Improvement strategies for yield potential, disease resistance and drought tolerance of Zimbabwean maize inbred lines

By

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Declaration

I declare that the thesis hereby submitted by me for the degree Philosophiae Doctor in Agriculture at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I further cede copyright of the thesis in favour of the University of the Free State.

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Thokozile Ndhlela Date

Dedication

To my late husband (Solomon), my sons (Eugene, Ginola, Winstone and Munashe), my father (Moses) and mother (Lillian).

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Abbreviations and symbols

AEC	An environment coordination
AFLP	Amplified fragment length polymorphism
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
ART	Agricultural Research Trust
bp	Base pairs
CA	Communal area
CIMMYT	International Maize and Wheat Improvement Center
cm	Centimetre (s)
CML	CIMMYT maize line
COI	Crossover interaction
CRS	Chiredzi Research Station
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
DR&SS	Department of Research and Specialist Services
E	Environment
EDTA	Ethylenediaminetetra acetate
ET	*Exhollium turcicum*
E x Y	Environment by year interaction
F_1	First filial generation
F_2	Second filial generation
F_3	Third filial generation
FAM	6-carboxyfluorescein
FAO	Food and Agriculture Organisation
FRET	Fluorescence resonance energy transfer
g	Gram (s)
G	Genotype
GA	Genetic advance
GCA	General combining ability
GCA_f	General combining ability due to females

GCA_m	General combining ability due to males
GCV	Genotypic coefficient of variation
GD	Genetic distance
G x E	Genotype by environment interaction
G x L x Y	Genotype by location by year interaction
GGE	Genotype and genotype by environment interaction
GLS	Grey leaf spot
G x Y	Genotype by year interaction
ha	Hectare (s)
h^2_B	Broad sense heritability
HP	High-parent
HPH	High-parent heterosis
H_2O	Water
HRS	Harare Research Station
IITA	International Institute of Tropical Agriculture
IPCA	Interaction principal component analysis
KASPar	KBioscience competitive allele-specific polymerase chain reaction
kg ha^{-1}	Kilogram per hectare
kg ha^{-1} yr^{-1}	Kilogram per hectare per year
KRI	Kadoma Research Institute
LSCFA	Large scale commercial farmers
m	Metre (s)
MARS	Marker-assisted recurrent selection
MAS	Marker-assisted selection
masl	Metre (s) above sea level
Max	Maximum
MET	Multi-environment trial data
MEYT	Multi-environment yield trial
$MgCl_2$	Magnesium chloride
Mg ha^{-1} $cycle^{-1}$	Megagram (s) per hectare per cycle
Min	Minimum

min	Minute
ml	Millitre
mm	Millimetre (s)
mM	Millimolar (s)
MP	Mid-parent
MPH	Mid-parent heterosis
MSV	Maize streak virus
MT	Metric ton
N	Nitrogen
NaCl	Sodium chloride
NARS	National Agriculture Research Systems
NCDII	North Carolina Design II
ng	Nanogram (s)
NPPES	Natal Potchefstroom Pearl Elite Selection
OPV	Open pollinated variety
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCV	Phenotypic coefficient of variation
pH	Soil acidity or alkalinity
PIC	Polymorphic information content
ppm	Parts per million
QTL	Quantitative trait loci
r	Pearson correlation coefficient
R^2	Coefficient of determination
RAPD	Random amplified polymorphic DNA
RARS	Rattray Arnold Research Station
RFLP	Restriction fragment length polymorphism
ROX	6-Carboxyl-X-Rhodamine, succinimdyl ester
rpm	Revolutions per minute
SAHN	Sequential agglomerative hierarchical nested cluster analysis

SC	Southern Cross
SCA	Specific combining ability
sec	Second (s)
SNP	Single nucleotide polymorphism
SREG	Site regression
SSR	Simple sequence repeat
SVD	Singular value decomposition
SV	Singular value
t ha^{-1}	Ton per hectare
Taq	*Thermus aquaticus*
TE	Tris/EDTA
Tris	2-amino-2-hydroxymethylpropane-1,3-diol
UK	United Kingdom
UPGMA	Unweighted pair-group method with arithmetic averages
USA	United States of America
v/v	Percent volume by volume
VIC	2´chloro-7´-phenyl-1,4-dichloro-6-carboxyfluorescein
w/v	Percent weight by volume
Y	Year
σ^2_g	Genotypic variance
σ^2_p	Phenotypic variance
σ^2_e	Error variance
$\sum$	Summation
%	Percent
µl	Microlitre
°C	Degrees Celcius

CHAPTER 1

General introduction

1.1 Importance of maize and production constraints in Africa

In eastern, central and southern Africa, maize (*Zea mays* L.) is the major staple food crop cultivated and consumed by most households. Approximately a quarter of a billion Africans depend on maize as their staple food and they eat on average a quarter of a kilo or more maize and maize products every day (African Press Agency, 2007). The successful and continuous production of maize is key to global food security (Edmeades *et al*., 2000) and any change leading to reduced production and subsequently reduced deliveries to the markets, result in hunger especially in the disadvantaged communities (African Press Agency, 2007). The two main abiotic stress factors that have hindered agricultural production in the past include drought stress and poor soil fertility (Beck *et al*., 1996) and will continue to have large negative effects on agricultural production in the coming years, mostly in Asia and Africa (Rijsberman, 2006). The Food and Agricultural Organisation (FAO) estimated that sub-Saharan Africa is the most severely affected region where almost half of the land surface is exposed to a high risk of meteorological drought (Ribaut *et al*., 2004). This effect is intensely influenced by continuing changes in the global climate (Hillel and Rosenzweig, 2002). As water continues to be a limiting factor in crop cultivation, breeding for drought tolerant genotypes becomes more and more imperative. Public and private plant breeders endeavour to incorporate breeding for abiotic stress tolerance into their breeding objectives in order to produce stress tolerant hybrids and open pollinated varieties.

1.2 Maize production in Zimbabwe

Maize is the principal food crop and is the main source of carbohydrates for the majority of the Zimbabwe populace. The country requires 1.8 million ton of maize for human and animal consumption and 300 000 ton as national strategic reserves per annum. It is produced by large and small scale commercial farmers for both food (grain and fresh green maize) and livestock feed (grain and silage). The requirement is divided into the following proportions; 64% for human consumption, 22% for livestock and poultry feed and 14% for other industrial uses (Mashingaidze, 2006). Zimbabwe's maize production trends are characterised

by extreme variability associated with the incidence of mid-season dry spells and high small scale contribution to national production (Figure 1.1a and 1.1b). It also varies annually according to input support programmes. As shown in Figure 1.2, national production has been oscillating up and down due to various constraints that farmers faced over the years. After 2001 the area under production continued to increase with the land reform programme but on the other hand production remained low (Figure 1.2). The communal sector continues to be the main producer of maize in the country (Figure 1.1a and 1.1b). These farmers are faced with many challenges such as biotic and abiotic stresses, unavailability of inputs and poorly adapted varieties. In the 2009/10 season the sector contributed 40% of the national production, whilst in 2010/11 it contributed 43%. Of the total land area in Zimbabwe approximately 50% is communal farming area, where about 70% of the population lives with an average of 2 ha per household set aside for crop cultivation.

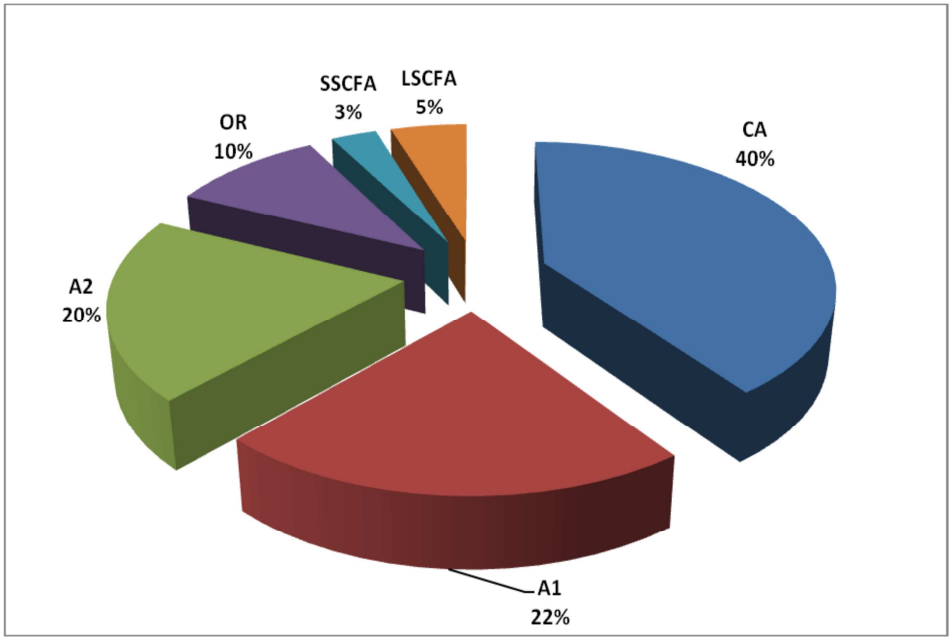

Figure 1.1a Sector contribution to national maize production in Zimbabwe in 2009/10 season.

OR=old resettlement; SSCFA=small scale commercial farmers; LSCFA=large scale commercial farmers; CA= communal area; A1 and A2=newly resettled under land reform programme.
Source: AGRITEX Crop and Livestock Assessment Report, 2011.

While commercial maize production increased by two thirds between 1979 and 1985, small scale production more than tripled (Rohrbach, 1989). The increase in small scale area under

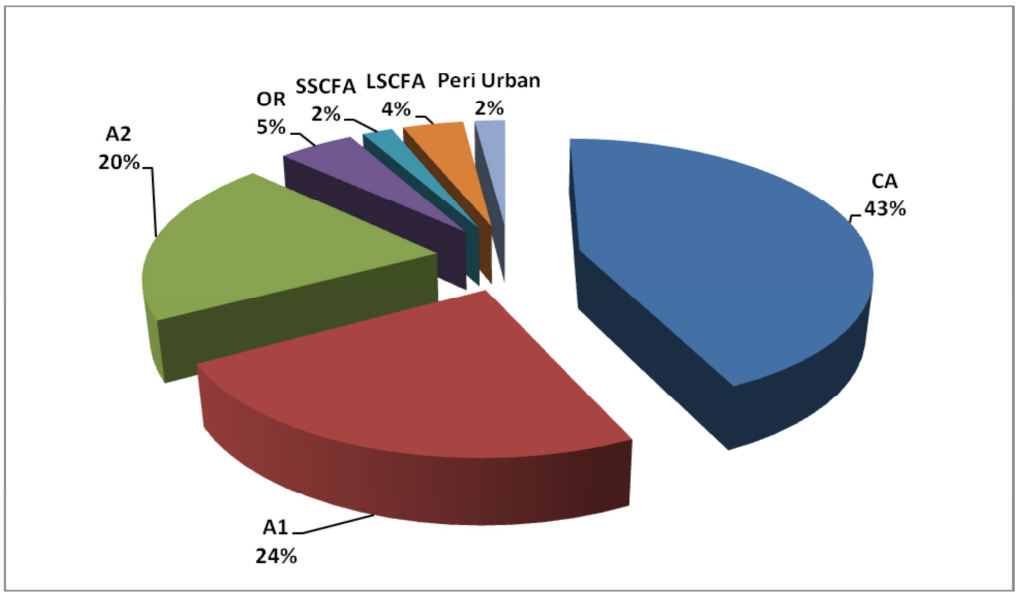

Figure 1.1b Sector contribution to national maize production in Zimbabwe in 2010/11 season.

OR=old resettlement; SSCFA=small scale commercial farmers; LSCFA=large scale commercial farmers; CA= communal area; A1 and A2=newly resettled under land reform programme.
Source: AGRITEX Crop and Livestock Assessment Report, 2011.

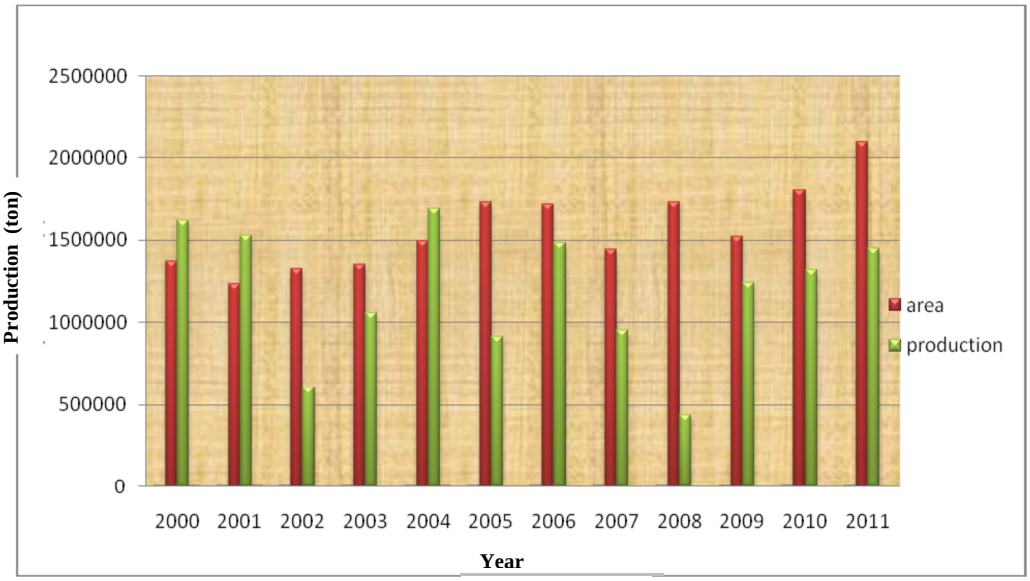

Figure 1.2 Maize production trends in Zimbabwe from 2000-2011.

Source: AGRITEX Crop and Livestock Assessment Report, 2011.

maize production was due to rapid expansion of government and private sector support for small scale farmers after independence, major investments in market infrastructure, expansion of a new smallholder credit programme, improved extension assistance and higher maize prices (Rohrbach, 1989). Announcement by the Zimbabwe government in late 1986 of a pre-planting producer price cut of 35% for deliveries of more than 91 metric ton (MT) saw a 50% reduction in maize area planted by the large scale commercial sector, whilst the small scale maize area remained roughly constant (Rohrbach, 1989). Small scale farmers had effectively been granted primary responsibility for the production and supply of the nation's main staple.

In effect, the post-1979 surge in small scale production transformed the communal sector from a relatively minor participant in the national maize economy to the principal source of national production growth (Rohrbach, 1989). Hence the variability in national maize production levels has increased with the growth of small scale maize production. Zimbabwe is currently facing some of the largest fluctuations in cereal grain production of any country in Africa. The 2010/11 maize production was estimated at 1 451 629 MT, from an area of 2 096 035 ha and an average yield of 0.69 t ha^{-1} (AGRITEX Crop and Livestock Assessment Report, 2011). The production estimate was about 9% more than the 2009/10 production estimate of about 1 327 572 MT. The maize area, yield estimate and production per province are presented in Table 1.1. Mashonaland West had the highest and Matebeleland South the lowest production. Generally high potential maize producing areas did not experience severe dry spells, whereas the southern parts of the country namely Masvingo, Matebeleland North, Matebeleland South and some parts of Manicaland, Midlands and Mashonaland East and Central were affected by severe mid-season dry spells, which adversely affected production in these areas (AGRITEX Crop and Livestock Assessment Report, 2011). There was an increase in yield estimates from the 2009/10 to 2010/11 for large scale commercial farmers (LSCFA), whilst for the rest of the sectors there was either a decrease or no change at all (Figure 1.3). The yield for communal area (CA) remained below 0.5 t ha^{-1} for both seasons and yet this is the sector contributing a large percentage to the total national production.

Table 1.1 Maize area, yield and production for the 2010/11 season as compared with the 2009/10 season

Province	Area (ha)			Production (MT)			Yield t ha^{-1}		
	2010/11	2009/10	% change	2010/11	2009/10	% change	2010/11	2009/10	% change
Manicaland	262 106	237 052	11	159 885	118 658	35	0.61	0.50	22
Mash Central	231 814	179 839	29	296 722	223 516	33	1.28	1.20	7
Mash East	247 511	243 995	1	148 507	181 994	-18	0.60	0.70	-14
Mash West	379 066	263 621	44	451 089	336 855	34	1.19	1.30	-8
Masvingo	276 105	229 887	20	80 070	56 201	43	0.29	0.20	45
Mat North	166 265	100 936	65	79 807	73 311	9	0.48	0.70	-31
Mat South	148 922	139 643	7	35 741	58 290	-39	0.24	0.40	-40
Midlands	384 246	408 569	-6	199 808	278 747	-28	0.52	0.70	-25
Total	**2 096 035**	**1 803 542**	**16**	**1 451 629**	**1 327 572**	**9**	**0.69**	**0.70**	**-1.4**

Mash=Mashonaland; Mat=Matebeleland.
Source: AGRITEX Crop and Livestock Assessment Report, 2011.

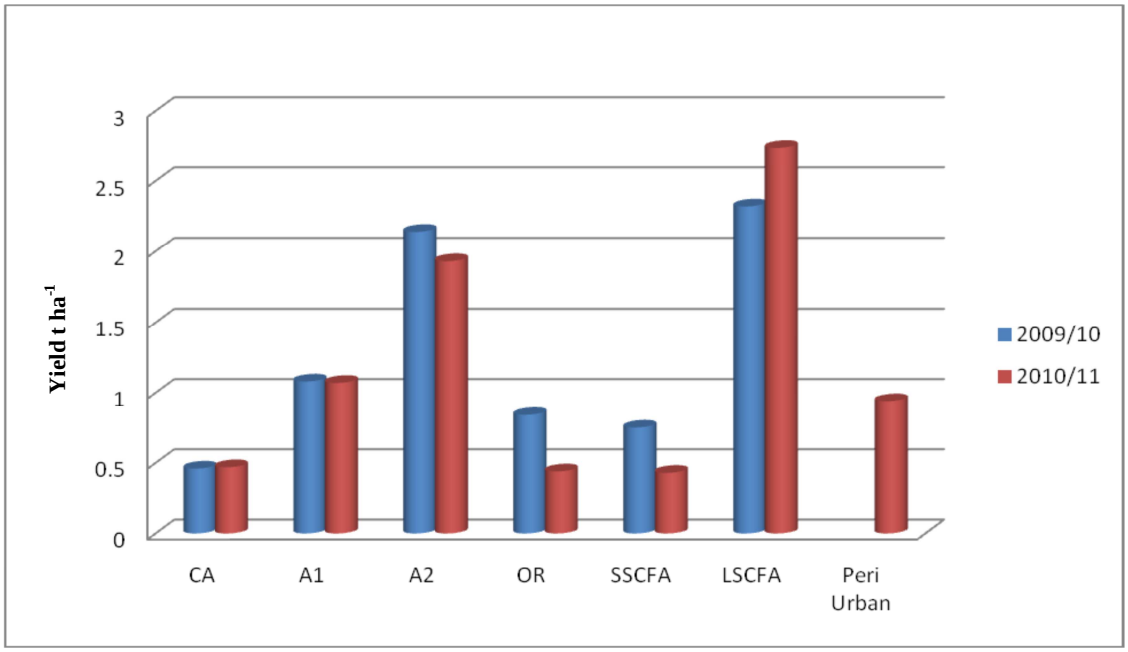

Figure 1.3 National yield comparison per sector in the 2009/10 and 2010/11 seasons in Zimbabwe.
OR=old resettlement; SSCFA=small scale commercial farmers; LSCFA=large scale commercial farmers; CA=communal area; A1 and A2=newly resettled under land reform programme.
Source AGRITEX Crop and Livestock Assessment Report, 2011.

1.3 Maize production constraints in Zimbabwe

The communal farmers are faced with a lot of challenges, amongst them the occurrence of dry spells, as they are mostly located in the drier parts of the country. The main maize production constraints in Zimbabwe include drought stress, low soil fertility and susceptibility to current major diseases. Downing (1992) found that with a temperature increase of 2ºC the wet regions of Zimbabwe (with a water surplus) declined by a third from 9% to about 2.5% and that the drier regions will be double in area in future. A further increase in temperature to 4ºC reduced the summer water surplus zones to less than 2% and a similar scenario was observed in the 1991/92 season drought (Downing, 1992). About 160 million ha of maize is grown under rain-fed conditions globally and annual yield losses attributed to drought are estimated at around 25% (Edmeades, 2008). The losses are expected to be greater in sub-tropical countries that rely on unpredictable and erratic rainfall (Mhike *et al*., 2011). When sub-Saharan Africa's recurrent droughts ruin harvests, lives and livelihoods are threatened and even destroyed and Zimbabwe is not exempted from these droughts. Maize is affected by drought mainly through reduction of the growing season and through erratic stress that occurs at any time during the growth of the crop. Annual maize production in Zimbabwe ranges between 1.8 to 2.1 million ton with an average yield of 1.2 t ha^{-1} in the small scale sector and 4.5 t ha^{-1} in the large scale commercial sector (Mhike *et al*, 2011).

International Maize and Wheat Improvement Center (CIMMYT) and International Institute for Tropical Agriculture (IITA), working closely with various partners in sub-Saharan Africa, have developed drought tolerant varieties that have benefited farmers, especially those located in the drier regions. Farmers realise higher economic returns to labour, other inputs and land with the use of drought tolerant varieties. Therefore development of new drought tolerant genotypes can contribute to food security worldwide. New drought tolerant maize varieties play a major role in alleviating the effects of drought that are expected to increase due to global warming. In developing countries maize is also grown under low nitrogen (N) conditions (McCown *et al*., 1992; Oikeh and Horst, 2001) and this is due to restricted N use and low N uptake in drought susceptible areas, high price ratios between fertiliser and grain, scarcity of fertiliser or lack of credit for farmers (Banziger *et al*., 1997). N availability is estimated to be the principal limiting factor in more than 20% of arable land (Lafitte and

Edmeades, 1988). N deprivation hastens senescence of lower leaves (Wolfe *et al*., 1988; Moll *et al*., 1994), reduces radiation use efficacy (Uhart and Andrade, 1995) and prolongs anthesis silking interval (Jacobs and Pearson, 1991; Edmeades *et al*., 2000). These factors result in maize being barren and eventually reduced yields.

Maize diseases of economic importance in Zimbabwe include maize streak virus (MSV), grey leaf spot (GLS) and *Exhollium turcicum* (Leornard and Snuggs) (ET). MSV is predominantly a disease of maize in Africa and the most devastating, although it has also been reported in South and South East Asia (Shepherd *et al*., 2007). According to researchers at IITA the disease was discovered in South Africa in 1901 (Shepherd *et al*., 2007). In Zimbabwe the disease is most prevalent in irrigation schemes although it can also be found in rain-fed crops and more specifically if the crop is planted late. MSV is transmitted by a species of leaf hoppers belonging to the genus *Cicadulina.* The leaf hoppers are minute and whitish in colour with a shape of an adult cockroach when observed under a magnifying lens (CIMMYT, 2004). Maize yield losses attributed to MSV vary from season to season and losses usually depend on the number of plants that are infected with the disease and the crop's growth stage when the infection took place. Approximately 50% of calories in local diets emanate from maize, therefore yield losses attributed to MSV result in starvation and overall food insecurity (Shepherd *et al*., 2007). Yield losses due to MSV range from close to zero to nearly 100% (Stevens, 2008).

Another major disease causing yield losses in maize worldwide is grey leaf spot (GLS). The disease is caused by the fungus *Cercospora zea-maydis* (Tehon and E.Y. Daniels). The occurrence of the pathogen in KwaZulu-Natal, South Africa, in the late 1980s was its first official report from the African continent and has since become pandemic, causing yield losses of up to 60% (Ringer and Grybauskas, 1995). The ideal conditions that are conducive for early season lesions and more severe disease attack include high early season rains and prolonged periods of high humidity between November and December (Ringer and Grybauskas, 1995). However, late season infections have been found to be more serious because they affect the upper canopy which contributes 75-90% of the photosynthate for grain filling (Allison and Watson, 1966).

1.4 Zimbabwe national maize breeding programme

Since its inception in 1909, the Zimbabwe National Maize Breeding Programme has managed to develop high performance germplasm adapted to tropical and mid-altitude growing regions roughly extending from 1 000-1 800 m above sea level (masl) and less than 23º from the equator (Doswell *et al*., 1996). Hybrid breeding in Zimbabwe started in 1932 and it was based on the populations Southern Cross, Salisbury White and to a lesser extent Hickory King (Olver, 1988). The Southern Rhodesian Department of Agriculture imported Hickory King, which was among a group of high yielding United States of America (USA) open pollinated varieties and distributed it to farmers between 1900 and 1905 (Weinmann, 1972). A commercial single cross hybrid SR52 based on inbred lines SC5522 (SC from Southern Cross) and N3-2-3-3 (N3 from Salisbury White) was released in 1960 (Doswell *et al*., 1996).

