investigations of maize (*Zea mays* L.) genotypes for tolerance to low Nitrogen and drought in Zambia. PhD thesis. University of KwaZulu-Natal, South Africa.

Ndhlela, T. 2012. Improvement strategies for yield potential, disease resistance and drought tolerance of Zimbabwean maize inbred lines. PhD Thesis. University of the Free State, South Africa.

Souza, A.R.R., G.V. Miranda, P.M. Gonzaga, and L.V. de Souza. 2009. Predicting the genetic gain in the Brazilian white maize landrace. *Ciencia Rural* 39: 19-24.

Tams, S.H., E. Bauer, G. Oettler, A.E. Melchinger, and C.C. Schon. 2006. Prospects for hybrid breeding in winter triticale: II. Relationship between parental genetic distance and specific combining ability. *Plant Breeding* 125: 331-336.

Yan, W., J. Fregeau-Reid, R. Martin, D. Pageau, and J. Mitchell-Fetch. How many test locations and replications are needed in crop variety trials for a target region?. *Euphytica* 202: 361-372.

Yu, J., H. Lu, and R. Bernardo. 2001. Inconsistencies between SSR grouping and genetic background of white corn inbreds. *Maydica* 46: 133-139.