Lines based on combining ability groups developed from material related to SC, N3 and K64r/M162W are now the main components of hybrid breeding efforts by a majority of national breeding programmes in eastern and the majority of southern African countries. K64r originated from Kansas and is a direct import from the USA, whilst M162W is an improved version of K64r (Mickelson *et al*., 2001). Gene introgression has been done in the national programme using germplasm mainly from CIMMYT, IITA and other National Agriculture Research Systems (NARS). Some authors have emphasised the use of exotic germplasm to widen the genetic base of germplasm used by maize breeders (Beck *et al*., 1991; Vasal *et al*., 1992; Ron Para and Hallauer, 1997). Introducing exotic germplasm is often suggested as a method to increase genetic diversity between populations in opposite heterotic groups, thereby increasing the magnitude of heterosis.

In Zimbabwe, the predominant maize inbred lines used in the most successful and current commercial hybrids and their derivatives were developed in the last century. Breeding gains have not been significant in the National Breeding Programme in the last few years mainly because of inadequate funding. As such, yield potential, disease resistance and drought stress tolerance are lacking in most of the current maize germplasm in the National Breeding Programme. There is thus an urgent need to improve the current elite lines used by the

National Breeding Programme to boost their yield potential and at the same time introduce resistance to current major diseases and general tolerance to drought stress.

Gene introgression of drought tolerance and disease resistance genes from CIMMYT germplasm into the National Breeding Programme elite inbred lines has been initiated at the Department of Research and Specialists Services (DR&SS) in Zimbabwe. In order to determine the best parents for this project, it is important to understand the heterotic relationships between the CIMMYT and National Breeding Programme lines with a view to selecting good parents to initiate crosses for pedigree, backcross and potential marker-assisted recurrent selection (MARS) populations for line extraction. The resultant new lines will then be used in developing new improved drought tolerant and disease resistant hybrids and open pollinated varieties (OPVs) for release. In addition, classification of inbred lines into heterotic groups will facilitate exploitation of heterosis which can contribute to hybrid performance.

1.5 Overall objective

The overall objective of the study was to identify improvement strategies for yield potential and tolerance to biotic and abiotic stress factors of Zimbabwean maize inbred lines

1.6 Specific objectives

(i) To estimate combining ability and heterosis for grain yield and other agronomic traits between DR&SS and CIMMYT white maize inbred lines under stress and non-stress environments

(ii) To analyse genotype by environment (G x E) interaction and stability of single cross hybrids for grain yield

(iii) To examine genetic diversity among DR&SS and CIMMYT white maize inbred lines using single nucleotide polymorphism (SNP) analysis and morphological traits

(iv) To assess the relationship between genetic diversity of DR&SS and CIMMYT parental inbred lines and F_1 performance, heterosis and specific combining ability (SCA) effects of hybrids under abiotic stress and non-stress environments

(v) To estimate test-cross performance of F_3 segregating populations developed from CIMMYT drought tolerant donors and DR&SS elite inbred lines under drought and non-drought conditions

(vi) To estimate performance and yield prediction of three-way hybrids from drought tolerant single cross hybrids.

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CHAPTER 2

Literature review

2.1 Introduction

Literature on principles of main concepts that are relevant in this research is reviewed in this chapter. These include i) understanding the effects of drought on maize, progress in breeding for drought, secondary traits used in selection for drought as well as managed drought screening, effects and breeding for low N conditions in maize, ii) combining ability, heterosis and heterotic groups, iii) genetic characterisation, molecular markers, SNPs and correlation between heterosis and genetic distances and iv) G x E interaction.

2.2 Major abiotic stress factors affecting maize production

Drought and low N are the two major abiotic stress factors affecting maize production in sub-Saharan Africa. In Zimbabwe the major maize producers are small scale farmers who are mainly located in dry regions of the country with low soil inherent fertility. Initial efforts in the National Breeding Programme were towards breeding maize varieties for high rainfall regions with optimum fertilisation. It is therefore important that efforts are made towards improving new maize varieties for tolerance to these stresses. Currently few drought and low N tolerant maize varieties have been released by the National Breeding Programme. Maize yields are mostly affected by drought through reduction of the growing season and erratic mid-season dry spells that take place at any time during the growth of the crop (Edmeades *et al.,* 1994). Maize is mainly susceptible to drought stress that takes place just before and during flowering when its yield potential is determined (Malosetti *et al*., 2007).

Drought is a water deficit in the plant's environment that has the potential to reduce crop yield (Cooper *et al.*, 2006). It has devastating economical and sociological effects. Drought incidents are predicted to increase due to long term effects of global warming (Cook *et al*., 2007). It is difficult to forecast manifestation of natural drought making it challenging or almost impossible to differentiate between stress and non-stress agricultural systems (Cooper *et al.*, 2006). In the semi-arid tropics the effect of drought is intensified by extremely erratic

rainfall, high temperatures, high levels of solar radiation and poor soil productiveness (Cook *et al*., 2007).

2.2.1 Effects of drought on maize

Maize inflorescence consists of separate male and female flowers making it more vulnerable to drought stress during flowering time (Prine, 1971; Grant *et al.*, 1989). Tassel development and pollen shed in maize are less sensitive to fluctuations in moisture availability compared to silk growth. The allocation of nutrients to ears, ovules and silks is reduced under drought as a result of the dominance effects of the apical tassel. Silk emergence in relation to male flowering is delayed when drought takes place just before flowering and this result in an increased anthesis silking interval (Bolanos and Edmeades, 1993a). When the anthesis silking interval is lengthened the pollen might arrive when silks have dried up (Bassetti and Westgate, 1993) or after ovaries have used up their starch reserves (Saini and Westgate, 2000; Zinselmeier *et al.*, 2000). This scenario results in retarded ear and silk growth and accelerated kernel and ear abortion (Westgate and Boyer, 1986; Edmeades *et al.*, 1993).

The maize crop has been found to be more susceptible to moisture stress one week before to two weeks after flowering (Grant *et al.*, 1989). Grain abortion normally takes place during the first 2-3 weeks after the emergence of silks (Westgate and Boyer, 1986; Schussler and Westgate, 1991). It is intensified by any stress that decreases canopy photosynthesis and movement of assimilates to the developing ear. This scenario results in the growing ear being deprived of the necessary nutrients (Stevens, 2008). Therefore the amount of assimilates reduces to below threshold levels required to sustain grain development and growth (Edmeades and Daynard, 1979; Tollenaar *et al.*, 1992). The decrease in photosynthesis can be due to a decrease in radiation interception associated with increased leaf rolling (Bolanos *et al.*, 1993). Reduction in photosynthetic rate decreases the volume of nutrients available for distribution to the sink organs (Kim *et al.*, 2000). The amount of stress that drought imposes on the maize crop results in modifications of photosynthetic pigments and constituents (Loggini *et al.*, 1999). It also causes damage to photosynthetic organs (Fu and Huang, 2001) and the calvin cycle enzyme activity is reduced (Monakhova and Cheryadév, 2004). Carbohydrate metabolism activity in the plant's reproductive organs is also negatively

affected (Liu *et al.*, 2004). Maize is more vulnerable to drought compared to sorghum as a result of its shallow root system, enlarged leaf surface area, increased transpiration rate, slower grain development rate and extended grain filling period (Sinclair and Muchow, 2001).

2.2.2 Breeding for drought tolerance in maize

Maize is considered the most susceptible cereal to drought stress, with the exception of rice (Banziger and Araus, 2007). Maize yields remain below 2 t ha^{-1} in most countries in sub-Saharan Africa and yields vary from year to year (FAO, 2011). Maize is the staple food crop of importance to over 300 million people in eastern and southern African countries (Heisey and Edmeades, 1999). Rainfall distribution and amount have been found to have a direct effect on maize productivity in these two regions. In southern Africa the 2002/03 drought left about 14 million people exposed to starvation and the food deficit was 3.3 MT (World Food Programme, 2003). The World Food Programme was expected to provide food aid to 7.8 million people in five East African countries (Somalia, Ethiopia, Djibout, Kenya and Uganda) as a result of consecutive seasons of drought (World Food Progamme, 2009). Again East Africa experienced a severe drought in 2011 that left more than 10 million people relying on food aid (World Food Programme, 2011). Therefore, in order for farmers to realise increased and stable yields and for seed merchants to be in a position to market a variety widely, it is critical that drought tolerance is incorporated in maize breeding strategies (Campos *et al.*, 2004). Hence, improvement or development of maize genotypes with high and constant yields under drought stress conditions is essential.

Among abiotic stresses, breeding for drought tolerance is one of the most challenging endevours, because selected germplasm ought to perform exceptionally well not only under drought stress but also under optimum conditions. Since water is a scarce resource, improving varieties for drought tolerance is an important approach in reducing this problem. It is important in breeding for drought tolerance to consider breeding for other stress factors as well (Beebe *et al.*, 2008). Progress in breeding for drought tolerance has been slow as a result of the complex nature of the trait and an improved understanding of the fundamental mechanisms of drought would hasten progress in breeding for the trait (Ribaut *et al.*, 2002).

In an effort to improve maize productivity, maize breeders have exerted enormous efforts to breed hybrids with drought tolerance (Bruce *et al.,* 2002). The efficiency in selection of germplasm for drought tolerance can be improved through use of managed drought environments. This can be done during the off-season (winter) with the use of controlled irrigation whereby the occurrence, extent and amount of drought stress on the crop are controlled (Banziger *et al*., 2000). As a result of significant G x E interaction, it is important that genotypes screened for drought tolerance are evaluated in the target locations before they are incorporated as parents in the breeding programmes.

Although progress in drought tolerance can be achieved through conventional selection methods, trials must usually be replicated across a large number of locations and across several years before the expression of the trait can be conclusively identified. Stevens (2008) reported that CIMMYT has been involved in breeding for drought tolerance in maize over the past three decades. A unique selection programme aimed at improving tolerance to drought using the lowland tropical maize populations Tuxpeño Sequía, LaPosta Sequia and Pool 26 Sequia was started at CIMMYT in the 1970s. Average maize yields under drought conditions were 126 kg ha^{-1} per cycle (Stevens, 2008). Progress in selection for drought tolerance of Tuxpeño Sequía resulted in average breeding gain of 108 kg ha^{-1} yr^{-1} with yield levels ranging from 1-8 t ha^{-1} (Bolanos and Edmeades, 1993a). A significant gain of 9.4% for ears per plant and reduction of anthesis silking interval from 34.2 days in the first cycle to 9.8 days in cycle eight were further reported by Bolanos and Edmeades (1993a). Bolanos and Edmeades (1993b) reported an increase in anthesis silking interval to 18.8 days under severe stress. The three populations outperformed their original cycles of selection and check varieties under drought conditions and yields ranged from 1.0-4.5 Mg ha^{-1} and 5.8-10.4 Mg ha^{-1} under drought and optimum conditions respectively (Edmeades *et al*., 1999). The authors further reported yield gains ranging from 0.08 - 0.29 Mg ha^{-1} $cycle^{-1}$ under drought conditions and 0.04 - 0.18 Mg ha^{-1} $cycle^{-1}$ under optimum conditions. Campos *et al*. (2004) reported 146 kg ha^{-1} gains annually when drought stress was introduced at flowering and 76 kg ha^{-1} when stress was introduced at mid-grain filling stage. Drought tolerant germplasm was introgressed into local African germplasm, producing varieties with stable and superior performance in southern and eastern Africa. Superior maize OPVs with yield performance

comparable to commercial hybrids under moderate to severe moisture stress were also produced.

Grain yield is considered the primary trait for selection under drought stress conditions. Nonetheless, reduced heritability and variance of yield components make selection based only on grain yield inefficient (Stevens, 2008). The major strategy in breeding for drought tolerance in maize has been direct selection for high yield. Grain yield under severe mid and late season moisture stress has been improved by 30-50% in three late maturing maize populations through recurrent selection at rates of up to 12% per selection cycle (Edmeades *et al.*, 1999). It has been reported that use of both secondary traits and grain yield in improving germplasm for drought stress tolerance has resulted in significant selection progress (Mhike *et al*., 2011).

2.2.3 Suitable secondary traits used in selection for drought tolerance

Appropriate secondary traits selected for under drought stress should be genetically related to grain yield, have high heritability estimates, be consistent and easy to select for and not too expensive. These traits must furthermore be measurable at or before flowering so that undesirable parents are selected against and these traits should not be accompanied by yield loss under optimum environments (Edmeades *et al.*, 1998). Critical secondary traits under drought identified at CIMMYT and Pioneer Hi-Bred include reduced prolificacy, anthesis silking interval, stay green and to a lesser extent leaf rolling (Banziger *et al.*, 2000). Anthesis silking interval is measured as the number of days between silk emergence and pollen shedding and increases under drought stress as a result of retarded ear and silk growth (Bolanos and Edmeades, 1993b). Work done at CIMMYT has revealed that the heritability estimate for anthesis silking interval is related to or greater than the heritability for grain yield. A high negative correlation of anthesis silking interval with grain yield and other related traits such as kernel number and number of ears per plant has been reported. Anthesis silking interval and ears per plant have been widely used in breeding for stress tolerance in maize (Bolanos and Edmeades, 1993b; Banziger *et al*., 2000). These two traits have shown good genetic variability under drought stress conditions and high heritability.

The variation in number of kernels has a major effect on maize grain yield under drought (Bolanos and Edmeades, 1996). Bolanos and Edmeades (1993a) observed a 90% drop in yield as anthesis silking interval increased from -0.4-10 days, whilst Du Plessis and Dijkhuis (1967) reported 82% drop in grain yield as anthesis silking interval increased from 0-28 days. In genotypes selected for short anthesis silking intervals and increased grain yield under drought the bulk of the carbohydrates are channeled towards development of the ear and less towards the growth of tassels and vegetative organs (Edmeades *et al.*, 1993). In tropical maize gains in selection have been linked with improved synchronisation in silking and pollen shedding, reduced barreness, reduced tassel size, increased harvest index, delayed leaf senescence and reduced root length density in the upper soil profile with no alterations in water uptake or biomass (Bolanos and Edmeades, 1993a; b; Bolanos *et al.*, 1993; Chapman and Edmeades, 1999). Genotypes are selected under managed drought stress based on grain yield performance and appropriate secondary traits.

2.2.4 Managed drought

Managed drought stress screening is usually done off-season (winter) with the use of irrigation. Drought stress on genotypes is induced either at flowering or at grain filling stage. At intermediate stress level average grain yield is targeted to reduce by 15-30% of yields expected under optimum conditions and the stress will be targeting grain filling. A yield reduction of 30-60% of yields realised under optimum conditions is targeted for severe stress levels and the stress affects both flowering and grain filling (Banziger *et al.*, 2000). Under severe stress, irrigation is scheduled such that drought stress coincides with anthesis and silk emergence, but supplementary irrigation is applied 14 days after the end of pollen shedding in order to facilitate adequate grain filling of the formed grain. In intermediate stress, drought stress is timed to coincide with grain filling. It is important to ensure that irrigation is uniformly applied before onset of stress as this will result in stress levels being uniform in all genotypes, more constant plant performance and eventually improved breeding progress (Banziger *et al.*, 2000).

2.2.5 Effects of low nitrogen on maize performance

Grain yield in tropical maize is negatively affected by the abiotic stress factors drought and low N (Pingali and Pandey, 2001). N is one of the major nutrients required by plants in large quantities. N use efficiency is always low in dry areas and has become one of the most limiting factors in crop yield improvement. It has been reported that besides providing nutrients for crop development, application of N may possibly lead to improved drought tolerance and enhanced yield (Zaman and Das, 1991; Xu *et al*., 2005). Kernel abortion in maize has been seen to be aggravated by N stress thereby leading to reduced grain number (Lemcoff and Loomis, 1986; Pearson and Jacobs, 1987; Uhart and Andrade, 1995a; b). Roughly 85% of kernel abortion takes place in the course of the first 20 days after silk emergence (Monneveux *et al.*, 2005). N plays a crucial role in the anti-oxidant defence enzyme and lipid peroxidation metabolism under stress environments (Sun *et al.*, 2001; Saneoka *et al.*, 2004). N deficiency has also been reported to negatively affect leaf expansion, emergence rate, radiation interception radiation use efficiency and assimilate distribution amongst vegetative and reproductive organs (Uhart and Andrade, 1995a). Reduction in kernel number and number of ears due to low N has been reported by Lemcoff and Loomis (1986); Pearson and Jacobs (1987); Uhart and Andrade (1995a; b) and Monneveux *et al.* (2005). Prolonged anthesis silking interval has also been reported as a result of N deficiency (Jacobs and Pearson, 1991) and accelerated senescence (Moll *et al.*, 1994). Low N affects maize growth throughout the life cycle of the crop compared to drought that occurs at any time during the growth of the crop (Banziger and Araus, 2007).

2.2.6 Breeding for low nitrogen tolerance in maize

Maize breeders have made enormous efforts towards screening maize germplasm for tolerance to low N conditions. However, headway in screening for low N tolerance has been slown down by pronounced genotype x season and genotype x location interactions (Ribaut *et al.*, 2007). Lafitte and Edmeades (1988) reported that N unavailability is projected to be the major limiting factor in more than 20% of arable land. The use of inorganic fertiliser in sub-Saharan Africa is on the lower side mainly as a result of unavailability of fertiliser and ever escalating costs. Cultivars tolerant to low N have been found to be efficient in utilising

available N, mainly as a result of increased uptake capacity or their ability to efficiently utilise absorbed N in grain production (Lafitte and Edmeades, 1994).

As a result of low heritability estimates for grain yield under low N environments, the utilisation of secondary traits in the selection process has frequently been recommended (Lafitte *et al.*, 2003). Anthesis silking interval, leaf senescence and ears per plant have been suggested as ideal secondary traits to select for when improving maize genotypes for low N conditions (Banziger and Lafitte, 1997; Banziger *et al.,* 2000). Selection indices centered around these traits were established and significantly improved the selection efficacy under low N stress environments (Banziger and Lafitte, 1997). It is simpler to breed for low N than for drought conditions mainly because unavailability of N affects plant growth in a more even manner unlike drought periods that occur randomly (Banziger *et al.*, 2000). It has been found that screening germplasm under severe low N conditions should be adequate to infer low N stress tolerance for different levels of N deficiency. Maize germplasm has been selected for both drought and low N tolerance using combining ability effects. Testers known to be drought or low N tolerant are crossed with selected lines and the progeny is evaluated for tolerance to the two stresses. Ideal parents are identified as having good general combining ability (GCA) under these environments; therefore it is critical to consider looking at combining ability effects of lines under evaluation in the current study.

2.3 Combining ability and gene action

Combining ability cannot be predicted from the parental phenotype and is assessed by progeny testing from controlled pollinations. It was initially a broad notion considered jointly for categorising an inbred line in relation to its hybrid performance, but was later refined as GCA and SCA. This had a major influence on inbred line evaluation and population development in maize breeding (Sprague and Tatum, 1942). Sprague and Tatum (1942) defined GCA as the average performance of a line in hybrid combination and SCA as those cases where some hybrid combinations are either superior or inferior than would be estimated on the average performance of the parental inbred lines.

SCA effects should be used in combination with hybrid performance and GCA of particular parents for selection (Shukla and Pandey, 2008). The GCA component is primarily a function of additive gene action while SCA variance is mainly a function of dominance variance. Evaluation of GCA effects of hybrid parents is essential to critic their appropriateness for hybrid development, since the mean performance of parental lines does not always translate to their GCA effects. GCA and SCA effects are important tools used by breeders in selecting superior parents for developing crosses (Shukla and Pandey, 2008). Favourable alleles are combined through hybrid combination with high *per se* performance with good SCA estimates and having at least one of the parents with high GCA (Marilia *et al.*, 2001). In choosing ideal parents and crosses and to estimate the combining abilities of parents in early generations, plant breeders have used the line x tester analysis method. Line x tester analysis provides an efficient approach for identification of suitable parents and crosses exhibiting good performance in traits under consideration (Ahuja and Dhayal, 2007).

GCA and SCA variances are used to deduce gene action. It is defined as the way genes express themselves in which case GCA effects represent additive gene action, whilst SCA effects represent non-additive gene action. Several mating designs have been used for estimating gene action amongst them the North Carolina Design II (NCDII) (Comstock and Robinson, 1948). The NCDII crosses are used for defining the cumulative gene effects of breeding populations and for estimating GCA and SCA effects. In the current study NCDII was used to form hybrids among 23 inbred lines that were to be evaluated for drought and low N tolerance and the method was chosen because the objective was mainly to estimate GCA and SCA variances. It is critical to understand gene action for grain yield and other secondary traits so that effective breeding strategies are developed for stress tolerance breeding without compromising on yield. Betran *et al*. (2003) reported additive gene action being more important under drought conditions and non-additive gene action being important under low N conditions. Their results suggested potential benefits of incorporating drought tolerance in both parental inbred lines in order to enhance performance of hybrids under drought conditions. A higher SCA variance compared to GCA was reported by Devi and Singh (2011), suggesting that non-additive gene action played a more important role in determining grain yield. Amount of heterosis realised in a cross depends among other things

on the differences of gene frequency between the crossed lines (Falconer, 1981). Once ideal parents are identified it is important to assess the levels of heterosis and possible heterotic relationships among these lines.

2.4 Heterosis and genetic diversity

2.4.1 Heterosis

The first step in the search for heterosis in crop improvement is a full characterisation of available genetic diversity, which forms the basis for the analysis of combining ability of inbred lines (Verbitskaya *et al.*, 1999; Diniz *et al.*, 2005). According to Falconer (1981) heterosis is the product of directional dominance and square of differences in gene frequency in parents. It is therefore obvious that the presence of both dominance and initial differences in gene frequency in parental lines causes heterosis in F_1 progeny. Heterosis will be significant when alleles in both parents are in a homozygous state (Falconer, 1981). Success of the formal seed sector involving maize and other crops has been attributed to heterosis/hybrid vigour. Manipulating heterosis in breeding facilitates yield improvement and helps augment many other necessary quantitative and qualitative traits in crops. Heterosis is observed in a situation where offspring obtained from crossing two inbred lines or populations perform above the mean of the two populations or lines for the trait under consideration. It is usually seen as increased growth rate, size, yield and other traits in F_1 individuals produced after crossing inbred lines (Melchinger and Gumber, 1998; Tollenaar *et al.*, 2004). Remarkable maize yield increases observed in the USA between the 1930s and 1970s came about through manipulation of heterosis (Duvick, 2001).

The magnitude of heterosis between lines or populations has assisted in determining the level of genetic diversity. It provides a base for choosing germplasm to be used as parents for developing segregating populations to be used in a reciprocal recurrent selection breeding programme (Ortiz *et al.*, 2008). Crosses produced from distantly related parents normally exhibit higher levels of heterosis compared to crosses produced from closely related parents and this can be partly explained by the level of genetic diversity that exists between the parents (Fabrizius *et al.*, 1998). The success of any hybrid breeding programme depends on

the existence of reasonable levels of heterosis and a favourable environment for economic hybrid seed production (Shukla and Pandey, 2008). The effective manipulation of intra- and inter-sub specific heterosis hinges on the genetic diversity of the parents, gene action, linked hybrid vigour and the biological achievability of hybrid seed production (Shukla and Pandey, 2008).

Results from studies of heterosis for grain yield in maize until 1979 were summarised by Hallauer and Miranda (1988) and mid-parent heterosis (MPH) ranged between -3.6% and 72.0%, whilst high-parent heterosis (HPH) ranged between -9.9% and 43.0%. Recently Betran *et al*. (2003) reported average MPH of 171.0% and HPH of 132.0% and heterosis increased as the severity of drought stress increased. In a study by Dhliwayo *et al*. (2009) average MPH of 5.14% was reported. George *et al*. (2011) reported MPH values of 114.0% and 130.0% and HPH values of 84.0% and 92.0% under high and low phosphorus soils, respectively. Maize hybrids normally yield two to three times better than their inbred parents, but ideal hybrids from the farmer's perspective are not necessarily the ones with high heterosis under optimum conditions but the hybrids with higher yield advantage under drought conditions (Duvick, 1997). Better performing hybrids in terms of yield owe their yield advantage not only to heterosis but also to other heritable elements that are not necessarily affected by heterosis.

2.4.2 Heterotic groups

Identification of heterotic groups and heterotic patterns is an important undertaking in hybrid maize breeding and contributes to substantial improvements in yield performance. Establishment of heterotic groups have assisted in improving breeding progress because hybrid performance can be determined without production of crosses or carrying out field experiments (Tracy and Chandler, 2006; Troyer, 2006). New inbred lines are established through selection of germplasm within the same heterotic group and the inbred lines are then evaluated by crossing them to an inbred line belonging to a different heterotic group. Exotic germplasm can be effectively exploited when the heterotic relationships among populations are understood. Heterotic patterns used in main maize production areas worldwide have been reviewed by several authors (Wellhausen, 1978; Ron Parra and Hallauer, 1997). Heterosis

can be exploited in maize breeding through classification of inbred lines into different heterotic groups thereby leading to improved hybrid performance (Bhatnagar *et al*., 2004).

Heterotic groups were not identified until extensive yield test data of different combinations of inbred lines in double crosses became available (Hallauer, 1997). Scientists from CIMMYT have conducted several combining ability studies to enable them to identify heterotic patterns among several maize populations and gene pools (Beck *et al*., 1990; 1991; Crossa *et al.,* 1990; Vasal *et al.,* 1992). Initially groups were identified by how lines performed in crosses i.e A x B crosses were superior to either A x A or B x B crosses where A and B represent different germplasm sources (Hallauer, 1997). Lines in genetically different heterotic groups are usually identified by positive SCA effects between them (Vasal *et al.,* 1992). Inbred lines in the same heterotic group have a tendency to exhibit negative SCA effects when crossed (Vasal *et al.,* 1992). In eastern and southern Africa, the heterotic groups are based on Southern Cross (SC), Salisbury White (N3), K64r/M162W and Natal Potchefstroom Pear Elite Selection (NPPES) varieties. The varieties SC, N3 and NPPES were developed from varieties imported from the USA, while K64r is a direct import from the USA (Mickelson *et al.,* 2001). CIMMYT has developed a number of heterotic groups from some of the above broad groups to suit its lowland tropical, sub-tropical and highland breeding programmes. In its programmes of southern and eastern Africa, there are two heterotic groups, A and B. Group A includes the following germplasm: Tuxpeno, Reid Yellow Dent and N3, whilst group B has ETO, Lancaster Sure Crop and SC germplasm (Mickelson *et al.,* 2001). Heterotic groups used in the National Breeding Programme in Zimbabwe are N3 and SC and these are equivalent to CIMMYT groups A and B, respectively. Lines in group A have been crossed with lines in group B and have shown high levels of heterosis. Grouping lines into heterotic groups has resulted in production of hybrids with high hybrid vigour and as a result high yielding varieties have been released into the market. It is critical to understand genetic diversity that exists among inbred lines as it is the basis for forming heterotic groups and helps in optimising the levels of heterosis during hybrid formation. In cases where defined heterotic groups do not exist, marker-based genetic distances can also be used to avoid making crosses between closely related lines. In the current study maize germplasm from two sources, namely the National Programme and

CIMMYT, was used, so it is important to determine the level of diversity through genetic characterisation of these lines.

2.4.3 Genetic diversity and characterisation

Genetic diversity is enhanced by germplasm collections from diverse sources and these should be well characterised for improved management and effective utilisation. Characterisation of elite material has received special attention since the late 1980s because of its strategic interest for breeders' rights protection and also because analyses of homogeneous material (inbreds) are easier than using unfixed material (Charcosset and Moreau, 2004). In order to optimise efficiency of hybrid combinations and to develop new inbred lines, existing maize inbred lines from various sources must be properly characterised. Germplasm organisation and variety protection in hybrid breeding programmes is enhanced through the knowledge of genetic relationships among inbred lines (Melchinger *et al.*, 1991; Bernardo, 2002). A better understanding of genetic relationships amongst genotypes is valuable in crop breeding programmes because it allows the organisation of germplasm, such as elite lines, and affords well-organised parental selection.

Newly developed inbred lines are usually separated into different heterotic pools using pedigree data (Messmer *et al.*, 1993). Nonetheless, pedigree information tracing back to more than two generations is difficult to find, therefore maize breeders now use genetic distance (GD) evaluation as an alternative method for germplasm selection. Morphological, biochemical and molecular analyses as well as heterosis and SCA revealed in different crosses in maize have been used to quantify GD among germplasm (Vasal *et al.*, 1992; Ajmone-Marsan *et al.*, 1998; Paterniani *et al.*, 2000; Menkir *et al.*, 2004; Laborda *et al.*, 2005). DNA marker-based diversity has given mixed results. Previous studies have confirmed that a high similarity for molecular markers was always associated with a high co-ancestry (Melchinger, 1999). Once possible parents have been identified based on an amalgamation of marker data and a trait of interest, the next step is to decide which crosses should be made to develop new breeding populations or hybrids (Charcosset and Moreau, 2004). Molecular markers are used to investigate the level of genetic diversity amongst maize inbred lines and this is mainly done through fingerprinting. Since homozygous lines were

used in this study, fingerprinting is most appropriate in assessing genetic diversity amongst them. The fingerprinting data can then be used in estimating genetic distances among inbred lines.

2.4.4 Molecular markers

A molecular marker is defined as a DNA sequence that is readily detected and whose inheritance can easily be examined. A marker must be polymorphic and occur in diverse forms such that a chromosome carrying the mutant gene can be distinguished from the chromosome with the normal gene by a marker it carries. Molecular markers can be used to better document the organisation of genetic diversity between possible parental materials of new breeding programmes and to establish the distinctiveness of new cultivars prior to their registration (Charcosset and Gallais, 2002). Plant breeders can by-pass traditional phenotype-based selection methods that include raising plants until they reach maturity and monitoring their physical make-up in order to infer underlying genetic make-up through use of molecular markers (Varshney *et al.*, 2004).

Molecular markers evolved from hybridisation based markers namely restriction fragment length polymorphism (RFLP) (Botstein *et al*., 1980). With the advent of the polymerase chain reaction (PCR) a number of markers were developed namely random amplified polymorphic DNA (RAPD) (Williams *et al*., 1990), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and SNP. However, each of these markers has their advantages and disadvantages. RFLP markers were successfully used in constructing linkage maps for different crops including maize and wheat (Hoisington, 2001). The limitations that RFLP markers had which included need for a suitable probe library, automation not being possible and technically demanding among others, prompted the development of RAPD markers. RAPD markers quickly gained popularity over RFLP markers because of their simplicity and reduced assay costs. They were also found to require small amounts of DNA compared to RFLPs and many possible primers were available. However, due to their dominant nature and not being reproducible RAPDs became less popular with reseachers. AFLPs were then developed and were found to combine specificity of restriction analysis with PCR amplification (Vos *et al*., 1995). AFLPs have a higher efficiency in detecting

polymorphism compared to RAPDs and RFLPs (Garcia-Mas *et al*., 2000). However, the AFLP technique was later found to be more labour intensive and time consuming compared to RAPDs. SSR markers were then applied to overcome the disadvantages of RFLPs, RAPDs and AFLPs. The SSR markers became popular mainly because of their co-dominant nature, large number of polymorphisms, their random distribution in the entire genome and being reproducible (Senior *et al*., 1993; Vos *et al*., 1995). It was also found that once SSR primers were developed, screening became less expensive. However, when primers were not available the organism had to be screened for SSRs first. The process of screening for SSRs was found to be expensive and practically complex and at times only a small number of SSRs could be detected. SNP markers were later developed and because of their abundance in different genomes and high levels of automation they have become the most popular markers.

Molecular markers have been widely used in assessing genetic diversity in maize (Melchinger *et al*., 1992; Betran *et al*., 2003; Jones *et al*., 2007; Dhliwayo *et al*., 2009; Van Inghelandt *et al*., 2010; Devi and Singh, 2011; George *et al*., 2011). Melchinger *et al*. (1992) used RFLP markers to assess genetic diversity among six flint and six dent maize inbred lines and narrow GDs were reported between flint x dent crosses (0.56-0.73) compared to flint x flint (0.14-0.66) and dent x dent (0.23-0.62). RFLP markers were again used to assess genetic diversity amongst tropical maize inbred lines and the GDs ranged from 0.20-0.84 (Betran *et al*., 2003). RAPD markers have been used to examine GD across varied species comprising segregating lines of maize (Ajmone-Marson *et al.*, 1993) and have also been used to assess genetic diversity among homozygous maize inbred lines (Lanza *et al*., 1997; Devi and Singh, 2011). The most widely used DNA marker for germplasm characterisation until now was SSR markers. SSR markers are easy to use, relatively cheap when primers are available and have a high degree of polymorphism provided by the large number of alleles per locus (Vignal *et al*., 2002). PCR based SSR markers have been widely used in fingerprinting of maize germplasm (Messmer *et al.*, 1992; Dubreuil *et al.*, 1996; Smith *et al.*, 1997; Senior *et al.*, 1998; Dhliwayo *et al*., 2009; George *et al*., 2011). A total of 89 SSR markers were found to perform better at clustering maize germplasm into populations than did a set of 847 SNPs or 554 SNP haplotypes (Hamblin *et al.*, 2007).

2.4.5 Choosing a marker

Given the innumerable DNA marker technologies available and the widespread range of applications they can be used for, an obvious problem that has to be faced is how to select the most ideal DNA marker for a particular analysis. A number of issues have to be considered and these include the technology itself, the problem under investigation and the circumstances of the investigator. Widely used markers have been categorised into different groups and these include hybridisation based markers such as RFLPs (Helentjaris *et al.*, 1986) and PCR-based markers such as SSRs (Senior *et al.*, 1993) and SNPs. A perfect marker system has been defined as one that is highly informative, evenly dispersed across the genome, co-dominant, accurate and have reproducible data that can be produced in a high-throughput and economical manner (Jones *et al.*, 2007; Yan *et al.*, 2010). RFLP and SSR possess the majority of these aspects but they have high developmental costs. SSR markers have been a marker of choice for the majority of crops but there have been complications in their use, especially challenges in accurately sizing SSR alleles due to PCR and electrophoresis artifacts (Hatcher *et al.*, 1993; Jones *et al.*, 1997; Bovo *et al.*, 1998; Fernando *et al.*, 2001; Heckenberger *et al.*, 2002; Davison and Chilba, 2003).

2.4.6 Single nucleotide polymorphism (SNP)

A SNP is a single change in the sequence of a section of DNA. It may come about as a result of a substitution of one nucleotide for another at the polymorphic site. A SNP can also be a single base insertion or deletion variant referred to as an indel. The majority of SNPs are biallelic; however they can also be tri- or tetra-allelic. Tri allelic SNPs involve the presence of three different nucleotides for the specific SNP whilst tetra-allelic involve all four nucleotides. Molecular markers based on SNPs are plenty, evenly dispersed throughout the entire genome and adequate to discriminate individuals in a population. SNP markers have been found to possess almost all attributes of an ideal marker, which include the potential for high throughput and low cost genotyping and they have since become the marker system of choice (Yan *et al.*, 2010). SNP markers received much consideration because of higher frequencies in the genome compared to SSR markers (Van Inghelandt *et al.*, 2010). SNP markers are usually biallelic and tend to be less informative than SSRs on a single marker basis, nevertheless this weakness can be compensated for by their abundance and the

capacity to utilise SNP haplotypes (Gupta *et al.*, 2001; Ching *et al.*, 2002; Rafalski, 2002). SNP markers have become the most widely used markers because they target single nucleotide differences between genotypes, showing more polymorphism compared to other types of markers (Jung *et al.*, 2010).

According to Lu *et al.* (2009) a large number of SNP markers would be required to substitute extremely polymorphic SSR markers in studies of diversity and relatedness. Jones *et al.* (2007) found a clear advantage for SNP markers when evaluating the repeatability of genotyping results and proportion of missing data for SSR and SNP markers. In a study by Van Inghelandt *et al.* (2010) the average number of alleles per SSR locus was higher than that for the SNP markers and this was due mainly to the fact that SNP markers are usually biallelic (Vignal *et al.*, 2002). The use of SNP markers in plants has been limited firstly by high developing and implementation costs and secondly because the development of SNP markers in plants is complex due to the presence (in polyploid plants) of homologues i.e. non-allelic versions of genes residing on homoelogous chromosomes. There are various SNP genotyping methods available but most of them are too costly for low- to medium-throughput academic laboratories and breeding programmes (Comai *et al.*, 2004; Lin *et al.*, 2009). These methods include the Illumina GoldenGate® genotyping and KBiosciences Allele Specific PCR technologies. It is also worth mentioning that the use of SNP markers produces a simple binary output that is appropriate for automatic data collection systems and therefore their use is gaining more popularity (Rostoks *et al.*, 2005; Varshney *et al.*, 2007).

SNP markers, specifically for cultivar identification, have in recent years been developed for use by the commercial sector following demand by the commercial sector (Reale *et al.*, 2006; Shirasawa *et al.*, 2006; Yoon *et al.*, 2007). Numerous SNP markers, mostly developed from DNA sequences of known genes, are now available for use in maize. Consequently SNP markers have become the marker of choice for various tasks in maize improvement that include genetic diversity analysis, linkage map construction, marker trait association or quantitative trait locus (QTL) mapping and marker-assisted selection (MAS) (Lu *et al.*, 2009). Ching *et al.* (2002) investigated the frequency of SNPs and distribution of DNA polymorphisms at 18 maize genes using 36 maize inbred lines. Tenaillon *et al.* (2001)

reported a SNP every 104 base pairs (bp) in coding regions, whilst Ching *et al.* (2002) reported a SNP every 31 bp in non-coding regions and a SNP every 124 bp in coding regions. In another study by Rafalsaki (2002) a SNP every 48 bp in non-coding regions and every 130 bp in coding regions was reported. SNP frequency in maize has been found to be high compared to other crops for example rice has a SNP frequency of 0.5-0.78%, wheat 0.5% and soybean 0.36% (Vroh *et al.*, 2006).

2.4.7 Correlation between genetic distance and heterosis

GD has been widely used to predict the level of heterosis in maize. The level of heterosis in F_1 populations is correlated with GD of the parental lines, and with more divergent parents the level of heterosis is higher and *vice versa* (Prasad and Singh, 1986; Duvick, 1999). In crops such as maize, oat, wheat and rice, GD based on molecular markers has been broadly correlated with heterosis but with diverse outcomes (George *et al.*, 2011). Some studies based on RFLP and SSR markers have concluded that heterosis is significantly correlated with GDs (Lee *et al.*, 1989; Smith *et al.*, 1990; Melchinger *et al.*, 1992; George *et al.*, 2011), whilst a low correlation was reported by other researchers (Godshalk *et al.*, 1990; Melchinger *et al.*, 1990). Boppenmeier *et al.* (1992), Ajmone-Marson *et al.* (1998), Menkir *et al.* (2004), Balestre *et al.* (2008), Legesse *et al.* (2008) and Dhliwayo *et al.* (2009) reported no significant correlation between GD among inbred lines and the agronomic performance of their F_1 hybrids. In general, linear correlation between DNA marker-based genetic distance and F_1 MPH seems to be higher among related and intra-group than among unrelated and inter-heterotic group lines (Melchinger, 1999). Conclusive results on the application of molecular based GD in predicting hybrid performance in maize have not been reported. Betran *et al.* (2003) found that drought significantly depressed correlations between GD and F_1 grain yield, MPH, HPH and SCA. Performance of genotypes is also influenced by G x E interaction so it is critical that the magnitude and pattern of G x E is examined. Genotypes will tend to perform differently in different environments resulting in changes in genotype rankings.

2.5 Genotype by environment interaction and assessment of stability

G x E interaction is the different performances of genotypes in different environments and consists of the following types of interaction: i) crossover interaction (COI) or genotypic rank changes across environments, the most crucial interaction in crop improvement and production (Baker, 1988; 1990), ii) non-COI or scale changes among environments and iii) a combination of both. G x E interactions may result from differences in manifestation and severity of moisture stress in different environments, differences in flowering time and mineral nutrient deficiencies and toxicities whose manifestation and rigorousness interrelate with moisture stress (Cooper *et al.*, 1999; Banziger and Cooper 2001). G x E interactions are critical only if they involve significant COI (Baker, 1988; 1990). G x E interaction plays an important role in the performance of different genotypes in various environments; therefore the choice of genotypes and test sites determines the level of stability estimates (Robert and Denis, 1996; Simic *et al.*, 2003). Genotypes are generally evaluated in several environments to select the best ones.

G x E interaction is a key concern in plant breeding mainly because it reduces progress from selection due to the build-up effects of the three components of interaction between genotype and environment, namely genotype x location, genotype x year and genotype x location x year. It also poses challenges in cultivar recommendation because it is statistically difficult to deduce the main effects (Kang and Magari, 1996). Relationships between phenotypic and genotypic variances is reduced by G x E interaction and this leads to best performing genotypes in one environment to perform poorly in another, compelling breeders to look at genotypic stability. Consequently large G x E interactions hinders advancement from selection and has important repercussions for testing and cultivar release. Once G x E interaction is identified as being significant it is important to then assess the response of varieties in different environments as well as assess their overall stability. This can be done through use of various statistical models some of which include additive main effects and multiplicative interaction (AMMI) and genotype and genotype by environment interaction (GGE) biplot analysis.

2.5.1 Additive main effects and multiplicative interaction

Cultivar responses in multi-environment trials have been predicted using various statistical models. Models such as AMMI, genotype site regression model (SREG) (Cornelius *et al.*, 1996) and the model that combines genotype, environment and attribute variables in regression models (GEAR) (Moreno-Gonzalez and Crossa, 1998) generally provide better estimates of a genotype's performance in specific environments than genotype-environment combination means. The AMMI model combines additive and multivariate methodologies (Nurminiemi *et al.*, 2002; Pinnschmidt and Hovmoller, 2002).

AMMI was found suitable to handle both the main effects and G x E interactions in multi-location yield trials more effectively and efficiently than any other statistical model (Gauch, 1993). The amalgamation of analysis of variance (ANOVA) and principal component analysis (PCA) in the AMMI model alongside with prediction assessment is an important tool in understanding G x E interaction and obtaining better yields. AMMI is represented by the following model:

$$Y_{ij} = \mu + G_i + E_j + \sum^{n}_{k=1} \lambda_k \alpha_{ik} \gamma_{jk} + e_{ij}$$

Where Y_{ij} is the yield of the i^{th} genotype in the j^{th} environment, μ is the grand mean, G_i and E_i are the genotype and environment deviations from the grand mean, respectively, λ_k is the eigen value of the PCA analysis axis k, α_{ik} and γ_{jk} are the genotype and the environmental principal component scores for axis k, n is the number of principal components retained in the model and e_{ij} is the error term.

G x E interaction is partitioned into several orthogonal axes namely interaction principal component analysis (IPCA) using PCA of AMMI. IPCA1 and IPCA2 are usually used in the construction of a biplot because higher IPCA axes are subject to noise and have no predictive value (Van Eeuwijk, 1995). Wallace *et al.* (1993) concluded that AMMI statistical analysis can separate and quantify G x E interaction effects on yield and other measured traits in different environments.

2.5.2 Genotype and genotype by environment interaction biplot analysis

GGE biplot methodology for graphical analysis of multi-environment trial data (MET) was developed by Yan *et al*. (2000). GGE denotes genotypic main effect (G) plus G x E interaction, and these are two main sources of variation that are relevant to cultivar assessment. The GGE biplot displays the GGE of MET data. The biplot is constructed by plotting the first two principal components (PC1 and PC2) and these are also referred to as primary and secondary effects respectively. The PC1 and PC2 values are derived from singular value decomposition (SVD) of the environment-centered data. GGE biplot analysis is used to identify some of the least discriminating locations and representative test locations (Fan *et al*., 2006). The same researchers implied that the GGE biplot methodology was a valuable tool for categorising sites that lead to optimum cultivar performance and efficient utilisation of limited resources available for the testing programmes.

2.6 Conclusions

As a result of climate change drought was predicted to occur frequently and Zimbabwe will not be spurred from these droughts. There is a shift from focusing on breeding varieties for optimum conditions to breeding stress tolerant varieties. Maize inbred lines developed in the past through Zimbabwe's National Breeding Programme were not bred for drought and low N stress tolerance. Generally, literature review revealed that there is very little published information for research conducted in Zimbabwe pertaining to germplasm tolerant to biotic and abiotic stress factors. There is a huge gap that still exists between maize yield potential and the actual yield in Zimbabwe, indicating that opportunities for grain yield improvement do exist. Therefore breeding for tolerance to drought and other stresses would contribute towards raising the national maize yield average. Yield losses due to drought are largest at flowering stage due to poor pollen-silk synchronisation and this further aggravates poor ear and kernel development. It is suggested that grain yield can be improved by selecting for short anthesis-silking interval and high number of ears per plant under stress environments. A few studies reviewed revealed that additive gene action was important in inheritance of grain yield under drought conditions. Literature therefore reveals that gene introgression in parental-inbred lines is bound to improve hybrid performance. Reviewed literature showed that G x E interaction was of great concern to plant breeders as it caused distortions in

performance of varieties in multi-location trials. Large G x E interaction reduces progress from selection and has important repercussions for testing and cultivar release. This suggests that there is need to evaluate hybrids in multi-location trials and identify the high yielding and stable ones before recommending them for release. Genetic diversity in a breeding programme can be enhanced by germplasm collections from divergent sources. However in order to maximise utilisation of new introductions there is need to characterise germplasm to determine genetic relationships among inbred lines. Heterosis is critical in a hybrid breeding programme and the amount of heterosis between lines has assisted in determining the level of genetic diversity. This suggests that heterosis can be enhanced by determining heterotic groupings such that crosses for hybrid development are made amongst lines in divergent groups.

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CHAPTER 3

Combining ability between Zimbabwean and CIMMYT maize inbred lines under stress and non-stress conditions

Abstract

Drought and low nitrogen (N) remain some of the major factors limiting maize production in Zimbabwe. It is therefore crucial for the National Breeding Programme to continue assessing the breeding values of potential stress tolerant parents for developing new and locally adapted hybrids. In this study 10 DR&SS and 13 CIMMYT parental lines were crossed using a NCDII mating scheme. The resultant 72 single cross hybrids together with eight local checks were evaluated under non-stress, drought and low N conditions using a 0.1 alpha lattice design with two replications across seven sites in the 2009/10 and 2010/11 seasons. The objective of this study was to estimate the combining ability effects among the CIMMYT and DR&SS elite white maize inbred lines. Significant GCA and SCA effects ($P \leq 0.001$) for grain yield, anthesis days and anthesis silking interval across all environments suggested the importance of both additive and non-additive gene effects respectively in the expression of these traits. However, additive gene action assumed a more important role as shown by higher GCA variances for most traits. Non-additive gene action was found to be important in the expression of grain yield, anthesis silking interval, ears per plant and senescence under stress environments. Larger general combining ability due to females (GCA_f) than general combining ability due to males (GCA_m) for grain yield under drought and for anthesis silking interval, ears per plant and senescence under both drought and low N suggested the importance of maternal effects in the expression of these traits. Tester identification was based on good GCA for grain yield and stability under diverse environments. Lines RS61P, NAW5885, CML444, CML539, CML442, CML537 and CML548 showed desirable GCA effects under both drought and low N conditions. In the SC heterotic group the single cross RS61P/CML444 and in the N3 heterotic group 2N3d/CML548 was identified as potential testers. The study therefore identified superior germplasm that will be put into use in germplasm improvement for stress environments.

3.1 Introduction

Maize accounts for roughly 15% of the daily intake of calories all over sub-Saharan Africa, though this varies from region to region as some countries obtain up to 50% of their every day calories from maize (Stevens, 2008). Temperatures are expected to rise and rainfall distribution to change in key production regions as a result of global climate change and this is anticipated to lead to significant yield losses in maize, mainly as a result of drought (African Press Agency, 2007). Significant yearly yield losses of between 9.3-15.5% are expected to be accountable to moisture stress (Wang *et al.,* 2006). Maize ranks first in terms of the number of producers, area grown and total cereal production in Zimbabwe. Drought reduced maize production in Zimbabwe by about 70% between 1981 and 1982 (Rukuni *et al*., 2006) and in 1991-1993 the country again registered the worst season for maize production. In 2001-2003, the drought left about seven million people malnourished and the nation imported more than two million tons of maize, hence the need to develop drought tolerant varieties (Rukuni *et al*., 2006).

Hybrid maize breeding has become the major driving force behind the success of seed systems worldwide, mainly due to good adaptation and superior yield performance of hybrids. As a result of hybrid breeding sufficient infrastructure has been developed mainly for seed production and seed supply chain linkages have been improved (Devi and Singh, 2011). It is essential for any breeding programme to evaluate the breeding value of prospective parental lines to be used for developing new, locally or extensively adapted varieties. NCDII crosses are used for assessing gene action in breeding populations and this is done by estimating GCA and SCA variances. GCA represents additive gene action and it is the average performance of a line in different hybrids, whilst SCA represents non-additive gene action and it is measured as the deviation of hybrid performance from parental performance. GCA and SCA are powerful tools used by breeders in selecting best parents for further crosses. Studies on combining ability help breeders in identifying parental lines with good GCA and in detecting hybrids with good SCA. The best performing genotypes ought to show steady performance across environments, as evaluated through multi-environment trials (Devi and Singh, 2011).

Heritability estimates allow breeders to develop more efficient selection strategies and to predict gain from selection (Allard, 1999). Variance components and heritability estimates have been extensively used by plant breeders in selection of promising genotypes and in prediction of percentage heritability of desirable traits (Morakinyo, 1996). While it is useful to have an estimate of the total genetic effects on a particular trait, such as broad-sense heritability, narrow-sense heritability provides a better estimate of the breeding value (Allard, 1999). It is important to realise that heritability is not only influenced by the trait under consideration but is also influenced by the population and environmental conditions which individuals are exposed to as well as the method of data collection used (Falconer and Mackay, 1996). It is advisable when selecting for grain yield of hybrids to do indirect selection for other yield related traits that show close correlation with yield and exhibit high heritability because yield is considered a polygenic trait.

Germplasm improvement for drought tolerance will remain a high priority for the Zimbabwe National Breeding Programme mainly because most of the maize in the country is produced under rain-fed conditions and is a major enterprise on small scale farms, where drought is considered to be the chief abiotic limitation to production. The Zimbabwe National Breeding Programme is one of the partners that have worked closely with CIMMYT in producing drought tolerant maize varieties. However, the predominant maize inbred lines used by the National Breeding Programme in the most successful and current commercial hybrids and their derivatives were developed in the last century and as such they were not screened for drought tolerance. To this effect the National Breeding Programme has acquired a number of inbred lines from CIMMYT for use in improvement of existing inbred lines, development of new inbred lines as well as constituting of new hybrids. However, little is known about the heterotic relationships between CIMMYT drought tolerant maize donors and Zimbabwe National Breeding Programme maize lines. In order to determine the best parents to use in the national breeding programme, it is important to understand the heterotic relationships between the CIMMYT and National Breeding Programme lines with a view of selecting good parents to initiate crosses for pedigree, backcross and potential marker assisted recurrent selection (MARS) populations for line extraction. In addition, the classification of the inbred lines into heterotic groups will facilitate exploitation of heterosis which can

contribute to hybrid performance. The objectives of this study were to estimate combining ability and heterotic patterns among 23 elite CIMMYT and DR&SS inbred lines for grain yield and other agronomic traits under optimal, low N and drought conditions.

3.2 Materials and methods

3.2.1 Germplasm

Ten DR&SS elite inbred lines susceptible to drought and diseases were crossed to 13 CIMMYT elite drought tolerant and disease resistant lines (Table 3.1) using a NCDII mating scheme. DR&SS lines were used as females and CIMMYT lines were used as males. Seventy-two single cross hybrids were successfully produced out of a potential 130 hybrids and the hybrids are presented in Appendix 1. The crosses were evaluated at six sites in the 2009/10 summer season and one managed drought site in the winter of 2010. The same evaluations were undertaken in the 2010/11 summer season and the winter of 2011.

Table 3.1 Maize germplasm used to produce the single cross hybrids

DR&SS lines	Heterotic group	CIMMYT lines	Heterotic group
N3.2.3.3.	N3	CML312-B	A
SC5522	SC	CML395-B	B
2Kba	SC	CML442-B	A
K64r	N3	CML444-B-B	B
NAW5885	N3	CML536	A
SV1P	SC	CML537	A
WCOBY1P	SC	CML538	A
2N3d	N3	CML539	A
RS61P	SC	CML548	A
RA214P	N3	CML545	A
		CML544	B
		CZL052	B
		CZL03007	B

3.2.2 Testing environments

Sites included one low N site, one mid-altitude site, four non-drought and high N sites and one managed drought site. The sites were the Agricultural Research Trust Farm (ART Farm)

(17°26'S, 31.5°E, 1 480 masl), Rattray Arnold Research Station (RARS) (17°40'S, 1 308 masl), Harare Research Station (HRS) (17.13°S, 31°E, 1 406 masl), Kadoma Research Institute (KRI) (18.32°S, 30.90°E, 1 155 masl), Chiredzi Research Station (CRS) (21.02°S, 31.58°E, 433 masl), and Chisumbanje Research Station (20°S, 33°E, 455 masl). The managed drought trial was planted at CRS during the winter. Trials were conducted in each site in the 2009/10 and 2010/11 summer seasons and 2010 and 2011 winter seasons at CRS only. ART Farm, RARS, CRS and Chisumbanje were non-stress sites, whilst Kadoma was non-stress within the mid-altitude and HRS was the low N site.

3.2.3 Management

General maize cultural practices were applied at all sites. Weeds were mostly controlled using herbicides at all sites, but hand weeding was also done when necessary. Managed drought trials were done in winter under irrigation, which was terminated two weeks before flowering to target stress during flowering. The total amount of rainfall received in all the sites in the 2009/10 and 2010/11 and the amounts of irrigation applied to managed drought trials are presented in Table 3.2. The level of stress applied was projected to achieve 15-20% (1-2 t ha^{-1}) of yields achieved under well watered conditions. This stress level delays silking and causes ear abortion in non-stress tolerant genotypes (Banziger *et al*., 2000). Such stress levels achieve an anthesis silking interval of between 4-8 days and 0.3-0.7 ears per plant (Banziger *et al*., 2000). The low N site used had already been depleted of N and this was achieved through growing summer maize and irrigated winter wheat continuously for six years. According to the soil analysis results the soil had the capacity to supply N since it contained 7 ppm in the top 30 cm of the soil and 7 ppm in the soil depth 30-60 cm. In terms of kg ha^{-1} this translates to 54 kg ha^{-1}. The 7 ppm was therefore considered as low N. Optimum sites constituted of the crop grown during the rainy season under rain-fed conditions with different N rates being applied to the crop in different sites. Maize fert (N-8, P-16, K-8) was applied as basal dressing with ART farm, Rattray Arnold, Chiredzi and Chisumbanje receiving 400 kg ha^{-1} and Kadoma 350 kg ha^{-1}. Different rates of ammonium nitrate (AN) were also applied with ART farm and Rattray Arnold receiving two split applications of 200 kg ha^{-1}, Chiredzi and Chisumbanje two split applications of 120 kg ha^{-1} and Kadoma two split applications of 100 kg ha^{-1}.

Table 3.2 Amount of rainfall received and irrigation applied in the 2009/10 and 2010/11 seasons

Site	Amount of rainfall(mm)		Irrigation applied (mm)	
	2009/10	2010/11	2009/10	2010/11
ART farm	714	529		
RARS	1074	907.5		
Harare	563	533.5		
Kadoma	593	500.5		
Chiredzi	332.3	341.3	220	220
Chisumbanje	335.2	466.5		

ART farm=Agricultural Research Trust farm; RARS=Rattray Arnold Research Station

3.2.4 Experimental design and data collection

Each site consisted of one hybrid and one parental trial and these were planted side by side. The hybrid trial consisted of 72 hybrids and eight hybrid checks whilst the line trial consisted of 23 lines and two inbred checks. Trials were planted in six sites during summer and one site during winter. Trials were planted using a 0.1 alpha lattice design with two replications for both the hybrid and line trials. The hybrid trials were one row each whilst the line trials constituted of two rows. The rows were 4 m long with an inter-row spacing of 0.75 m and in-row spacing of 0.25 m. A maximum of four seeds were planted per planting station and plants were thinned to one plant per station at three weeks after emergence to achieve the targeted plant population of 53 000 plants ha^{-1}. Different traits were measured and derived as described in Table 3.3. The data were collected following the standard practise at CIMMYT.

3.2.5 Statistical analysis

ANOVA for each environment and combined ANOVA were computed using the PROC MIXED procedure of SAS (SAS Institute, 2002). Genotypes were considered fixed effects whilst replications and incomplete blocks were considered random effects. The SAS programme for the line x tester analysis was used to compute the GCA and SCA effects following the procedure presented by Singh and Chaudhary (1977). The following line x tester model was used:

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

Where:

Y_{ijk} = mean value of a character measured on cross i x j in k^{th} replication

g_i = GCA effect of i^{th} parent

g_j = GCA effect of the parent j

s_{ij} = SCA effect of cross i x j

r_k = replication effect

e_{ijk} = environmental effect peculiar to $(ijk)^{th}$ individual

μ = population mean effect

Estimation of GCA effects:

Lines: $g_i = x_i\ldots/tr - y\ldots/lrt$

Testers: $g_t = x_{.j.}/lr - x\ldots/ltr$

Estimation of SCA effects:

$s_{ij} = x_{ij.}/r - x_i\ldots/tr - x_{.j.}/lr - x\ldots/ltr$

Where:

l = number of lines

t = number of testers

r = number of replications

Estimation of standard errors:

S.E. (GCA for line) = $(M_e/r \times t)^{½}$

S.E. (GCA for tester) = $(M_e/r \times l)^{½}$

S.E. (SCA effects) = $(M_e/r)^{½}$

S.E. $(g_i - g_j)$ line = $(2M_e/r \times t)^{½}$

S.E. $(g_i - g_j)$ tester = $(2M_e/r \times l)^{½}$

S.E. $(s_{ij} - s_{kl})$ = $(2M_e/r)^{½}$

For each cross combination (P_1 x P_2) MPH was calculated as the difference between the F_1 hybrid mean and the average of its parents (Falconer and Mackay, 1996) as follows:

MPH = [(F_1-MP)/MP] x 100

Where F_1 is the mean of the F_1 hybrid performance and MP = (P_1 + P_2)/2 in which P_1 and P_2 are the means of the inbred parents, respectively.

Heritability of all the traits was calculated using the narrow sense formula according to Hallauer and Miranda (1988).

h^2 = gca/ gca + sca + error

Where: h^2 = narrow sense heritability

gca = General combining ability

sca = Specific combining ability

3.3 Results

3.3.1 Analysis of variance and hybrid mean performance across all environments

The means for different traits presented in tables in this chapter are for best 10 and poorest 10 hybrids in terms of grain yield and the complete data for all the entries is found in Appendices 2-5. The data for GCA effects for lines and testers presented in figures is also presented in Appendices 6 and 7. ANOVA and means for grain yield and other agronomic traits across the 14 sites in the 2009/10 and 2010/11 seasons are presented in Tables 3.4 and 3.5. Sites were highly significantly different for all traits (Table 3.4, P≤0.001). Entries were highly significantly different for grain yield, anthesis date, anthesis silking interval, plant height and ear height (P≤0.001) and significantly different for ears per plant (P≤0.05). The general combining ability attributable to females (GCA_f) mean squares were highly significant (P≤0.001) for grain yield, anthesis days, anthesis silking interval, plant height and ear height, whilst ears per plant were only significant at P≤0.05. GCA_f*site was highly significant for grain yield, anthesis silking interval, plant height and ear height. The GCA_m was highly significant (P≤0.001) for grain yield, anthesis days, anthesis silking interval, plant height and ear height and was not significant for ears per plant.

Table 3.3 Agronomic traits that were measured and derived

Trait	Procedure
Anthesis days (AD) /silking date (SD)	Taken as number of days after planting to when 50% of plants started shedding pollen or had extruded silks of at least 5cm
Anthesis silking interval (ASI)	Derived from anthesis date and silking date as follows: ASI=SD-AD
Ears per plant (EPP)	Calculated as a ratio of the number of ears with at least one fully developed grain divided by the number of harvested plants
Plant height (PH)	Measured as the height between the base of a plant and the insertion of the first tassel branch
Ear height (EH)	Measured as the height between the base of a plant to the insertion of the top ear
Ear position (EPO)	Calculated as EH divided by PH
Root lodging (RL)	Measured as a percentage of plants that showed lodging by being inclined 45°
Stem lodging (SL)	Measured as a percentage of plants that were broken below the ear
Leaf senescence (SEN)	Measured on a score of 1-10 with 1 having no signs of senescence and 10 100% senescence
Ear rot (ER)	Measured as the number of ears affected then converted to percentage
Ear aspect (EA)	Measured as the appearance of the ear with 1 being excellent and 5 being very poor
Grain texture (TEX)	Measured on a scale of 1-5 with 1 being flint and 5 being dent
Grain yield (GYD)	Calculated from shelled grain weight per plot adjusted to 12.5% grain moisture
Foliar diseases	Measured on a scale 0f 1-5 where 1 is completely free from disease and 5 severely affected

The GCA_f and GCA_m mean squares were greater than SCA mean squares for all traits. SCA was significant for grain yield, anthesis silking interval and plant height ($P \leq 0.001$) and significant for anthesis days ($P \leq 0.05$). SCA*site was highly significant ($P \leq 0.001$) for anthesis days, plant height ($P \leq 0.01$) and anthesis silking interval ($P \leq 0.05$). The mean values for grain yield, anthesis days, anthesis silking interval, ears per plant, plant height and ear height across 14 sites were 4.07 t ha^{-1}, 70.6 days, 1.4 days, 0.84 ears, 238.5 cm and 122.4 cm respectively (Table 3.6).

Senescence is another important trait especially under drought and low N conditions. Entries were significantly different for this trait ($P \leq 0.001$) as well as for grey leaf spot, common rust and leaf blight turcicum (Table 3.5). The sites were significantly different for senescence, ear rot, grey leaf spot, common rust and leaf blight turcicum. The GCA_f mean squares were larger than GCA_m mean squares for senescence, grey leaf spot and leaf blight turcicum and on the other hand, the overall GCA mean squares were larger than the SCA mean squares. A check variety, entry 74 (SC727), out-yielded all experimental hybrids as well as the check varieties and had a mean yield of 5.62 t ha^{-1} (Table 3.6). The second best performer was entry 61 (RS61P/ CML548) with a mean yield of 5.31 t ha^{-1} and it performed above the mean. The poorest performing hybrid was entry 17 (2Kba/CML444) with a mean yield of 2.29 t ha^{-1}. Generally the hybrids had good disease scores for GLS, RUST and ET except for entry 48 (2N3d/CML444), which had a GLS score of 3.9 (Table 3.6).

Table 3.4 Combined analysis of variance of 14 sites in the 2009/10 and 2010/11 seasons for grain yield and other agronomic traits

Source	DF	GYD	AD	ASI	EPP	PH	EH	EPO
Site	13	547.24***	20561.34***	558.42***	2.12***	207100.97***	86941.07***	0.617***
Entry	69	9.70***	192.96***	16.61***	0.15*	4165.09***	2417.32***	0.012***
GCA_f	7	48.42***	887.08***	62.34***	0.23*	16331.75***	8475.14***	0.038***
GCA_m	11	18.93***	646.52***	25.18***	0.17	12050.78***	9060.36***	0.029***
GCA_f*site	79	2.90***	0.00	5.30***	0.15*	706.29***	430.18***	0.006
GCA_m*site	119	0.29	0.00	7.16***	0.13*	661.57***	344.137***	0.006**
Site*entry	765	1.35*	0.65	4.25***	0.12	448.98***	246.32**	0.004
SCA	52	2.69***	5.87**	8.46***	0.13	785.61***	185.72	0.005
SCA* site	566	1.33	5.52***	3.48*	0.11	368.55**	197.25	0.004
Error	843	1.34	3.24	2.92	0.11	301.78	192.6	0.004
Heritability (narrow sense) %		60	73	40	66	73	93	74

*****P≤0.001; **P≤0.01; *P≤0.05; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; EPP=ears per plant; PH=plant height; EH=ear height; EPO=ear position; DF=degrees of freedom; GCA_f=general combining ability attributable to females; GCA_m=general combining ability attributable to males; SCA=specific combining ability.**

Table 3.5 Combined analysis of variance across 14 sites for senescence and diseases in the 2009/10 and 2010/11 seasons

Source	DF	SEN	DF	ER	DF	GLS	RUST	ET
Site	4	1207.22***	11	5552.69***	2	70.71***	1.57***	4.20***
Entry	69	1.26***	69	105.95**	69	1.71***	0.18***	0.37***
Site*entry	254	0.12	627	80.71*	94	0.68***	0.10	0.24*
GCA_f	7	5.32***	7	201.76**	7	7.59***	0.28**	0.86***
GCA_m	11	3.21***	11	108.16	11	3.28***	0.51***	0.85***
GCA_f*site	26	0.43	65	123.85**	10	0.14	0.09	0.52**
GCA_m*site	40	0.00	97	89.94*	14	1.86***	0.28***	0.29*
SCA	52	0.65***	52	102.12*	52	0.57**	0.10	0.19
Site*line*tester	187	0.08	464	71.61	69	0.52**	0.06	0.19
Error	328	0.30	704	69.21	166	0.33	0.10	0.18
Heritability (narrow sense) %		59		49		43	67	92

*****P≤0.001; **P≤0.01; *P≤0.05; SEN=senescence; ER=ear rot; GLS=grey leaf spot; RUST=common rust; ET=leaf blight turcicum; DF=degrees of freedom; GCA_f=general combining ability attributable to females; GCA_m=general combining ability attributable to males; SCA=specific combining ability.**

Combined ANOVA across six optimum sites and the hybrid mean performances are presented in Table 3.7. Entry 74 (SC727) was the best performer (7.86 t ha^{-1}) across optimum conditions followed by entry 68 (RA214P/CML538) (Table 3.7). Entry 61 (RS61P/CML548) was still among the top 10 best performing hybrids and had an anthesis silking interval of -0.1 together with entry 23 CML545/2Kba-B) and entry 30 (K64r/CML442). Entries 57 (RS61P/CML444), 48 (2N3d/CML444) and 5 (N3.2.3.3/CML444) had the best ears per plant values of 1.00, 1.08 and 1.01 respectively. Entry 57 was the third best hybrid across all sites and maintained the same position under optimum conditions. Entries 74, 61, 57, 48, 52, 79 and 68 were amongst the best ten hybrids across all sites and under optimum conditions they were also amongst the best 10 hybrids, although rankings varied.

The mean performance for grain yield and other agronomic traits under managed drought conditions are presented in Table 3.8. The data presented is for the best 10 hybrids and the poorest 10 hybrids. The best ten hybrids performed well above the mean (2.09 t ha^{-1}). Entry 52 (CML548/2N3d) was the best performing hybrid under managed drought over two seasons with a mean yield of 3.26 t ha^{-1}.

Table 3.6 Performance of hybrids for grain yield and other agronomic traits across 14 sites in the 2009/10 and 2010/11 seasons

	Entry	GYD	AD	ASI	PH	EH	RL	SL	EPP	ER	GLS	RUST	ET	SEN
		t ha^{-1}	d	d	cm	cm	%	%	#	%	1-5	1-5	1-5	1-10
Best 10 hybrids	74	5.62	75.3	1.4	266.7	138.6	5.7	5.0	0.80	8.4	1.5	1.3	2.2	1.9
	61	5.31	69.7	0.6	230.7	119.5	11.0	3.7	0.92	5.0	2.7	1.0	1.5	2.1
	57	4.94	72.4	1.5	240.5	133.3	6.9	6.7	0.91	5.2	1.6	1.0	2.6	1.9
	54	4.89	70.2	1.6	242.2	138.7	8.3	4.6	0.87	6.9	2.1	1.0	1.8	1.9
	48	4.88	73.0	1.1	267.9	147.0	7.7	3.7	0.96	8.1	3.9	1.4	1.1	1.8
	63	4.78	69.8	1.2	227.3	121.1	19.4	6.7	0.85	4.7	1.3	1.0	1.6	2.1
	52	4.76	71.0	1.0	244.4	125.3	9.4	1.8	0.86	12.7	3.1	1.3	2.0	2.2
	79	4.74	75.1	0.9	243.3	135.6	5.4	5.5	0.83	4.4	1.9	1.0	1.5	1.8
	45	4.74	70.2	1.9	239.6	122.5	7.7	7.9	0.84	6.3	2.7	1.0	1.5	2.1
	68	4.69	70.7	1.5	235.5	114.6	10.0	0.6	0.89	7.4	1.8	1.0	1.5	2.3
Poorest 10 hybrids	69	3.48	71.7	1.6	191.4	91.6	6.2	20.8	0.72	7.3	2.6	1.0	1.0	2.4
	18	3.45	71.0	1.7	249.4	133.7	1.9	6.6	0.86	6.4	2.3	1.4	1.9	2.3
	39	3.41	71.4	0.7	251.1	132.2	6.0	0.7	0.87	8.2	3.3	1.5	1.0	1.7
	50	3.41	70.7	1.5	232.2	116.2	14.7	3.3	0.69	12.7	3.1	1.3	1.6	2.2
	14	3.28	75.6	2.6	257.7	142.8	7.0	11.4	0.74	6.1	1.6	1.2	1.8	1.9
	37	3.07	68.4	0.4	222.6	107.8	17.3	14.5	0.70	9.0	1.7	1.1	1.8	2.2
	73	2.91	72.3	4.2	250.8	136.2	20.7	2.9	0.55	13.2	2.7	1.2	2.0	2.3
	12	2.79	73.7	2.4	251.5	134.7	16.2	4.7	0.70	7.1	2.9	1.0	2.1	2.2
	29	2.37	71.2	3.3	238.3	127.3	18.6	21.5	0.74	9.8	2.8	1.3	2.7	2.0
	17	2.29	74.4	2.8	239.8	124.9	16.3	11.9	0.67	5.9	1.9	1.4	1.2	2.0
	Mean	**4.07**	**70.6**	**1.4**	**238.5**	**122.4**	**10.3**	**6.1**	**0.84**	**7.6**	**2.5**	**1.1**	**1.8**	**2.1**
	LSD	**0.68**	**0.9**	**0.9**	**10.1**	**8.0**	**9.2**	**11.0**	**0.11**	**4.4**	**0.9**	**0.5**	**0.9**	**0.4**
	MSE	**1.04**	**2.9**	**2.1**	**285**	**163.4**	**129.1**	**60.6**	**0.02**	**35.0**	**0.5**	**0.1**	**0.2**	**0.1**

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; RL=root lodging; SL=stem lodging; EPP=ears per plant; ER=ear rot; GLS=grey leaf spot; RUST=common rust; ET=leaf blight turcicum; SEN=senescence; LSD=least significant difference; MSE=mean square error; DF=degrees of freedom.

Table 3.7 Performance of hybrids for grain yield and other agronomic traits across six optimum sites in the 2009/10 and 2010/11 seasons

	Entry	GYD	AD	ASI	PH	EH	RL	SL	EPP	HC	ER	GLS	RUST	ET
		t ha^{-1}	d	d	cm	cm	%	%	#	%	%	1-5	1-5	1-5
Best 10 hybrids	74	7.86	73.1	0.6	296.5	148.5	4.9	5.0	0.97	22.4	4.5	1.5	1.3	2.2
	68	6.79	67.8	0.1	257.1	120.6	10.0	0.6	0.95	5.2	7.3	1.8	1.0	1.5
	57	6.63	70.0	0.5	262.5	142.5	11.1	6.7	1.00	11.1	2.7	1.6	1.0	2.6
	48	6.63	69.2	0.5	291.1	151.6	6.0	3.7	1.08	8.7	9.2	3.9	1.4	1.1
	61	6.62	67.5	-0.1	247.8	120.5	13.1	3.7	0.98	19.3	3.7	2.7	1.0	1.5
	7	6.61	67.5	1.0	266.5	122.7	9.8	5.6	0.86	8.8	6.5	1.8	0.9	1.8
	79	6.55	71.5	0.5	264.6	141.0	9.0	5.5	0.96	3.3	3.4	1.9	1.0	1.5
	5	6.54	69.6	0.7	272.4	142.7	10.8	13.1	1.01	13.1	2.9	3.4	1.1	2.3
	51	6.52	67.1	-0.9	265.6	116.9	8.0	7.6	0.98	16.0	7.3	3.6	1.0	1.6
	52	6.45	68.5	0.2	261.9	125.6	6.0	1.8	0.95	24.8	4.4	3.1	1.3	2.0
Poorest 10 hybrids	13	4.49	70.2	1.6	276.6	136.1	20.3	6.7	0.85	5.8	4.8	2.4	1.9	2.8
	23	4.46	63.8	-0.1	249.9	119.5	4.5	9.0	0.92	16.1	12.9	2.1	1.3	2.3
	30	4.44	65.4	-0.1	246.1	110.1	6.9	2.0	0.86	16.1	5.9	3.8	1.2	2.5
	22	4.33	64.3	0.1	250.0	120.2	12.1	4.4	0.92	16.5	3.0	3.0	1.0	1.0
	73	4.26	68.9	3.0	278.2	142.7	23.7	2.9	0.66	9.0	7.7	2.7	1.2	2.0
	18	4.17	68.8	0.6	270.5	134.1	-1.1	6.6	0.98	22.1	6.8	2.3	1.4	1.9
	37	3.67	65.3	0.1	240.9	111.6	25.2	14.5	0.87	7.9	6.7	1.7	1.1	1.8
	12	3.54	70.4	0.9	282.9	137.1	17.3	4.7	0.78	3.7	4.1	2.9	1.0	2.1
	17	3.10	71.2	1.6	270.4	125.2	14.0	11.9	0.90	19.5	8.2	1.9	1.4	1.2
	29	2.91	67.8	2.1	257.7	129.1	17.9	21.5	0.81	7.0	6.1	2.8	1.3	2.7
	MEAN	**5.39**	**67.7**	**0.6**	**260.2**	**125.5**	**9.9**	**6.1**	**0.94**	**13.0**	**5.3**	**2.5**	**1.1**	**1.8**
	LSD	**0.99**	**1.2**	**1.0**	**14.5**	**9.4**	**14.0**	**11.0**	**0.12**	**9.6**	**4.2**	**0.9**	**0.5**	**0.9**
	MSE	**1.24**	**2.1**	**1.4**	**263.7**	**112.6**	**147.6**	**60.6**	**0.01**	**92.1**	**13.4**	**0.5**	**0.1**	**0.2**

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; RL=root lodging; SL=stem lodging; EPP=ears per plant; HC=husk cover; ER=ear rot; GLS=grey leaf spot; RUST; common rust; ET=leaf blight turcicum; LSD=least significant difference; MSE=mean square error; DF=degrees of freedom.

Table 3.8 Performance of hybrids for grain yield and other agronomic traits across two managed drought sites in the 2009/10 and 2010/11 seasons

	Entry	GYD	AD	ASI	PH	EH	RL	EPP	SEN
		t ha^{-1}	d	d	cm	cm	%	#	1-10
Best 10 hybrids	52	3.26	98.0	1.1	236.9	121.6	5.5	0.95	3.7
	27	3.07	95.8	0.9	216.6	100.7	0.2	1.07	3.3
	19	3.01	91.7	1.9	230.5	105.7	0.7	0.90	3.2
	38	3.01	94.6	3.0	222.9	110.3	7.5	0.92	2.9
	59	2.84	94.8	-0.1	210.4	102.3	3.5	0.91	3.8
	36	2.79	90.6	-0.7	215.9	98.2	0.6	0.96	3.6
	61	2.79	94.1	1.9	230.1	114.4	2.3	0.97	3.6
	66	2.77	98.7	4.4	220.8	105.7	5.2	0.84	3.9
	3	2.74	97.1	3.0	228.5	119.4	31.4	0.79	3.7
	30	2.71	93.7	1.3	226.4	112.9	2.1	0.96	3.5
Poorest 10 hybrids	17	1.40	102.3	4.5	214.2	115.9	19.4	0.30	3.5
	68	1.40	96.8	4.0	219.3	102.1	-1.1	0.76	4.1
	65	1.36	102.8	3.9	238.7	120.6	0.4	0.19	3.8
	43	1.36	97.7	3.6	232.4	111.7	5.6	0.67	3.9
	13	1.35	97.7	0.1	238.2	112.6	4.5	0.10	3.8
	14	1.18	104.0	1.2	237.9	114.1	6.4	0.43	3.3
	51	1.17	97.2	1.0	227.6	104.3	9.5	0.68	3.5
	73	1.15	99.2	5.8	247.4	124.2	-0.7	0.21	4.0
	56	1.04	99.3	2.8	209.3	96.1	23.3	0.44	3.7
	69	0.59	95.6	2.9	166.9	66.4	-3.1	0.30	4.1
	Mean	**2.09**	**96.4**	**1.7**	**223.7**	**109.3**	**4.5**	**0.77**	**3.6**
	LSD	**0.92**	**2.6**	**2.3**	**20.9**	**23.0**	**15.7**	**0.45**	**0.73**
	MSE	**0.42**	**3.3**	**2.8**	**220**	**267.5**	**62.5**	**0.05**	**0.3**

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; RL=root lodging; EPP=ears per pant; SEN=senescence; LSD=least significant difference; MSE=mean square error; DF=degrees of freedom.

Entry 61 (RS61P/ CML548) and entry 52 (CML548/2N3d-B) were amongst the best 10 hybrids across sites (Table 3.6) as well as under managed drought (Table 3.8). Entry 59 (CML538/RS61P-B) and entry 36 CML545/K64r-B) had good anthesis silking interval values of -0.1 and -0.7 respectively, which are desirable under drought conditions. Entry 69 (CML539/RA214P-B) was the poorest amongst all hybrids with a mean yield of 0.59 t ha^{-1}. Entry 27 (K64r-B/CML536) had 1.07 ears per plant under managed drought and was the second best performing hybrid in terms of grain yield. The number of days to anthesis increased under managed drought conditions with a mean of 96.4 days compared to 67.7 days under optimum conditions.

Mean hybrid performance for grain yield and other secondary traits under low N conditions are presented in Table 3.8. Entry 76 (CZH0829), an experimental hybrid from CIMMYT, was the best performing hybrid, followed by entry 16 CZL03007/SC5522-B). Generally the mean yields were low under low N conditions with a mean of 0.47 t ha^{-1}. Entries 61, 54 and 57 remained within the ten best performing hybrids. Plant heights were reduced under low N conditions with a mean of 217.0 cm. The days to anthesis increased with anthesis-silking interval with mean values of 72.9 and 7.3 days, respectively. The ear rot values were also high with entry 2 recording 59.9% ear rot. Ears per plant values were also lower than under low N with a mean of 0.67 compared to 0.77 under managed drought.

3.3.2 Performance *per se* of inbred lines

In across site analysis the inbred line CML548 was the best performer (2.91 t ha^{-1}) and the poorest performer was SC5522 (0.51 t ha^{-1}) (Table 3.10). CML548 was also the best performer under optimum conditions and the poorest performer was again SC5522. Under managed drought 2Kba was the best yielder (3.57 t ha^{-1}) followed by K64r (3.12 t ha^{-1}). The CIMMYT inbred lines generally displayed better performance across all sites compared to the DR&SS inbred lines.

3.3.3 Combining ability and heritability

GCA variances had predominance over the SCA variances for all traits. In ears per plant SCA variance was 52% of the GCA variance. Ear height had the highest heritability value (93%) and anthesis-silking interval had the lowest heritability value (40%) (Table 3.11). Grain yield had a narrow sense heritability of 60%. Three traits anthesis-silking, ear rot and GLS had heritability estimates below 50%.

Table 3.9 Performance of hybrids across two low nitrogen sites in the 2009/10 and 2010/11 seasons

	Entry	GYD	AD	ASI	PH	RL	EPP	ER
		t ha^{-1}	d	d	cm	%	#	%
Best 10 hybrids	76	0.81	71.4	4.7	210.9	2.6	0.76	8.8
	16	0.77	73.4	5.9	228.0	1.9	0.56	12.6
	3	0.76	71.5	10.3	217.7	2.0	0.76	10.1
	1	0.75	73.4	4.6	222.3	0.8	0.79	11.7
	54	0.73	73.7	4.1	210.4	5.0	0.70	24.6
	61	0.73	70.7	2.3	177.7	7.2	0.76	9.3
	8	0.72	70.3	6.8	217.1	1.0	0.78	12.5
	78	0.71	74.1	7.9	192.3	6.3	0.65	27.9
	57	0.68	73.6	8.6	221.9	1.7	0.79	16.2
	26	0.67	70.4	6.3	239.7	3.1	0.71	26.0
Poorest 10 hybrids	30	0.26	69.7	8.8	185.1	7.1	0.86	26.8
	70	0.22	77.2	17.6	205.8	10.4	0.36	43.9
	73	0.22	74.8	12.2	215.0	15.2	0.59	29.6
	45	0.21	71.4	11.4	220.8	6.2	0.66	14.4
	2	0.2	74.6	8.6	184.9	4.7	0.44	59.9
	50	0.16	74.4	7.8	207.6	6.5	0.47	38.7
	37	0.15	73.7	9.5	185.7	8.3	0.38	30.6
	25	0.14	72.5	12.4	205.3	0.3	0.44	21.3
	11	0.10	71.9	12.6	234.5	31.6	0.67	16.8
	69	0.08	75.5	8.8	208.3	6.0	0.51	24.3
	Mean	**0.47**	**72.9**	**7.3**	**217.0**	**5.0**	**0.67**	**19.3**
	LSD	**0.34**	**3.9**	**5.0**	**46.6**	**22.3**	**0.22**	**21.8**
	MSE	**0.03**	**7.5**	**6.3**	**546.9**	**125**	**0.02**	**119.6**

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; RL=root lodging; EPP=ears per plant; ER=ear rot; LSD=least significant difference; MSE=mean square error; DF=degrees of freedom.

3.3.4 Correlation between grain yield and secondary traits

Grain yield was significantly negatively correlated ($P \leq 0.01$) with the secondary traits anthesis date (r=-0.314), anthesis silking interval (r=-0.299) and positively correlated with ears per plant (r=0.57) (Table 3.12). There was significant correlation ($P \leq 0.01$) of anthesis date with anthesis silking interval, plant height and ear height. Anthesis silking interval was negatively but significantly correlated with ears per plant. Grain yield was negatively correlated with senescence although the correlation was not significant.

Table 3.10 Performance of inbred parents for grain yield (t ha^{-1}) across different environments in the 2009/10 and 2010/11 seasons

Inbred line	Across	Optimum	Managed drought	Low N
N3233-B	1.52	1.94	2.98	0.83
SC5522-B	0.51	0.63	1.26	0.28
2Kba-B	1.17	1.55	3.57	0.35
K64r-B	1.37	1.77	3.12	0.02
NAW5885-B	1.30	1.67	2.61	0.02
SV1P-B	1.05	1.21	1.50	0.65
WCOBY1P-B	1.19	1.91	1.21	0.54
2N3d-B	1.98	2.65	2.29	1.11
RS61P-B	2.53	3.32	2.77	0.53
RA214P-B	1.75	2.31	1.58	0.78
Group Mean	**1.44**	**1.90**	**2.29**	**0.51**
CML312-B	1.68	2.25	2.41	0.80
CML395-B	2.39	3.20	2.48	0.71
CML442-B	2.27	2.91	2.34	0.76
CML444-B-B	2.58	2.60	1.29	1.43
CML536	1.81	2.37	1.94	0.65
CML537	2.32	3.16	0.94	0.52
CML538	2.37	2.98	2.85	1.21
CML539	1.99	2.54	2.02	1.15
CML548	2.91	3.87	1.51	1.34
CML545	2.58	3.04	2.99	2.30
CML544	1.95	2.42	1.92	1.53
CZL052	1.52	1.99	2.69	0.44
CZL03007	1.91	2.49	2.43	0.47
CML448	1.60	2.08	2.29	0.49
CML449	2.83	3.44	2.86	1.74
Group Mean	**2.18**	**2.75**	**2.54**	**1.10**
Mean	**1.88**	**2.41**	**2.63**	**0.83**
LSD	**0.49**	**0.62**	**1.22**	**1.32**
MSE	**0.34**	**0.36**	**0.51**	**0.41**
Min	**0.51**	**0.63**	**0.94**	**0.02**
Max	**2.91**	**3.87**	**3.57**	**2.3**

Table 3.11 General and specific combining ability variances and heritability estimates for the measured traits across sites

Trait	GCA variance	SCA variance	Heritability %
GYD	75.72	10.76	60
AD	3548.32	23.48	73
ASI	249.36	13.92	40
EPP	0.92	0.52	66
PH	48203.12	3142.44	73
EH	33900.56	742.88	93
SEN	21.32	2.60	74
ER	807.04	408.48	59
GLS	30.36	2.28	49
RUST	2.04	0.4	67
ET	3.44	0.76	92

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; EPP=ears per plant; PH=plant height; EH=ear height; SEN=senescence; ER=ear rot; GLS= grey leaf spot; RUST=common rust; ET=leaf blight turcicum; GCA=general combining ability; SCA=specific combining ability.

Table 3.12 Correlation coefficients between grain yield and other secondary traits under managed drought conditions

	GYD	AD	ASI	PH	EH	RL	EPP
AD	-0.31**						
ASI	-0.30**	0.30**					
PH	0.00	0.56**	0.14				
EH	0.07	0.51**	0.04	0.84			
RL	-0.68	0.24*	0.17	0.11	0.09		
EPP	0.57**	-0.47	-0.42**	-0.17	-0.09	-0.22	
SEN	-0.20	-0.21	0.13	-0.12	-0.23*	-0.03	-0.12

**** P≤0.01; *P≤0.05; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; RL=root lodging; EPP=ears per plant; SEN=senescence.**

Grain yield was negatively but significantly correlated (P≤0.01) with anthesis silking interval (r=-0.538), root lodging (r=-0.346) and ear rot (r=-0.434) under low N conditions (Table 3.13). It was also highly and positively correlated with ears per plant. Anthesis days were significantly (P≤0.01) and positively correlated with anthesis silking interval and negatively correlated with ears per plant. Anthesis silking interval was correlated with root lodging (P≤0.05), ears per plant and ear rot (P≤0.01). The correlation with ears

per plant and root lodging was negative. As expected, ears per plant were significantly and negatively correlated with ear rots.

Grain yield was significantly (P≤0.05) and negatively correlated with root lodging (r=-0.27) and stem lodging (r=-0.28) under optimum conditions (Table 3.14). It was also significantly (P≤0.01) and positively correlated with ears per plant (r=0.48). Anthesis days were significantly (P≤0.01) and positively correlated with anthesis silking interval and ear and plant height. Plant height was highly and positively correlated with ear height (r=0.85).

3.3.5 Relative contribution of general combining ability and specific combining ability sums of squares to variation

GCA sum of squares contributed a larger percentage of variation to grain yield across sites, and for optimum and low N conditions, whilst under managed drought conditions SCA contributed a higher percentage of the variation (Table 3.15). Again GCA made a larger contribution in all sites for anthesis days, plant height and ear height.

Table 3.13 Correlation coefficients between grain yield and other secondary traits under low nitrogen conditions

	GYD	AD	ASI	PH	RL	EPP
AD	-0.20					
ASI	-0.54**	0.30**				
PH	0.13	0.28*	0.07			
RL	-0.35**	0.03	0.27*	-0.20		
EPP	0.47**	-0.38**	-0.38**	0.22	-0.36**	
ER	-0.43**	0.07	0.35**	-0.17	0.12	-0.37**

****P≤0.01; *P≤0.05; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; RL=root lodging; EPP=ears per plant; ER=ear rot.**

Table 3.14 Correlation coefficients between grain yield and other secondary traits under optimum conditions

	GYD	AD	ASI	PH	EH	RL	SL
AD	0.01						
ASI	-0.21	0.49**					
PH	0.01	0.69**	0.50**				
EH	0.15	0.70**	0.49**	0.85**			
RL	-0.27*	0.03	0.28*	0.05	0.07		
SL	-0.28*	0.07	0.14	-0.11	-0.01	0.25*	
EPP	0.48**	0.01	-0.31**	-0.02	0.03	-0.34**	-0.10

****P≤0.01; *P≤0.05; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; RL=root lodging; SL=stem lodging; EPP=ears per plant.**

SCA contributed more to variation for ears per plant (prolificacy) in all sites as well as for anthesis silking interval under drought and low N sites. GCA was partitioned into GCA_f and GCA_m (Table 3.15). GCA_f dominated GCA_m for grain yield across sites (52 vs 31%), under optimum conditions (53 vs 28%) and under low N conditions (44 vs 28%), whereas under drought they contributed the same percentage (22%). GCA_m was generally higher than GCA_f for anthesis days. Anthesis silking interval was controlled by GCA_f across sites and under low N sites, whilst under drought and optimum conditions it was controlled by GCA_m.

3.3.6 Importance of maternal and paternal effects

In across site analysis GCA_f mean squares were higher than GCA_m mean squares for grain yield, anthesis days, anthesis silking interval, plant height, ears per plant, senescence, ear position, GLS and ear rot (Table 3.16). Under optimum conditions GCA_f mean squares were higher than GCA_m mean squares for grain yield, anthesis days, anthesis silking interval, plant height and GLS. GCA_f and GCA_m mean squares for grain yield under drought conditions were both not significant. GCA_f mean squares were again higher for grain yield under low N conditions as well as for anthesis silking interval. The mean squares for senescence were both not significant under drought conditions.

Table 3.15 Relative contribution (∑SS) for GCA and SCA across environments

		% Sum of Squares		
Trait	**Environment**	**Female GCA**	**Male GCA**	**SCA**
GYD	Across	52	31	17
	Optimum	53	28	19
	Drought	22	22	56
	Low N	44	28	28
AD	Across	26	53	21
	Optimum	38	55	7
	Drought	39	51	10
	Low N	30	47	23
ASI	Across	38	24	38
	Optimum	34	36	30
	Drought	12	21	67
	Low N	29	22	49
EPP	Across	15	18	67
	Optimum	15	30	55
	Drought	24	15	61
	Low N	19	19	62
SEN	Drought	20	25	55
PH	Across	40	46	14
	Optimum	43	47	10
EH	Across	34	60	6
	Optimum	32	59	9

GCA=general combining ability; SCA=specific combining ability; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; EPP=ears per plant; SEN=senescence; PH=plant height; EH=ear height.

3.3.7 General combining ability effects across environments

The inbred line GCA effects across sites for grain yield, anthesis days, anthesis silking interval, ears per plant, plant height, ear height, root lodging, stem lodging and senescence are presented in Figures 3.1, 3.2 and Table 3.17.

The line with the best GCA value for grain yield (0.72) (Figure 3.1) and ears per plant (0.064) (Table 3.17) across all environments was RS61P. SC5522 had the poorest GCA effect for grain yield (-0.95) (Figure 3.1). The ideal line GCA values for anthesis days would be the negative values and in this case the line with the best GCA value for anthesis days was 2Kba (-1.99) (Figure 3.2).

Table 3.16 Mean squares for general combining ability due to female and male effects under different environments

Trait	Across		Optimum		Managed drought		Low N	
	GCA_f	GCA_m	GCA_f	GCA_m	GCA_f	GCA_m	GCA_f	GCA_m
GYD	48.42***	18.93**	37.88***	12.76***	3.21	2.02	3.98***	1.62***
AD	887.08***	646.52***	213.55***	194.83***	210.91***	177.88***	76.78***	76.98***
ASI	62.34***	25.18***	22.68***	15.39***	10.38**	11.46***	48.91***	23.14**
PH	16331.75***	12050.78***	11775.29***	8195.45***	1880.71***	1632.11***	3563.03***	1941.45***
EH	8475.14***	9060.36***	4574.82***	5346.24***	1366.65***	1399.11***	541.46*	577.16**
EPP	0.23*	0.17	0.048*	0.062***	0.18**	0.07	0.12***	0.07**
SEN	5.32***	3.21***	nm	nm	0.74	0.6	nm	nm
EPO	0.038***	0.029***	0.022***	0.018***	0.009*	0.007*	0.005	0.006
GLS	7.59***	3.28***	7.58**	3.27***	nm	nm	nm	nm
RUST	0.28**	0.51***	0.28*	0.51***	nm	nm	nm	nm
ET	0.86**	0.85***	0.86***	0.84***	nm	nm	nm	nm
ER	201.76**	108.16	61.66**	96.74***	nm	nm	nm	nm

*****P≤0.001; **P≤0.01; *P≤0.05; GCA_f=female general combining ability; GCA_m=male general combining ability; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPP=ears per plant; SEN=senescence; EPO=ear position; GLS=grey leaf spot; RUST=common rust; ET=leaf blight turcicum; ER=ear rot; nm=not measured.**

SC5522 had the highest positive GCA effect for anthesis days (5.19) which is an indication that it is a very late line. K64r had the best GCA value for anthesis silking interval (-0.69), plant height (-9.5) and ear height (-7.7) (Table 3.17).

The tester GCA effects for grain yield are presented in Figure 3.3. The tester with the best GCA effect for grain yield was tester 11 (CML548). Testers with the poorest GCA effect for grain yield were testers 1 (CML395) and 12 (CZL052) (Figure 3.3). Testers 3 and 7 had good anthesis days values whilst tester 10 had the highest positive GCA value for anthesis days, indicating that it is very late (Figure 3.4). The tester with the best GCA effect for anthesis silking interval was tester 12, while tester 1 had the poorest GCA effect for anthesis silking interval. The tester GCA effects for other agronomic traits are presented in Table 3.18.

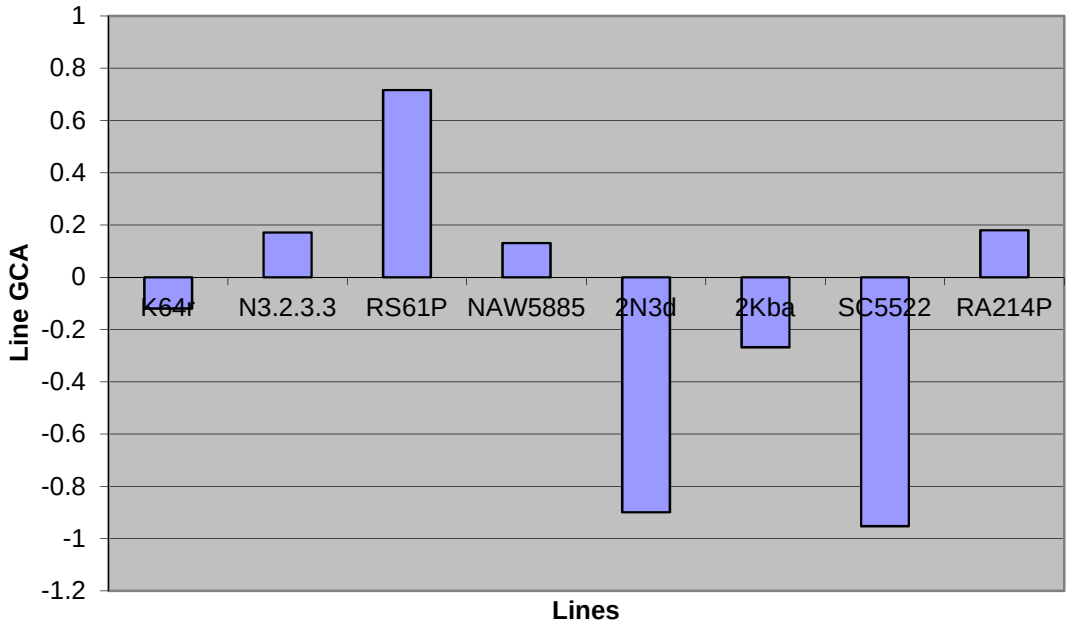

Figure 3.1 Line general combining ability (GCA) values for grain yield for all environments.

Table 3.17 Line general combining ability values for other agronomic traits for all environments

Line	ASI	EPP	PH	EH	RL	SL	SEN
K64r	-0.69*	0.001	-9.5**	-7.7**	1.8	1.2	0.4*
N3.2.3.3	0.34	-0.026	6.2*	5.1*	0.5	-0.3	1.2***
RS61P	-0.57*	0.064	-6.6*	1.7	-0.9	-5.6*	-1.9***
NAW5885	0.71**	0.015	2.3	-2.7	1.0	1.3	0.2*
2N3d	-0.29	-0.011	14.0	7.2**	-6.7*	2.8	1.5***
2Kba	-0.05	-0.009	-5.6*	-3.9	2.4	-0.1	-0.5*
SC5522	0.70**	-0.062	20.9***	16.8***	-1.5	0.1	-0.8*
RA214P	0.38	-0.016	0.7	-1.0	-2.3	0.3	0.1
LSD (0.05)	**0.015**	**0.00056**	**1.54**	**0.98**	**1.43**	**4.33**	**0.004**
SE	**0.348**	**0.067**	**3.545**	**2.83**	**3.28**	**4.19**	**0.111**

*****P≤0.001; **P≤0.01; *P≤0.05; ASI=anthesis silking interval; EPP=ears per plant; PH=plant height; EH=ear height; RL=root lodging; SL=stem lodging; SEN=senescence; LSD=least significant difference.**

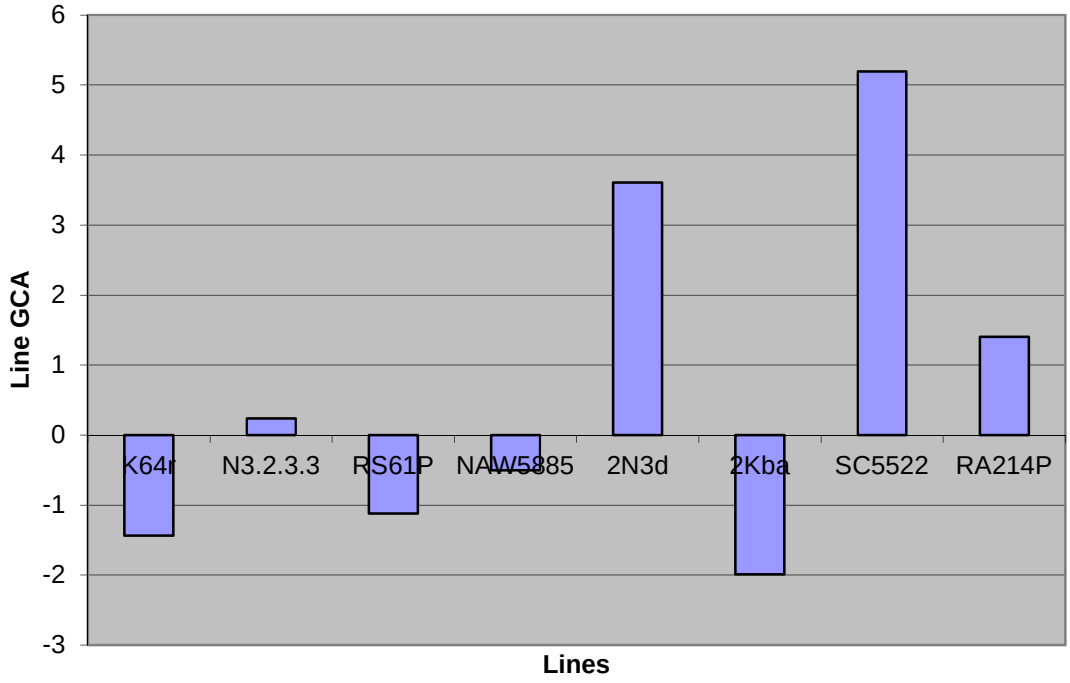

Figure 3.2 Line general combining ability (GCA) values for anthesis days for all environments.

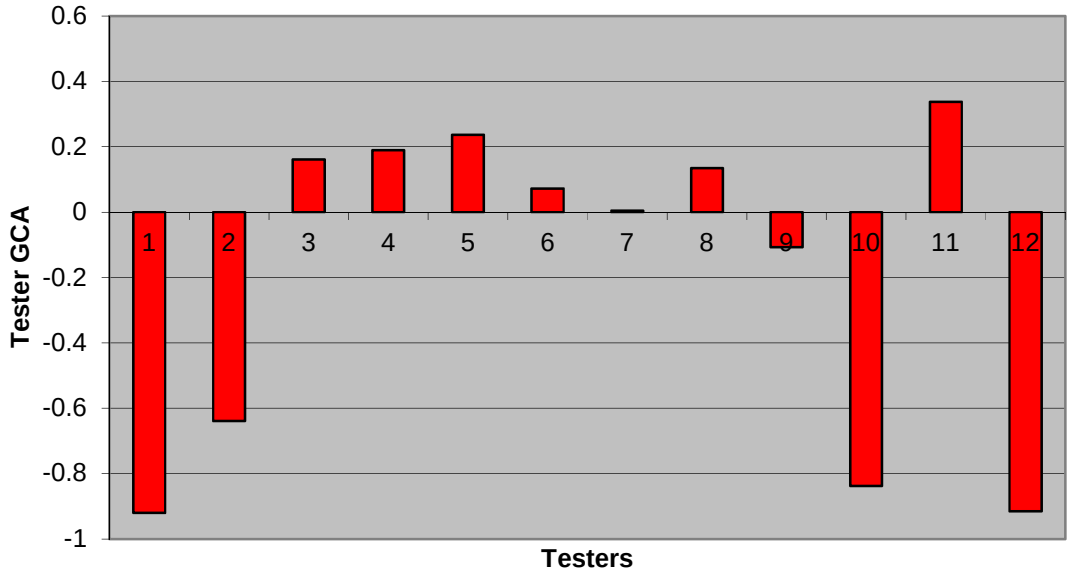

Figure 3.3 Tester general combining ability (GCA) effects for grain yield for all environments.

Figure 3.4 Tester general combining ability (GCA) effects for anthesis days for all environments.

Table 3.18 Tester general combining ability effects for other agronomic traits under all environments

Tester	ASI	EPP	PH	EH	RL	SL	SEN
1	1.00**	-0.078	19.0***	15.7***	2.2	1.8	0.5**
2	-0.50*	0.022	-9.3**	-6.0*	-8.4*	-1.8	-0.3*
3	-0.20	0.005	-13.2***	-11.1***	0.1	0.4	-0.3*
4	0.10	0.003	-0.3	-3.3	-0.8	-1.5	-0.7**
5	0.10	0.000	2.4	-0.1	4.0	-4.0	0.7**
6	-0.10	-0.005	-6.0*	-3.8	2.3	3.8	-1.6***
7	-0.60*	0.034	-4.0	-1.0	-1.1	-2.2	1.1***
8	-0.20	-0.032	-3.2	-4.9	-1.0	-2.4	0.3*
9	0.30	-0.026	11.3***	11.7***	-1.0	-1.8	0.2
10	-0.10	0.027	20.0***	15.6***	-0.1	14.7***	-0.7**
11	0.20	0.058	-2.3	-1.6	-1.5	-0.9	0.8**
12	-1.00**	0.026	-6.4*	0.2	-9.8**	1.4	-3.3***
LSD (0.05)	**0.02**	**0.00056**	**1.54**	**0.98**	**1.43**	**4.33**	**0.004**
SE	**0.43**	**0.082**	**4.34**	**3.47**	**4.02**	**5.13**	**0.136**

*****P≤0.001; **P≤0.01; *P≤0.05; ASI=anthesis silking interval; EPP=ears per plant; PH=plant height; EH=ear height; RL=root lodging; SL=stem lodging; SEN=senescence; LSD=least significant difference.**

3.3.8 General combining ability effects under optimum conditions

The line GCA effects for grain yield were significant (P≤0.05). The line with the best positive GCA effect for grain yield was RS61P (0.64) followed by N3.2.3.3 (0.59) (Figure 3.5). Four lines K64r, 2N3d, 2Kba and SC5522 had negative GCA effects for grain yield under optimum conditions with SC5522 having the poorest GCA effect of -1.32. GCA effects for the other agronomic traits under optimum conditions are presented in Table 3.19. There were five lines with negative GCA effect for anthesis days, which is an indication of their earliness. K64r recorded a GCA effect for anthesis days of -1.68 followed by 2Kba with a GCA effect of -1.43. SC5522 had the highest positive GCA effect for anthesis days of 3.75. Anthesis silking interval is another important trait to consider in selection and lines K64r, RS61P, 2N3d and 2Kba recorded negative GCA effects for anthesis silking interval of -0.64, -0.42, -0.29 and -0.14 respectively. Generally RS61P displayed good GCA effects for traits such as plant height, ears per plant, root and stalk lodging and diseases with the exception of ear height. SC5522 had the highest positive GCA effect for plant height (25.49), ear height (18.24) and root lodging (5.59). K64r had positive GCA effects for all diseases recorded.

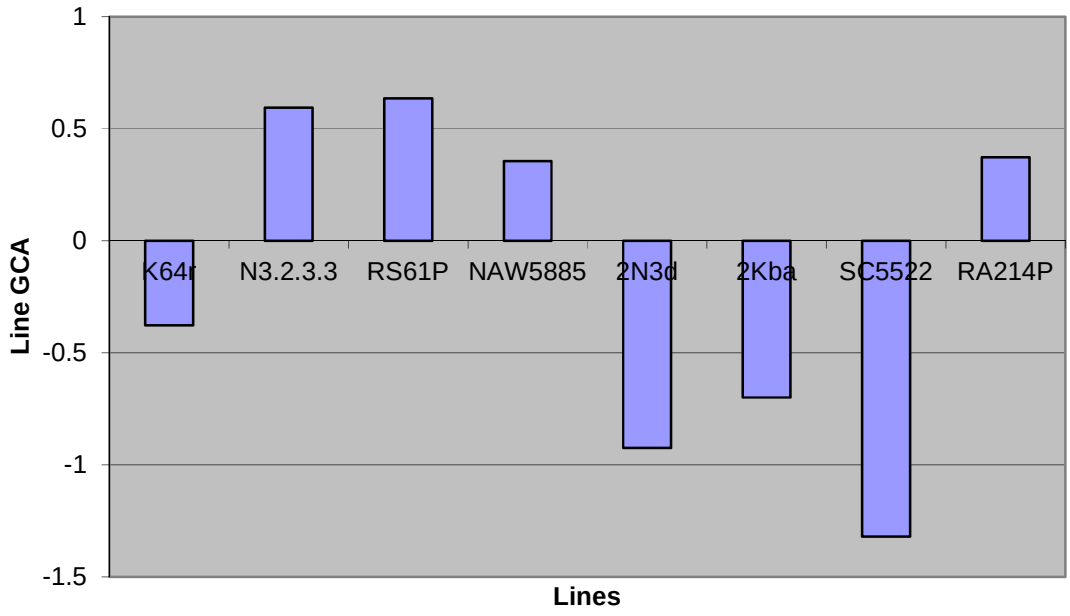

Figure 3.5 Line general combining ability (GCA) effects for grain yield under optimum conditions.

Table 3.19 Line general combining ability effects for anthesis days and other agronomic traits under optimum conditions

Line	AD	ASI	EPP	PH	EH	RL	SL	ER	ET	GLS	RUST
K64r	-1.68***	-0.64**	-0.014	-12.76***	-9.60***	0.38	-0.95	1.19***	0.20**	0.01	0.05
N3.2.3.3	0.45*	0.40*	0.009	11.39***	8.30***	2.55	0.62	-0.42	0.03	0.39*	0.01
RS61P	-0.64**	-0.42*	0.039	-11.13***	1.38	-1.13	-1.73	-1.30***	-0.01	-0.38*	-0.12*
NAW5885	-0.11	0.64**	-0.017	4.99*	-1.33	0.06	0.46	0.08	-0.23**	0.05	0.02
2N3d	1.58***	-0.29	-0.001	14.32***	4.07**	0.97	0.36	-0.17	-0.03	1.36***	0.15*
2Kba	-1.43***	-0.14	-0.013	-6.53*	-4.90**	-0.64	1.32	0.79*	0.04	-0.11	0.00
SC5522	3.75***	0.96***	-0.054	25.49***	18.24***	5.59*	-0.26	-4.78***	0.24**	-0.10	0.22*
RA214P	1.83***	0.15	0.014	1.78	0.07	-4.57*	0.13	0.47	-0.09	-0.39*	-0.06
LSD (0.05)	**0.027**	**0.017**	**0.00019**	**2.64**	**1.57**	**2.51**	**1.35**	**0.21**	**0.004**	**0.0078**	**0.002**
SE	**0.309**	**0.242**	**0.0258**	**3.03**	**2.34**	**2.95**	**1.76**	**0.69**	**0.084**	**0.1153**	**0.061**

*****P≤0.001; **P≤0.01; *P≤0.05; AD=anthesis days; ASI=anthesis silking interval; EPP=ears per plant; PH=plant height; EH=ear height; RL=root lodging; SL=stem lodging; ER=ear rot; ET=leaf blight turcicum; GLS=grey leaf spot; RUST=common rust; LSD=least significant difference.**

Tester GCA effects for grain yield and other agronomic traits are presented in Table 3.20. Tester 11 (CML548) had the highest positive GCA effect for grain yield and the tester with the poorest GCA effect was tester 10 (CML536) with a negative GCA effect. Tester 2 (CML312) had the best GCA effects for both anthesis days and anthesis silking interval, whilst tester 10 (CML536) had the highest positive GCA effect for anthesis days.

3.3.9 General combining ability effects under managed drought conditions

Lines K64r and RS61P had the highest significant ($P \leq 0.05$) positive GCA effects for grain yield under drought conditions of 0.37 followed by NAW5885 with a GCA effect of 0.12 (Figure 3.6). The rest of the lines had negative GCA effects for grain yield under drought conditions with SC5522 having the poorest GCA effect. The GCA effects for the secondary traits anthesis days, anthesis silking interval, ears per plant and senescence are presented in Table 3.21. Two lines K64r and RS61P, had negative GCA effects for both anthesis days and anthesis silking interval. These two lines also recorded good GCA effects for ears per plant and senescence. Lines SC5522 and RA214P had poor GCA effects for anthesis days and other secondary traits.

3.3.10 General combining ability effects under low nitrogen conditions

Three lines had significant positive GCA effects for grain yield under low N conditions with RS61P having the best GCA effect of 0.58 (Figure 3.7). Line 2N3d had the poorest GCA effect of -0.70 followed by SC5522 with a GCA effect of -0.63. Tester 3 (CML 539) had the best GCA effect for grain under low N conditions (0.30) while tester 1 (CML 395) had the lowest GCA effect (-0.68) (Figure 3.8). Lines 2Kba and RS61P showed significant negative GCA effects for anthesis days of -1.6 and -1.4 respectively whilst lines 2N3d and SC5522 showed significant positive GCA effects of 2.7 and 4.1 respectively (Table 3.22). Line RS61P had a significant negative GCA effect for anthesis silking interval. RS61P and N3.2.3.3 had positive significant GCA effects for ears per plant. Tester GCA effects for other traits are presented in Table 3.23.

Table 3.20 Tester general combining ability effects for grain yield and other agronomic traits under optimum conditions

Tester	GYD	AD	ASI	PH	EH	RL	SL	EPP	ER	ET	GLS	RUST
1	-1.19***	2.7***	1.4***	20.4***	15.2***	9.7***	-2.2	-0.073	-4.9***	0.6**	0.3*	0.4*
2	-0.97**	-3.3***	-1.2***	-21.5***	-12.2***	-9.6***	9.5***	0.004	-3.3***	-0.3*	-0.6**	-0.1
3	0.22	-1.6**	-0.4*	-15.7***	-10.7***	-4.1	2.6*	-0.001	-0.8*	-0.1	0.3*	-0.1
4	-0.02	-0.4	0.2	-1.0	-4.8*	-1.7	-1.9	-0.009	-0.1	0.1	0.5**	0.0
5	0.16	0.5	0.2	2.9*	-0.7	3.6	-1.8	0.029	0.4	-0.1	0.3*	-0.1
6	0.02	-0.6	-0.2	-4.6*	-3.2*	3.2	1.6	-0.011	-1.7**	-0.1	-0.5**	0.0
7	0.10	-2.2***	-0.6*	-6.3*	-3.2*	-5.9*	-1.3	0.040	2.7**	0.0	-0.1	-0.1
8	0.25	-0.4	-0.2	-4.6*	-5.9*	-1.3	-2.9*	-0.048	1.4**	0.1	-0.4*	-0.1
9	0.01	3.0***	0.2	15.9****	16.7***	-0.4	4.3**	0.025	1.4**	0.0	-0.1	0.2*
10	-1.34***	3.6***	-0.2	29.6***	22.7***	13.2***	-3.7*	0.016	-3.5***	0.2*	-0.1	0.3*
11	0.48*	0.1	0.3	-0.1	-1.1	-3.9	-1.0	0.003	-0.9*	-0.2*	0.0	0.0
12	-1.10***	-3.0***	-1.0***	-23.1***	-4.7*	-3.5	-1.6	0.004	-3.3***	0.6**	-0.3*	0.2*
LSD (0.05)	**0.024**	**0.027**	**0.017**	**2.64**	**1.57**	**2.51**	**1.35**	**0.00019**	**0.27**	**0.004**	**0.0078**	**0.002**
SE	**0.25**	**0.379**	**0.297**	**3.71**	**2.86**	**3.61**	**2.16**	**0.03162**	**0.87**	**0.1030**	**0.1414**	**0.075**

*****P≤0.001; **P≤0.01; *P≤0.05; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; RL=root lodging; SL=stalk lodging; EPP=ears per plant; ER=ear rot; ET= leaf blight turcicum; GLS=grey leaf spot; RUST=common rust; LSD=least significant difference.**

Tester 3 (CML539) had the best significant negative GCA effect for anthesis days (-2.2), whilst tester 12 (CZL052) had a significant negative GCA effect for anthesis silking interval (-1.7). Tester 4 (CML 442) had the best GCA effect for ears per plant under low N conditions (Table 3.23), whilst tester 5 (CML 537) had the poorest GCA effect. Testers 1 (CML395), 2 (CML 312), 6 ([CML445/ZM621B]-2-1-2-3-1-B*8), 10 (CML536) and 11 (ZM523A-16-2-1-1-B*5) showed good GCA effects for both plant height and ear height.

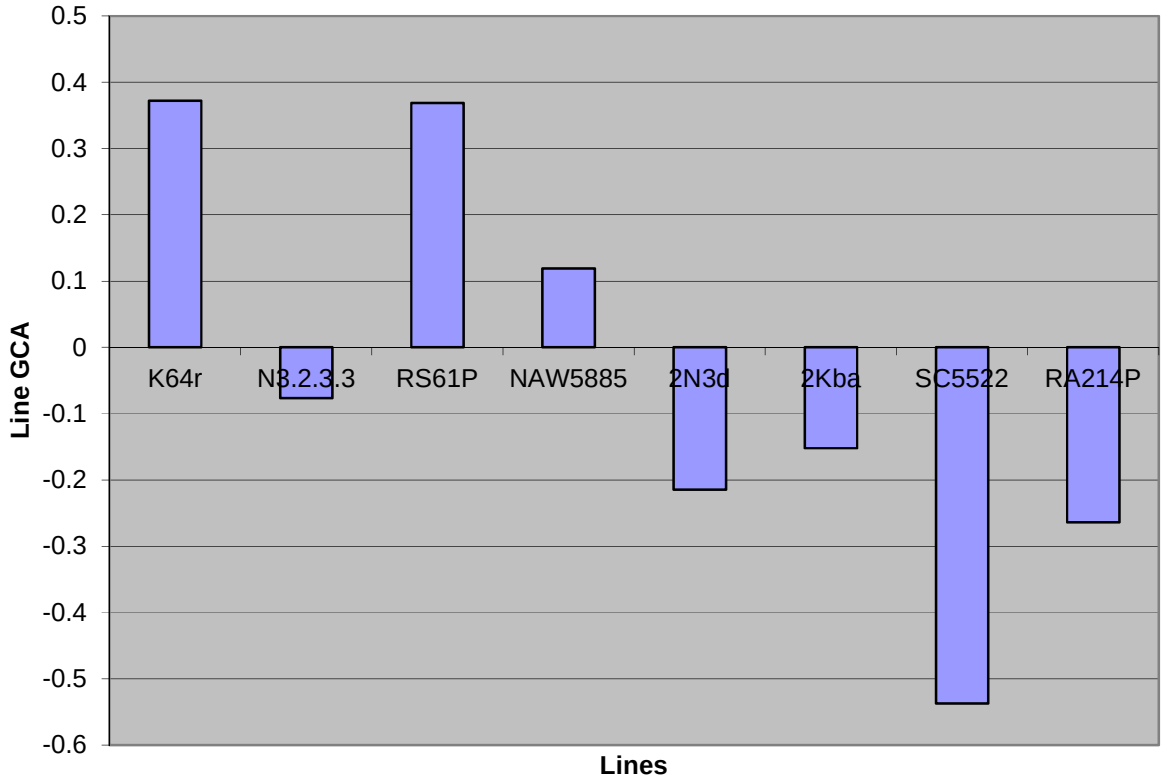

Figure 3.6 Line general combining ability (GCA) effects for grain yield under managed drought conditions.

3.3.11 Specific combining ability effects across all environments

The SCA effects for crosses are presented in Table 3.24. The line and tester GCA effects are also presented in the same table in order to determine whether lines with good GCA effects also produced crosses with good SCA effects or *vice versa*.

Table 3.21 Line general combining ability effects for anthesis days and other secondary traits under managed drought conditions

Line	AD	ASI	EPP	SEN
K64r	-2.0***	-0.7*	0.07	-0.02
N3.2.3.3	1.4**	0.4	-0.06	0.13
RS61P	-2.2***	-0.4	0.06	-0.21*
NAW5885	0.3	0.4	0.07	-0.23*
2N3d	2.8***	-0.5	-0.01	0.01
2Kba	-3.4***	0.3	0.01	0.09
SC5522	2.3***	0.1	-0.13	0.09
RA214P	2.7***	0.7*	-0.07	0.15
LSD (0.05)	**0.12**	**0.13**	**0.0019**	**0.01**
SE	**0.373**	**0.39**	**0.046**	**0.117**

*****P≤0.001; **P≤0.01; *P≤0.05; AD=anthesis days; ASI=anthesis silking interval; EPP=ears per plant; SEN=senescence; LSD=least significant difference.**

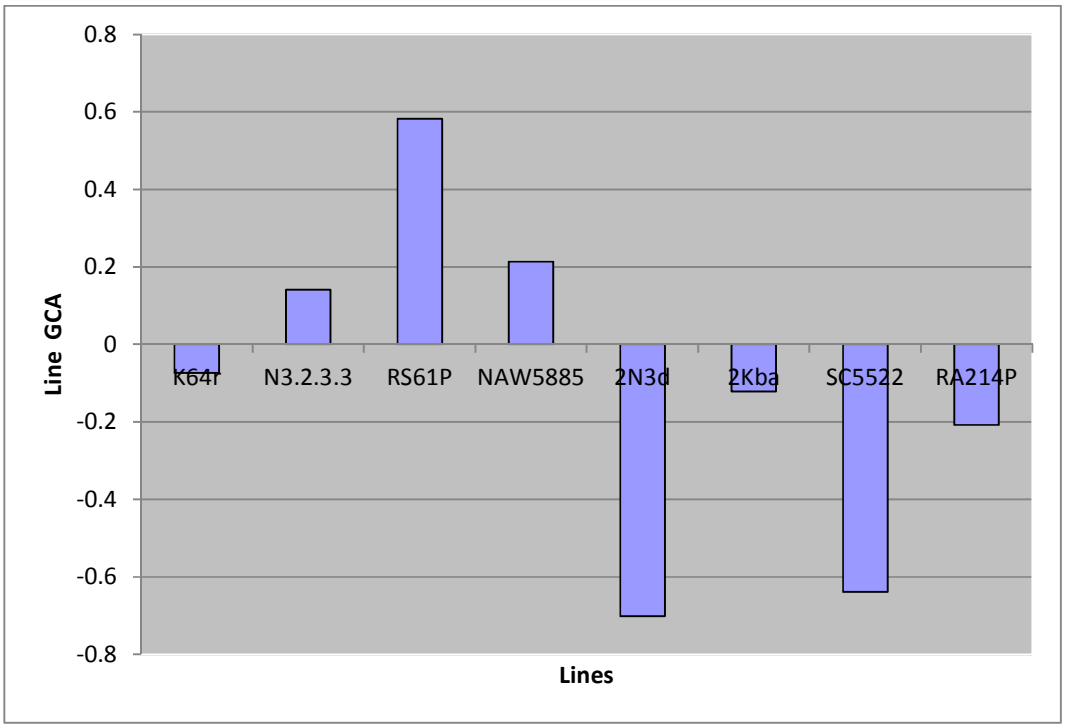

Figure 3.7 Line general combining ability (GCA) effects for grain yield under low nitrogen conditions.

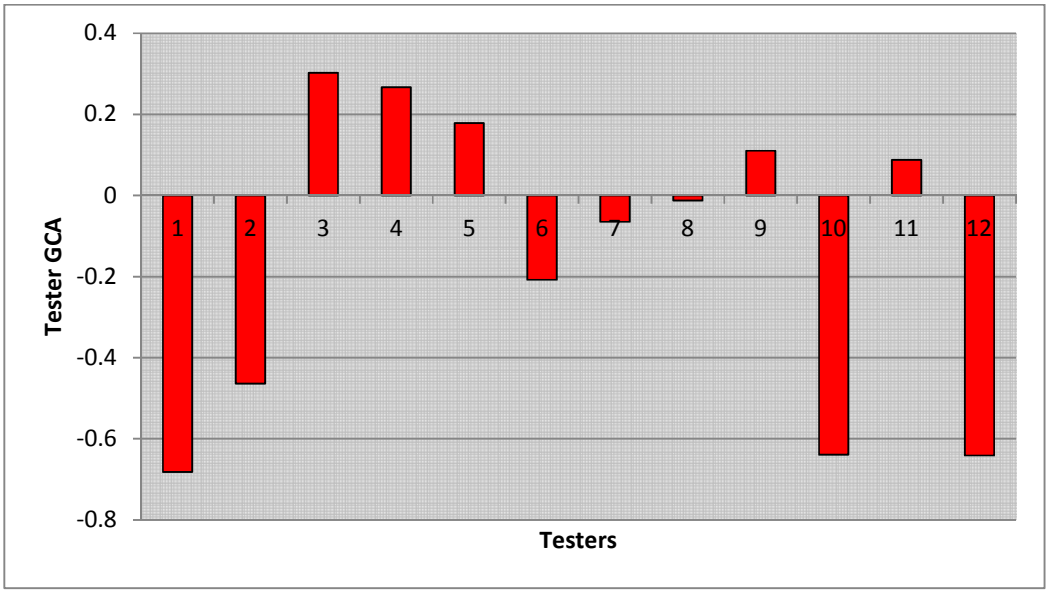

Figure 3.8 Tester general combining ability (GCA) effects for grain yield under low nitrogen conditions.

Table 3.22 Line general combining ability effects of other agronomic traits under low nitrogen conditions

Line	AD	ASI	EPP	PH	EH
K64r	-0.4	-0.9*	-0.0296	-6.9	-3.5
N3.2.3.3	0.2	0.8*	0.0230	3.3	1.2
RS61P	-1.4**	-2.1***	0.1314	8.8*	6.8*
NAW5885	-0.7	0.3	-0.0335	8.5*	1.0
2N3d	2.7***	1.5**	0.0087	-30.0***	-7.2**
2Kba	-1.6**	-0.4	-0.0298	4.3	2.1
SC5522	4.1***	1.9**	-0.0711	-16.0***	-7.5**
RA214P	1.4**	1.3**	-0.0427	2.4	-1.5
LSD (0.05)	**0.26**	**0.34**	**0.00089**	**24.05**	**8.04**
SE	**0.55**	**0.63**	**0.032**	**5.26**	**3.05**

*****P≤0.001; **P≤0.01; *P≤0.05; AD=anthesis days; ASI=anthesis silking interval; EPP=ears per plant; PH=plant height; EH=ear height; LSD=least significant difference.**

Table 3.23 Tester general combining ability effects of other agronomic traits under low nitrogen conditions

Tester	AD	ASI	EPP	PH	EH
1	3.6***	1.9**	-0.0972	-23.6***	-9.5**
2	-1.8**	1.1*	0.0849	-26.5***	-8.3**
3	-2.2***	-0.1	0.0703	7.2	-2.7
4	-1.3*	-1.3*	0.0972	3.0	-0.1
5	1.1*	-0.2	-0.0005	5.1	4.9*
6	-0.8	1.6**	-0.0793	-8.9*	-5.1*
7	-1.5*	-1.4**	0.0278	4.2	4.7*
8	-0.7	-0.4	-0.0093	2.0	-0.9
9	2.5***	0.5	-0.0283	10.1*	8.0**
10	5.5***	-0.7	0.0566	-25.3***	-12.8***
11	-0.2	0.7	-0.0534	-1.6	-1.1
12	-1.1*	-1.7**	0.0274	-11.2**	0.4
LSD (0.05)	**0.26**	**0.34**	**0.00089**	**24.05**	**8.04**
SE	**0.67**	**0.77**	**0.03924**	**6.45**	**3.73**

*****P≤0.001; **P≤0.01; *P≤0.05; AD=anthesis days; ASI=anthesis silking interval; EPP=ears per plant; PH=plant height; EH=ear height; LSD=least significant difference.**

The cross with the highest SCA (0.79) for grain yield was between line 3 (RS61P) and tester 9 (CML444) whilst the poorest (-0.76) was between line 8 (RA214P) and tester 3 (CML539). RS61P (line 3) had the best positive GCA effect and CML444 (tester 9) had negative GCA. The second best cross was between line 6 (2Kba) and tester 11 (CML548). Both the line and the tester had positive GCA effects. The poorest cross was between line 6 (2Kba) and tester 9 (CML444) and both these lines had negative GCA effects for grain yield. SC5522 had significant positive SCA effects of 0.77 and 0.75 respectively (data not shown) with CML395 and CML 536 of 0.77 despite the fact that it had the poorest GCA effect for grain yield.

Line 6 (2Kba) and tester 11 (CML548) produced a cross with the best SCA effects for anthesis days (-5.73) (Table 3.25). 2Kba (line 6) had a negative GCA effect (-1.99) for anthesis days whilst CML548 (tester 11) also had a negative GCA effect (-0.29) for anthesis days. The same line 2Kba produced a cross with a positive SCA effect (2.66) with tester 9 (CML444), which had a positive GCA effect of 2.86. Line 4 (NAW5885) with a negative

Table 3.24 Specific combining ability effects for grain yield across all environments

	Line							
Tester	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**GCA**
3	0.50*	0.15	-0.06	-0.52*	0.07	0.35	-0.76**	**0.16**
4	0.02	-0.23	-0.65*	0.51*	0.06	0.18	-0.17	**0.19**
5	0.36	-0.09	-0.26	-0.32	-0.45	0.27	0.03	**0.24**
7	0.14	0.50*	-0.20	-0.19	-0.50*	0.12	0.13	**0.01**
8	-0.09	0.03	-0.07	-0.17	-0.26	-0.22	0.36	**0.13**
9	0.35	0.32	0.79**	-0.30	0.33	-0.92**	-0.27	**-0.11**
11	0.03	-0.44	0.08	-0.50*	-0.16	0.68*	0.19	**0.34**
GCA	**-0.12**	**0.17**	**0.72**	**0.13**	**-0.9**	**-0.27**	**0.18**	

Lines 1=K64r; 2=N3.2.3.3; 3=RS61P; 4=NAW5885; 5=2N3d; 6=2Kba; 8=RA214P; Testers 3=CML539; 4=CML442; 5=CML537; 7=CML545; 8=CML538; 9=CML444; 11=CZL052; GCA=general combining ability. SE=0.82; LSDs:V(s_{ij})=0.014* and 0.018 for testing significance of SCA effects from zero; *P≤0.05; **P≤0.01.**

Table 3.25 Specific combining ability effects for anthesis days across all environments

	Line							
Tester	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**GCA**
3	-0.60	0.87	-0.56	0.03	0.81	-0.16	1.31*	**-2.11**
4	-0.32	-0.34	1.61*	-0.44	0.55	-0.02	0.55	**-0.78**
5	-0.47	-0.08	-0.71	1.52*	0.57	-0.03	0.80	**0.51**
7	-0.30	0.42	0.28	0.23	0.02	-0.14	-0.96	**-2.13**
8	-0.05	-0.51	0.35	1.48*	0.93	0.08	-0.53	**-0.91**
9	-0.54	-0.96	-0.25	-1.97*	-1.16	2.66***	-0.20	**2.86**
11	0.15	-0.49	0.40	2.07**	-0.57	-5.73***	0.88	**-0.29**
GCA	**-1.44**	**0.24**	**-1.12**	**-0.50**	**3.60**	**-1.99**	**1.40**	

Lines 1=K64r; 2=N3.2.3.3; 3=RS61P; 4=NAW5885; 5=2N3d; 6=2Kba; 8=RA214P; Testers 3=CML539; 4=CML442; 5=CML537; 7=CML545; 8=CML538; 9=CML444; 11=CZL052; GCA=general combining ability. SE=1.27; LSDs:V(s_{ij})=2.10* and 2.96 for testing significance of SCA effects from zero; *P≤0.05; **P≤0.01; ***P≤0.001.**

GCA effect (-0.50) produced three crosses with high positive SCA effects of 2.07, 1.52 and 1.48 with testers 11(CML548), 5 (CML537) and 8 (CML538) respectively.

SCA effects for anthesis silking interval are presented in Table 3.26. Crosses with negative SCA effects are ideal. The cross with the best SCA effect (-1.53) was between line 4 (NAW5885) and tester 9 (CML444) and both the line and the tester had positive GCA effects of 0.71 and 0.26 respectively. The same cross had the best GCA effect for anthesis days. The poorest cross (1.31) was between line 6 (2Kba) and tester 9 (CML444) with GCA effects of -0.05 and 0.26, respectively. The second best cross was between line 2 (N3.2.3.3) and tester

11 CML548 with a SCA effect of -0.66 and the two parents had positive GCA effects of 0.34 and 0.26, respectively.

Table 3.26 Specific combining ability for anthesis silking interval across all environments

	Line							
Tester	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**GCA**
3	-0.27	0.01	0.35	0.46	-0.15	-0.60	0.25	**-0.16**
4	-0.25	0.54	-0.36	0.33	-0.48	-0.23	0.29	**0.12**
5	-0.14	-0.13	0.14	0.03	0.30	-0.04	0.05	**0.08**
7	-0.17	-0.09	0.11	1.21*	-0.52	-0.06	-0.60	**-0.60**
8	-0.01	0.40	-0.58	-0.26	0.65	0.39	-0.22	**-0.16**
9	0.20	-0.30	0.25	-1.53*	-0.30	1.31*	-0.36	**0.26**
11	0.28	-0.66	-0.41	0.30	-0.23	-0.05	0.74	**0.24**
GCA	**-0.69**	**0.34**	**-0.57**	**0.71**	**-0.29**	**-0.05**	**0.38**	

Lines 1=K64r; 2=N3.2.3.3; 3=RS61P; 4=NAW5885; 5=2N3d; 6=2Kba; 8=RA214P; Testers 3=CML539; 4=CML442; 5=CML537; 7=CML545; 8=CML538; 9=CML444; 11=CZL052; GCA=general combining ability. SE=1.21; LSDs: V(sij)=1.99* and 2.82 for testing significance of SCA effects from zero. *P≤0.05**

3.3.12 Specific combining ability effects under optimum conditions

The cross between line 3 (RS61P) and tester 9 (CML444) had the highest positive SCA effect for grain yield under optimum conditions (0.99) and both parents had positive GCA effects for grain yield (Table 3.27). The second best cross was between line 1 (K64r) and tester 3 (CML 539) and one of the parents K64r had a negative GCA effect for grain yield (-0.38). The cross between line 3 (RS61P) and tester 4 (CML 442) had the highest negative SCA effect (-1.10). Line 6 (2Kba) and tester 4 (CML 442) produced a cross with a positive SCA effect (0.47) when they both had negative GCA effects for grain yield of -0.70 and -0.02 respectively. Line 4 (NAW5885) had positive GCA effect for grain yield but it produced crosses with negative SCA effects with testers 3 (CML 539), 5 (CML 537), 7 (CML545), 8 (CML 538), 9 (CML 444) and 11 (CML548) (Table 3.26).

3.3.13 Specific combining ability effects under managed drought conditions

Line 5 (2N3d) and tester 9 (CML 444) had negative GCA effects of grain yield of -0.21 and -0.42 respectively but they produced a cross with the highest significant positive SCA effect under managed drought (1.16) (Table 3.28). Tester 4 (CML 442) had the highest GCA effect but had crosses with negative SCA effects of -0.80 with line 5 (2N3d) and -0.74 with line 3 (RS61P) which also had a positive GCA effect. The parental lines with positive GCA effects

sometimes produced hybrids with negative SCA, whilst on the other hand some parental lines with negative GCA effects produced hybrids with positive GCA effects.

Table 3.27 Specific combining ability for grain yield under optimum conditions

	Line							
Tester	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**GCA**
3	0.94**	0.16	-0.01	-0.44	0.35	-0.19	-1.05**	**0.22**
4	-0.33	-0.07	-1.10**	0.57	0.42	0.47	-0.13	**-0.02**
5	0.38	-0.35	-0.05	-0.18	-0.26	0.11	-0.19	**0.16**
7	0.13	0.37	0.12	-0.26	-0.71	-0.07	0.13	**0.10**
8	-0.37	0.51	-0.49	-0.57	-0.18	-0.17	0.76*	**0.25**
9	0.62	0.67	0.99**	-0.68	-0.06	-0.86*	-0.54	**0.01**
11	-0.05	-0.48	0.12	-0.36	-0.55	0.72*	-0.07	**0.48**
GCA	**-0.38**	**0.59**	**0.64**	**0.36**	**-0.92**	**-0.70**	**0.37**	

Lines 1=K64r; 2=N3.2.3.3; 3=RS61P; 4=NAW5885; 5=2N3d; 6=2Kba; 8=RA214P; Testers 3=CML539; 4=CML442; 5=CML537; 7=CML545; 8=CML538; 9=CML444; 11=CZL052; GCA=general combining ability. SE=0.71; LSDs: (Vs_{ij})=1.17* and 1.65 for testing significance of SCA effects from zero; *P≤0.05; **P≤0.01.**

Table 3.28 Specific combining ability effects under managed drought conditions

	Line							
Tester	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**GCA**
3	0.32	0.32	0.78**	-0.46	-0.06	-0.41	-0.77**	**-0.13**
4	0.11	-0.13	-0.74**	0.66*	-0.80**	0.50*	0.25	**0.59**
5	-0.34	-0.87**	0.17	0.79**	-0.51*	0.03	0.58*	**-0.04**
7	0.50*	0.62*	-0.56*	-0.59*	-0.34	0.64*	0.06	**-0.16**
8	0.17	-0.60*	0.20	0.53*	-0.18	-0.20	-0.08	**-0.10**
9	-0.37	-0.36	0.06	0.24	1.16***	-0.69*	0.27	**-0.42**
GCA	**0.37**	**-0.08**	**0.37**	**0.12**	**-0.21**	**-0.15**	**-0.26**	

Lines 1=K64r; 2=N3.2.3.3; 3=RS61P; 4=NAW5885; 5=2N3d; 6=2Kba; 8=RA214P; Testers 3=CML539; 4=CML442; 5=CML537; 7=CML545; 8=CML538; 9=CML444; 11=CZL052; GCA=general combining ability. SE=0.42; LSDs: V(s_{ij})=0.69* and 0.97 for testing significance of SCA effects from zero. *P≤0.05; **P≤0.01; ***P≤0.001.**

3.3.14 Specific combining ability effects under low nitrogen conditions

Parental lines generally produced hybrids with negative SCA effects (Table 3.29). Line 1 (K64r) and tester 4 (CML442) produced a hybrid with the highest significant positive SCA effect (0.73). Line 3 (RS61P) with the highest GCA effects produced hybrids with negative SCA effects for grain yield with testers 4 (CML442), 5 (CML537) and 7 (CML545). Line 3 (RS61P) and tester 3(CML539) produced a cross with the second best (0.66) SCA effect.

Table 3.29 Specific combining ability effects under low nitrogen conditions

	Line							
Tester	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**GCA**
3	-0.11	0.10	0.66**	-0.15	-0.41**	0.16	-0.64**	**0.30**
4	0.73**	-0.15	-0.55**	0.05	-0.12	-0.09	-0.11	**0.27**
5	0.13	0.43**	-0.41**	-0.57**	-0.16	0.41**	-0.09	**0.18**
7	0.18	-0.07	-0.28	0.23	0.34*	-0.12	-0.01	**-0.07**
8	-0.30*	-0.06	0.26	-0.56**	-0.31*	0.21	0.43**	**-0.01**
9	0.26	0.07	0.49**	0.06	-0.07	-0.53**	-0.10	**0.11**
11	0.09	0.03	0.10	-0.16	-0.19	-0.18	-0.12	**0.09**
GCA	**-0.07**	**0.14**	**0.58**	**0.21**	**-0.70**	**-0.12**	**-0.21**	

Lines 1=K64r; 2=N3.2.3.3; 3=RS61P; 4=NAW5885; 5=2N3d; 6=2Kba; 8=RA214P; Testers 3=CML539; 4=CML442; 5=CML537; 7=CML545; 8=CML538; 9=CML444; 11=CZL052; GCA=general combining ability. SE=0.17; LSDs: V(sij)=0.28* and 0.40 for testing significance of SCA effects from zero; *P≤0.05; **P≤0.01.**

3.4 Discussion

The ANOVA revealed significant differences for grain yield and other agronomic traits among the inbred parents and their single cross hybrids in different environments. The environments under which evaluations were done were diverse and this was proved by the significant differences among them. The differential performance of genotypes over environments, as found in the current study, has implications for breeding, presenting the question of whether to breed for specific or general adaptation. On the other hand such information is useful in identifying a suitable genotype for specific environments. Entry 74 (SC727), a late maturing single cross hybrid, out-yielded all experimental hybrids as the other hybrids were among the medium maturity group. Late maturing hybrids always tend to give higher yields because when the season length is ideal they have more time to capture and utilise the sun's energy when forming their grain (Smith *et al*., 2009). Vasal *et al*. (1992) reported mean grain yields of 4.59 t ha^{-1} under sub-tropical environments and 4.35 t ha^{-1} under temperate environments. The average grain yield reported in this study for all crosses, across all environments, was 4.07 t ha^{-1} and this was again similar to the result reported by George *et al*. (2011). The four hybrids (entries 54, 57, 61 and 63) that were amongst the top 10 best performing hybrids in across site analysis had one common parent, RS61P. The highest yielding hybrid among the experimental hybrids, entry 61, was from a cross between heterotic groups CIMMYT A x DR&SS SC (B) and the lowest yielding hybrid entry 17 was between CIMMYT B x DR&SS SC (B) heterotic groups, while some of the best performing

hybrids (entries 54, 57 and 61) were among CIMMYT B x DR&SS SC (B). Results show that there was no consistency in hybrid performance based on predefined heterotic groups. Similar results were reported by Dhliwayo *et al*. (2009). Breeders at CIMMYT initially put more emphasis on population improvement at the expense of hybrids and they are now extracting lines from these broad populations (Kassa *et al*., 2012). Therefore heterotic grouping used at CIMMYT is too broad and it is difficult to divide lines into heterotic groups when the lines were developed from the same original pool without considering their racial origin or heterotic pattern. Again the designation of A and B groups at CIMMYT might have done in an arbitrary manner for the convenience of managing germplasm lines in hybrid-oriented programmes.

Lines and testers differed significantly for anthesis days under different environments. The number of days to anthesis was expressed differently by the lines across the tested environments. Mungoma and Pollak (1988) and Betran *et al.* (2003) reported significant differences for anthesis days under different environments and their results agree with findings in this study. The mean days to anthesis were higher under stress conditions (drought and low N) due to the negative effects the stress had on the growth of the maize crop. Vasal *et al*. (1992) recorded mean days to anthesis of 71 under temperate environments and 54 under sub-tropical environments. This has an implication on breeding, in that selection for anthesis days has to be done under optimum conditions to cater especially for seed production.

The trial mean yield of 2.09 t ha^{-1} reported in this study under drought was 61% lower than the trial mean (5.39 t ha^{-1}) under optimum conditions. According to Banziger *et al*. (2000) the level of yield reduction observed in the current study was associated with severe drought and results are within previously reported yield reduction ranges of drought stress levels to screen maize genotypes (Camphos *et al*., 2006). Betran *et al*. (2003) reported yield reductions of 13 and 50% under intermediate and severe drought stress, respectively, in one site and reductions of 5 and 48% in another site during the same season. Banziger *et al*. (2000) however, reported yield reductions under moderate drought of 15-20%. Negative anthesis silking-interval values reported in this study were indicative of ideal varieties under drought

conditions. This showed that the varieties were able to synchronise pollen shedding with silk emergence. Edmeades *et al*. (1993) concluded that a reduced anthesis silking interval was a sign of improved apportioning of assimilates to ears around flowering time. This scenario assists drought tolerant selection cycles to reach silking earlier and have a better ear biomass at anthesis. Entry 27 had an average number of ears per plant of 1.07 and this contributed to its good performance.

Highly significant and negative correlations between grain yield, anthesis days and anthesis silking interval under stress conditions were reported in this study and results are indicative of the fact that an increase in any of these traits results in a corresponding decrease in grain yield. These findings are consistent with findings by other investigators (Banziger *et al*., 1997; Betran *et al*., 2003; Zaidi *et al*., 2004; Derera, 2005; Gissa, 2008; Pswarayi and Vivek, 2008). A significant correlation (r=-0.39) of anthesis silking interval with grain yield for hybrids across all environments was found (data not shown) and this is consistent with findings of Betran *et al.* (2003), Zaidi *et al*. (2004) and Derera (2005) of -0.33 to -0.45 under moisture stress conditions. These results suggest that yield under stress can be improved by selecting for early silk emergence. The correlation coefficients reported in this study under stress conditions for anthesis days were -0.20 to -0.32 and for anthesis silking interval was -0.39 to -0.54. Again these values are in agreement with findings of Zaidi *et al*. (2004) of -0.22 to -0.56. Banziger *et al*. (1997) reported a correlation of -0.47 for anthesis silking interval with grain yield under low N, yet in this study a correlation of -0.54 was reported. However, Gissa (2008) reported a highly significant negative correlation between grain yield and anthesis silking interval of -0.60. A highly significant and positive correlation of ears per plant and grain yield reported in this study is consistent with findings by Banziger *et al*. (1997) and Gissa (2008). These results show that an increase in ears per plant results in a corresponding increase in grain yield under optimum, drought and low N conditions. Anthesis-silking interval and ears per plant are considered important secondary traits used in selection of drought tolerant materials (Bolanos and Edmeades, 1996; Vasal *et al*., 1997; Banziger and Lafitte, 1997; Edmeades *et al*., 1997; Banziger *et al*., 2000, Mhike *et al*., 2011b).

A number of investigators have reported variability in the performance of maize under low N conditions (Banziger *et al*., 1997; 2000; Betran *et al*., 2003; Gissa, 2008; Mhike *et al*., 2011a). The yield performance of hybrids in this study was 15% of the grain yield under optimum conditions, however this was lower than what Banziger *et al*. (1997) recommended. They reported that grain yield under low N stress should be between 25 and 35% of the average yield under optimum conditions. Betran *et al*. (2003) reported values as high as 65%. However some scientists indicated that varied magnitudes of grain loss can be expected under low N conditions (Banziger *et al*., 1999; Smallberger and Toit, 2004; Monneveux *et al*., 2005). Low yields under low N reported in the study might have been due to mid-season dry spells that were experienced at the site.

GCA effects are associated with additive gene action while SCA effects are associated with non-additive gene action. Significant GCA and SCA effects for traits such as grain yield, anthesis days, anthesis silking interval, plant height, senescence and GLS across all environments suggest the importance of both additive and non-additive gene action in the inheritance of these traits. Gissa (2008) reported similar findings. GCA effects, however, were more important than SCA effects. Betran *et al*. (2003) reported that additive genetic effects across environments accounted for 61% of total genetic variation in grain yield and they assumed importance over non-additive variances. GCA variances that are higher than SCA variances indicate that additive genetic effects are more important (Gethi and Smith, 2004). This has an implication in breeding in that good parents can be identified using the GCA effects and then crossed to produce high yielding hybrids. Early testing of inbred lines becomes more effective and good hybrids can be identified in the early stages of breeding using GCA effects (Melchinger *et al*., 1998). A high contribution of SCA to grain yield, anthesis silking interval and ears per plant under stress environments in this study suggested that non-additive gene action assumed a crucial role in the expression of these traits under these conditions. These findings are contrary to the findings by Gissa (2008), where GCA assumed a more important role in most of the traits under low N conditions. Expression of grain yield, anthesis silking interval and ears per plant under stress environments being controlled by non-additive genes in this study has an implication in breeding in that good parents cannot be identified using these traits, instead good specific combiners are the ones

that can be selected for. Long *et al*. (2004) found both GCA and SCA effects to be significant for grain yield but SCA effects were more important than GCA effects. The inbred lines used in this study can therefore be exploited for grain yield under drought conditions through targeting the SCA effects. In this study anthesis silking interval was mainly controlled by non-additive gene effects.

GCA_m and GCA_f contributed differently to the expression of different traits in this study. Derera *et al*. (2008) reported different contributions of GCA_m and GCA_f for grain yield under drought conditions (44 versus 32%) and similar contributions under non-drought environments (29 vs 31%). Results in this study are contrary to these findings. In this study GCA_f and GCA_m for grain yield contributed similarly (22%) under drought conditions, indicating that both parents made similar contributions to grain yield in hybrids under drought. However, under non-drought environments GCA_f dominated GCA_m (53 vs 28%). GCA_m contributed more to anthesis days under optimum, drought and low N conditions, indicating that inbred lines can be used in selecting for earliness or lateness in the maturity of hybrids.

In this study GCA_f mean squares for grain yield across all environments, under low N conditions and under optimum conditions were higher than GCA_m mean squares, suggesting the importance of maternal effects in the expression of grain yield under these conditions. The expression of grain yield under drought conditions was also affected by maternal effects. Similar results were reported by Derera *et al*. (2008). The maternal effects were also important in the expression of anthesis silking interval under low N and optimum conditions and the paternal effects assumed an important role in the expression of ear height across all trials. Senescence, an important secondary trait under stress conditions, was also influenced by maternal effects. Prolificacy (ears per plant) under drought and low N conditions was influenced by maternal effects. Khehra and Bhalla (1976) studied reciprocal dissimilarities under optimum environments and reported that cytoplasmic effects were not significant for grain yield, which is consistent with the observations in the current study. Largely maternal effects, if unrestrained, would inflate GCA variance for yield and secondary traits; and as a result heritability is overvalued which might deceive breeders in implementing a wrong

selection strategy (Derera *et al*., 2008). GLS and leaf blight turcicum diseases of economic importance, especially under optimum conditions, were influenced by maternal effects, whilst rust and ear rots were influenced by paternal effects.

GCA_f x E, GCA_m x E and SCA x E were significant for most traits in across site analysis in this study and results were consistent with the findings by other scientists (Derera *et al*., 2008; Gissa, 2008; Machida, 2008; Mhike *et al*., 2011a). It appears that G x E effects would present challenges in the breeding of materials for different environments. Significant G x E interactions highlights the need to use several environments in the estimation of genetic effects. Crossover type of G x E was observed in the current study. Genetic component estimates based on data from single environments were found to be unreliable. Results show that GCA effects associated with the parents and SCA effects associated with crosses were not consistent over environments. Banziger *et al*. (2000) reported that stress environments produce high G x E interactions.

The lines and testers exhibited different GCA effects for different traits in this study. Line 3 (RS61P) showed consistency in its performance exhibiting good GCA effects for grain yield across environments (0.72), optimum conditions (0.64), drought conditions (0.37) and under low N conditions (0.58). This line is therefore a good general combiner for grain yield across all environments and this was further confirmed by four of its hybrids that appeared among the 10 best performing hybrids across all environments. This line was also a good general combiner for other traits such as anthesis days, anthesis silking interval, plant height, root and stalk lodging and senescence. A combination of a negative GCA effect for anthesis days and anthesis silking interval would be good in the early maturing maize breeding programme. On the other hand testers 9 (CML444) and 11 (CML548) were consistent in their GCA effects, again proving to be good general combiners for grain yield and other agronomic traits. The other lines and testers that showed good and consistent GCA effects were NAW5885, CML539, CML537 and CML442.

The presence of significant SCA is a consequence of fluctuations in dominance relationships among parents (Wassimi *et al*., 1986). Line 3 (RS61P) was a good specific combiner as

indicated by significant positive SCA effects for grain yield in different environments. Hybrids RS61P/ CML444 and 2N3d/CML548 were among the best performing hybrids across all environments, optimum conditions and 2N3d/CML548 was the overall best performer under drought conditions with a mean yield of 3.26 t ha^{-1}. However RS61P did not combine well with other testers. Results from previous studies as well as the current study indicate that a parent having a good GCA effect does not automatically produce better hybrids all the time (Tyagi and Lal, 2005). A parent with poor GCA might produce better hybrids (Tyagi and Lal, 2005) and this agrees with some of the findings in this study where poor general combiners produced good hybrids with the testers. For example SC5522 had poor GCA for grain yield but it produced hybrids with good SCA with CML395 (0.77) and CML536 (0.75). The results suggest that SC5522 displayed a dominance effect whereby it contributed non-additive genes towards expression of grain yield. Thus the role of dominance in conferring heterosis was displayed by the results. This has an implication in breeding in that lines should be selected based on both GCA and SCA effects. If GCA is insignificant it is advisable to select lines based only on SCA (Narro *et al*., 2003).

Variance components and heritability estimates have been extensively used by plant breeders in selection of promising genotypes and prediction of desirable traits (Morakinyo, 1996). The relatively high heritability estimates in this study are an indication that the studied traits are mainly controlled by additive genes. Hallauer and Miranda (1981) reported heritability estimates that were lower in magnitude than the ones found in the current study. Mhike *et al*. (2011a) reported heritability estimates that were above 50% for anthesis days, ear height, anthesis silking interval and ear position and the rest were below 50%. A heritability estimate of 68% for grain yield was reported in this study and this was higher than values reported by Bolanos and Edmeades (1996) (60% under optimal and 40% under drought conditions) and Mhike *et al*. (2011a) (21%). However, the magnitudes of heritability estimates are products of the population being tested, environments within which the testing is done and traits being measured (Falconer and Mackay, 1996). To this end therefore the differences in magnitudes observed here is a manifestation of the differences in these three determinants of the heritability estimates. It should, therefore, be understood that heritability values reported for

a given trait, are specific to a particular population under particular conditions (Hallauer and Miranda, 1981).

3.5 Conclusions

Abiotic stress factors such as drought and low N, are among the critical factors affecting maize production in Zimbabwe. Results from this study revealed that there is a high level of genetic variability and there is a possibility of selecting good hybrids for grain yield and other agronomic traits under both drought and low N conditions. The NCDII was effective and ideal in the identification of lines with good GCA effects and potential single cross testers. The lines identified as good potential parental lines in the stress breeding programme include RS61P, NAW5885, CML444, CML539, CML442, CML537 and CML548. On the other hand the single crosses RS61P/CML444 (SC) and 2N3d/CML548 (N) were identified as the highest potential single cross testers, however further studies have to be done to confirm validity of these testers. However RS61P performed best under all conditions. GCA and SCA effects were significant for most traits across environments and this showed the significance of both additive and non-additive genes in the expression of these traits. GCA variances were, however, larger than SCA variances for the majority of traits, resulting in high heritability estimates. The parental materials used in this study can therefore be used in selecting good parents for future use in the development of stress tolerant genotypes as most traits are controlled by additive genes. However, a higher SCA contribution to grain yield under drought and ears per plant (prolificacy) and anthesis silking interval under both drought and low N is an indication that non-additive gene action was important in the expression of these traits. Under drought conditions specific hybrids with high mean yields and high prolificacy can be selected. Maternal effects were important in modification of grain yield and other traits in all the environments.

3.6 References

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CHAPTER 4

Genotype by environment interaction and stability analysis for grain yield of single cross hybrids

Abstract

Maize is the most important cereal crop in Zimbabwe and is grown by both large and small scale farmers who are located in different agro-ecological zones of the country. The development and dissemination of adapted and high yielding maize varieties to these agro-ecological zones involves conducting multi environment trials (METs). This study was conducted with the objective of assessing genotype by environment (G x E) interaction and stability of single cross hybrids grown in stress and non-stress environments. Yield data of 80 maize single cross hybrids tested across seven environments during the 2009/10 and 2010/11 seasons were analysed using AMMI and GGE biplot methods. However, the analysis was later narrowed to 20 hybrids for better graphical visualisation and better conclusions. In the combined ANOVA the environment (E) explained 85% of the total (G + E + G x E) variation, whereas genotype (G) and G x E interaction captured 7.0% and 13.1%, respectively. The largest proportion of the total variation was explained by environmental effects as a result of inclusion of environments with varying stress conditions. Higher yielding and adapted genotypes were determined by PC1 scores >0, whilst non-adapted and lower yielding genotypes were determined by PC1 scores <0. PC2 scores of approximately zero identified stable genotypes, whilst larger PC2 scores detected unstable genotypes. Good hybrids had high PC1 scores (high yield) and small absolute PC2 scores (high stability). On the other hand the environmental PC1 scores were related to non-crossover G x E interaction, whilst PC2 scores were related to the crossover type. The two methods detected similar high yielding and stable genotypes. The higher yielding and stable genotypes were G52 (CML548/2N3d-B) and G57 (CML444-BB/RS61P-B). Agricultural Research Trust farm was the most powerful site in discriminating genotypes and the most representative environment. Results showed that there were three mega environments within the test environments, which can be utilised for multi-environment yield trials.

4.1 Introduction

The most important cereal crop in Africa is maize and in Zimbabwe, maize production accounts for 80% of the total cereal production. It is grown by both large and small scale farmers for both food and feed in different agro-ecological zones. Newly improved hybrid maize cultivar candidates from different breeding programmes must be evaluated at many sites and for a number of years before being recommended to be grown in given locations. Evaluation of genotypic performance of hybrid maize cultivar candidates in many environments generates valuable data to ascertain how stable and adapted genotypes are (Crossa, 1990). The multi-location evaluation, however, result in G x E interactions that often complicate the interpretation of results obtained and reduce efficiency in selecting the best genotypes (Annicchiarico and Perenzin, 1994). The existence of G x E interaction may mean that a superior variety in one location is not necessarily the best in another environment. Kang *et al*. (1991) indicated that selection based on yield only may not always be adequate when G x E is significant. The analysis of G x E interaction, thus, turns out to be a significant strategy used by breeders for assessing varieties for adaptation and also for selecting parents for base populations (Aina *et al*., 2007).

Plant breeders usually look for non-crossover G x E or rather the absence of G x E interaction when selecting genotypes for general adaptation and crossover G x E interaction for specific adaptation (Matus-Cadiz *et al*., 2003). ANOVA only defines if G x E is significant or not, but it does not provide information as to which varieties or locations contribute to the interaction (Samonte *et al*., 2005). Various approaches have been established to reveal forms of G x E interaction and these include joint regression developed by Finlay and Wilkinson (1963), Eberhart and Russel (1966) and Perkins and Jinks (1968), AMMI developed by Gauch (1992) and GGE developed by Yan *et al*. (2000).

It is usually challenging to define how genotypes respond, without graphically presenting the data, when many varieties are evaluated across many locations, seasons and years (Yan *et al*., 2001). There are two types of biplots that have been extensively used to visualise G x E interactions and these are the AMMI and GGE biplots (Gauch, 1988; Gauch and Zobel, 1997; Yan *et al*., 2000; Ma *et al*., 2004). Yan and Kang (2003) postulated that a GGE biplot

is an effective tool for mega environment analysis ("which-won-where"). GGE biplots have also been found to be effective in depicting genotype mean performance and stability and the ability of environments to distinguish genotypes in target locations. In the AMMI model, ANOVA for varieties and environment main effects is combined with PCA of the G x E interactions (Gauch and Zobel, 1996). Dissimilarities among the two approaches are that GGE biplot analysis is founded on location centred PCA, whilst AMMI analysis is denoted as double centred PCA. However it is not always easy to visualise "which-won-where" in the AMMI graph, particularly when a number of varieties and locations are involved (Ebdon and Gauch, 2002) and at times it could be deceptive (Yan *et al*., 2007).

Hence the AMMI graph is regarded as a better tool for presenting conclusions rather than as a tool for determining "which-won-where". In this study 80 genotypes were evaluated across seven environments in the 2009/10 and 2010/11 seasons. Given the number of genotypes used and number of environments there should be better a presentation of the G x E interaction if both GGE and AMMI biplots are used and this should assist in reaching better conclusions. The objectives of this study were therefore to i) analyse G x E interaction and stability of single cross hybrids generated using CIMMYT elite drought tolerant lines and DR&SS elite drought susceptible lines for grain yield across stress and non-stress environments and ii) to observe the pattern of grouping of environments based on grain yield responses of hybrids.

4.2 Materials and methods

4.2.1 Germplasm

Seventy two experimental single cross hybrids and eight check single cross hybrids were evaluated in a total of 14 environments in the 2009/10 and 2010/11 seasons. The experimental hybrids constituted of cross progeny of 10 DR&SS and 13 CIMMYT elite inbred lines and their single cross hybrids. Details of the germplasm used in the study are given in section 3.2.1. A total of 72 single cross hybrids out of a possible 130 hybrids were used in the study because they were the successful ones and had enough seed for multi-location trials.

4.2.2 Sites

The site details are given in the materials and methods section of Chapter 3 (section 3.2.2). Details of annual average rainfall and soil type of these sites are shown in Table 4.1.

Table 4.1 Site annual average rainfall and soil type

Site	Environment code	Management	Annual average rainfall (mm)	Soil type	Latitude, longitude and altitude
ART Farm	E1	Optimum	891	*Rhodustalf* greater group with texture code ICG	17°26'S, 31.5°E, 1 480 masl
Harare	E2	Low N	820	*Rhodustalf* greater group with texture code ICG	17.13°S, 31°E, 1 406 masl
Kadoma	E3	Optimum	727	*Haplustox* code FRr14 and texture code DCE	18.32°S, 30.90°E, 1 155 masl
Chiredzi winter	E4	Managed drought	450	*Haplustalf* code LXh7 and texture code ICH	21.02°S, 31.58°E, 433 masl
Rattray Arnold	E5	Optimum	820	Clay loam	17°40'S, 1 308 masl
Chiredzi	E6	Random drought	450	*Haplustalf* code LXh7 and texture code ICH	21.02°S, 31.58°E, 433 masl
Chisumbanje	E7	Random drought	420	Deep vertisols	20°S, 33°E, 455 masl

ART=Agricultural Research Trust; Low N= low nitrogen.

4.2.3 Experimental design and data collected

Trials were planted in one row plots with 0.75 m inter-row spacing and 0.25 m in-row spacing using a 0.1 alpha lattice design. Grain yield and other agronomic data such as anthesis days, anthesis silking interval, plant height, ear height, root lodging, stem lodging, ears per plant and senescence were collected during the growth period of the crop as described in Table 3.2. Scores for diseases of economic importance in maize in Zimbabwe were also taken and these include MSV, GLS, *Exhohilium turcicum* and *Puccinia sorghi*.

4.2.4 Statistical analysis

The data was first subjected to ANOVA in order to determine the effects of G x E interaction for grain yield. ANOVA performed on plot basis and pooled over locations and seasons using AGROBASE version II (2005). Broad sense heritability estimates were calculated for the two seasons as described in section 3.2.5.

The AMMI statistical model in GenStat 14th Edition (2011) was used to analyse the yield data.

The following AMMI model was used:

$Y_{ger} = \mu + \alpha_g + \beta_e + \sum\lambda_n Y_{gn} \delta_{en} + \rho_{ge} + E_{ger}$

Where:

Y_{ger} = Yield of genotype *g* in environment *e* for replicate *r*

μ = Grand mean

α_g = Genotype mean deviations (genotype means minus grand mean)

β_e = Environment mean deviation

n = Number of PCA axes retained in the model

λ_n = Singular value for PCA axis n

Y_{gn} = Genotype eigenvector values for PCA axis n

δ_{en} = Environment eigenvector values for PCA axis n

ρ_{ge} = Residuals

E_{ger} = Error term

GGE biplot analysis was conducted using the "GGE biplot" software (Yan and Tinker, 2005). The model for a GGE biplot (Yan, 2002) based on SVD of the first two principal components was used:

$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \varepsilon_{ij}$

Where Y_{ij} = the measured mean (DBH) of genotype *i* in environment *j*

M = grand mean

β_j = main effect of environment *j*,

$\mu + \beta_j$ = the mean yield across all genotypes in environment *j*

λ_1 and λ_2 = singular values (SV) for the first and second principal component (PC1 and PC2) respectively

ξ_{i1} and ξ_{i2} = eigenvectors of genotype I for PC1 and PC2 respectively

η_{j1} and η_{j2} = eigenvectors of environment *j* for PC1 and PC2 respectively

ε_{ij} = residual associated with genotype *i* in environment *j*.

Spearman's rank correlation (r) amongst environments was calculated from means across environments and statistical calculations were executed with SPSS 15.0 version for Windows (2006). Hierarchical cluster analysis of environments was done in NTSYSpc 2.21n software (Rohlf, 1993) using trait means.

4.3 Results

4.3.1 Analysis of variance within years and across years

Results for ANOVA for within years and across years are presented in Tables 4.2 and 4.3. In both the 2009/10 and 2010/11 seasons the environments, genotypes and G x E were highly significant (P≤0.001). The broad sense heritability values for yield for the years were 65% (2009/10) and 46% (2010/11) and the r values were 0.90 and 0.83 respectively.

Table 4.2 Analysis of variance for grain yield across environments in the 2009/10 and 2010/11 seasons

		2009/10	2010/11
Source	**DF**	**MS**	**MS**
Environments	6	1172.27***	216.54***
Genotype	79	8.74***	1.79***
G x E	474	3.03***	0.97**
Residual	553	1.66	0.78
LSD		0.96	0.65
CV		28.2	34.2
R^2		0.90	0.83
Herit.		0.65	0.46

*****P≤0.001; **P≤0.01; G x E=genotype by environment interaction; LSD=Least significant difference; CV=coefficient of variation; Herit=heritability; R^2=coefficient of determination; DF=degrees of freedom; MS=mean square.**

The combined ANOVA indicated that maize grain yields were significantly affected by environment, which explained 85% of the total (G + E +G x E) variation, whilst genotype and G x E explained 7.0% and 13.1% of variation respectively (Table 4.3). The mean squares for years, E x Y, G x Y and G x E x Y were all highly significant.

Table 4.3 Combined analysis of variance for grain yield of 80 genotypes across seven environments

Source	DF	SS	MS
Enviroments	6	6073.676	1012.27***
Genotype	79	535.531	6.779***
Year	1	2237.381	2237.38***
G x E	474	1000.657	2.11***
E x Y	6	2259.167	376.53***
G x Y	79	295.793	3.74***
G x E x Y	474	893.205	1.88***
Residual	1114	1346.403	1.21
Total	2239	14893.493	
CV	30.75		
R^2	0.91		

*****P≤0.001; G x E=genotype by environment interaction; E x Y=environment by year interaction; G x Y=genotype by year interaction; G x E x Y=genotype by environment by year interaction; CV=coefficient of variation; R^2=coefficient of determination; DF=degrees of freedom; SS=sums of squares; MS=mean squares.**

4.3.2 Additive main effect and multiplicative interaction analysis

The partitioning of G x E through AMMI analysis showed that PC1 and PC2 were significant (P≤0.001) (Table 4.4), explaining 48.27% and 27.82% of the total variation and together they explained 76.1% of G x E interaction (Table 4.5). The mean yields of genotypes in all environments are presented in Appendix 8. The highest yielding genotypes for each environment were as follows: G74 in Agricultural Research Trust farm, Harare low N and Rattray Arnold Research Station, G61 in Kadoma, G52 in Chiredzi winter, G57 in Chiredzi summer and G53 in Chisumbanje. In the AMMI biplot genotypes were clustered around the zero point except for a few genotypes that were distinct (Figure 4.1). G74 proved that it was high yielding but did not show good stability, whilst G59, G10 and G63 were high yielding and more stable. Genotypes G29, G17, G73 and G12 were low yielding and more stable. G74 was mostly associated with high yielding environments.

Genotype means were plotted against the IPCA scores across all environments and G74 was the highest yielder but very unstable, whilst G60 and G61 were high yielding and more stable (Figure 4.2). G57 was also high yielding and relatively stable. G68, G4, G52, G5 and G63 were high yielding but very unstable. G73 was low yielding but very stable. Environments Agricultural Research Trust farm (optimum), Chisumbanje (random drought), Rattray Arnold Research Station (optimum) and Kadoma (optimum) were higher yielding; whilst

environments Harare low N, Chiredzi winter (managed drought) and Chiredzi (random drought) were lower yielding (Figure 4.3).

4.3.3 Genotype and genotype by environment interaction biplot for all 80 genotypes

Positioning of genotypes on the GGE biplot are presented in Figure 4.4. The GGE biplot for all 80 genotypes explained 61.7% of the genotype main effect and the G x E interaction (Figure 4.4). Primary (PC1) and secondary (PC2) scores were significant and explained 47.5% and 14.2% of the genotype main effect and G x E interaction respectively. A moderate percentage variability of GGE (61.7%) was explained by the biplot and this suggests some strong and complex G x E interaction in the MET data. Genotypes G61, G52, G4, G57 and G74 were mostly associated with Kadoma and Harare low N.

Table 4.4 Analysis of variance for additive main effects and multiplicative interaction model for grain yield across seven environments for the 2009/10 and 2010/11 seasons

Source	DF	MS
Environments	13	912.61***
Genotype	79	13.80***
G x E	891	2.44***
IPCA1	91	9.59***
IPCA2	89	5.65***
IPCA3	87	2.99***
IPCA4	85	2.02**
IPCA error	539	0.68
Residual	972	1.19

*****P≤0.001; **P≤0.01; IPCA=interaction principal components axes; G x E=genotype by environment interaction; DF=degrees of freedom; MS=mean squares.**

Table 4.5 Additive main effects and multiplicative interaction analysis of yield data of 80 maize genotypes tested across seven environments in the 2009/10 and 2010/11 seasons

IPCA Axis	Eigenvalue	% G x E explained	Cumulative %
1	436.183	48.27	48.27
2	251.411	27.82	76.1
3	130.352	14.43	90.52
4	85.652	9.48	100

IPCA=interaction principal component axes; G x E=genotype by environment interaction.

G38, G59 and G27 were associated with Chiredzi, whilst G67 and G60 were associated with Chisumbanje and Chiredzi winter. G74 was once again the highest yielding genotype. G48 was more closely associated with environments Rattray Arnold Research Station and Agricultural Research Trust farm. Graphical presentation of stability of genotypes using GGE biplot analysis is presented in Figure 4.5. Genotypes G5, G26, G62, G6, G57, G74 and G45 were high yielding as well as stable since their absolute PC2 scores were near zero, whereas genotypes G67, G60, G51, G7 and G68 were high yielding and unstable as they had larger absolute PC2 scores (Figure 4.5). G74 was the highest yielding genotype (large PC1 score) but unstable in different environments (large PC2 score) (Figure 4.5). G17 and G37 were the poorest performing genotypes (low PC1 scores), whereas G73 and G21 were amongst the poorest performing genotypes but very stable (near zero PC2 score). G61 and G52 were very stable and their average yields were larger than all other genotypes except G74. G74 is a commercial long season hybrid which was included in the trials as a control.

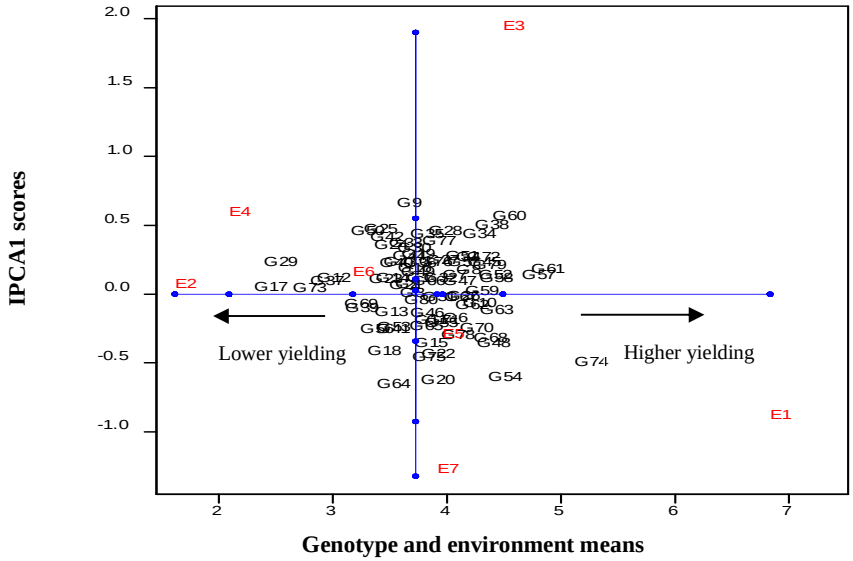

Figure 4.1 Additive main effects and multiplicative interaction biplot for genotype grain yield in seven environments for two seasons combined.

Genotypes are labelled with a G followed by a number; environments are labelled with an E followed by a number. E1=Agricultural Research Trust farm; E2=Harare low nitrogen; E3=Kadoma; E4=Chiredzi winter; E5=Rattray Arnold Research Station; E6=Chiredzi summer; E7=Chisumbanje.

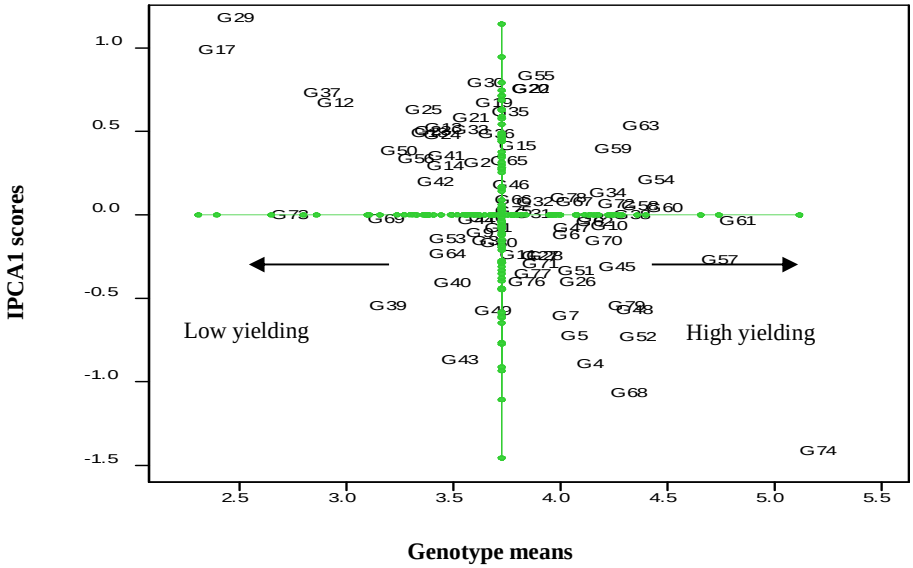

Figure 4.2 Additive main effect and multiplicative interaction biplot for genotype grain yield across environments across two seasons.

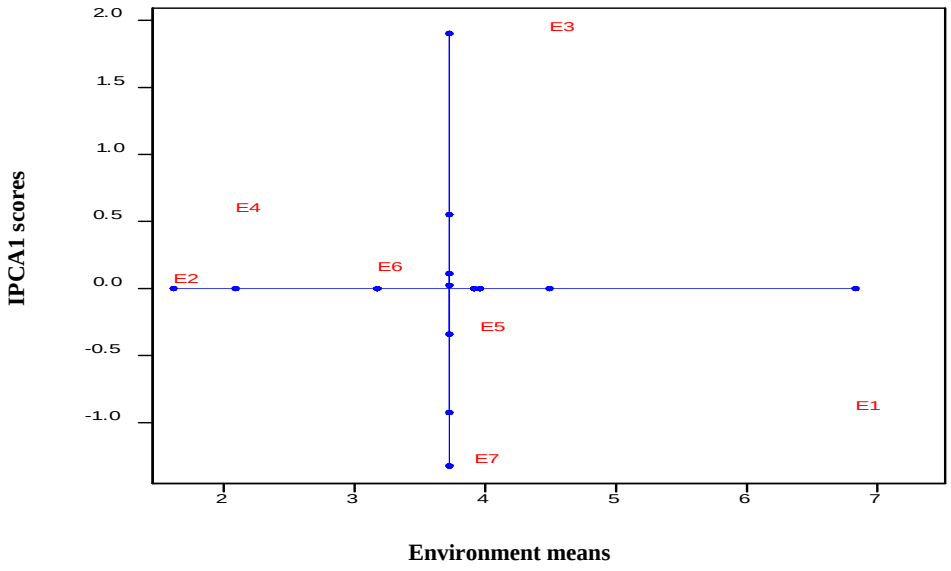

Figure 4.3 Additive main effects and multiplicative interaction biplot for environment means across two seasons.

E1=Agricultural Research Trust farm; E2=Harare low nitrogen; E3=Kadoma; E4=Chiredzi winter; E5=Rattray Arnold Research Station; E6=Chiredzi summer; E7=Chisumbanje.

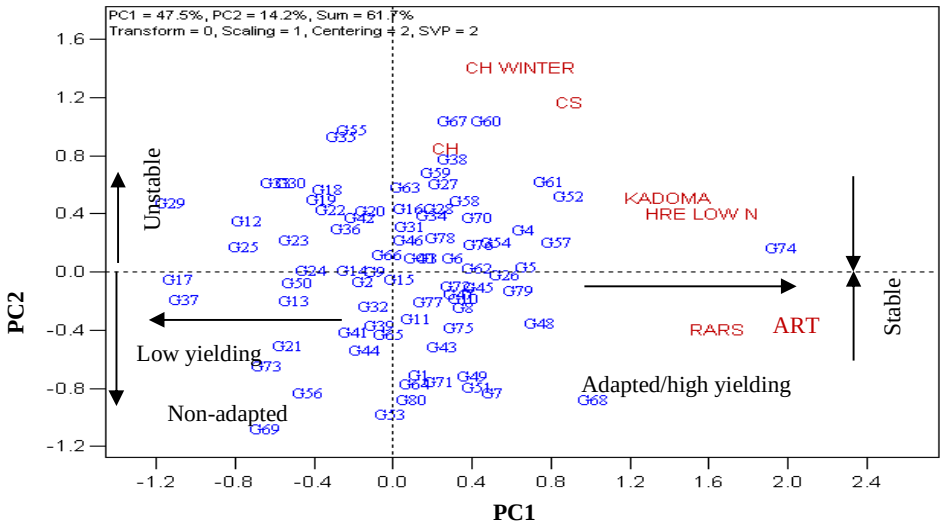

Figure 4.4 Genotype and genotype by environment interaction biplot analysis of yield across seven environments and two seasons.

CH WINTER=Chiredzi winter; CH=Chiredzi summer; CS=Chisumbanje; HRE Low N=Harare low nitrogen; RARS=Rattray Arnold Research Station; ART=Agricultural Research Trust farm.

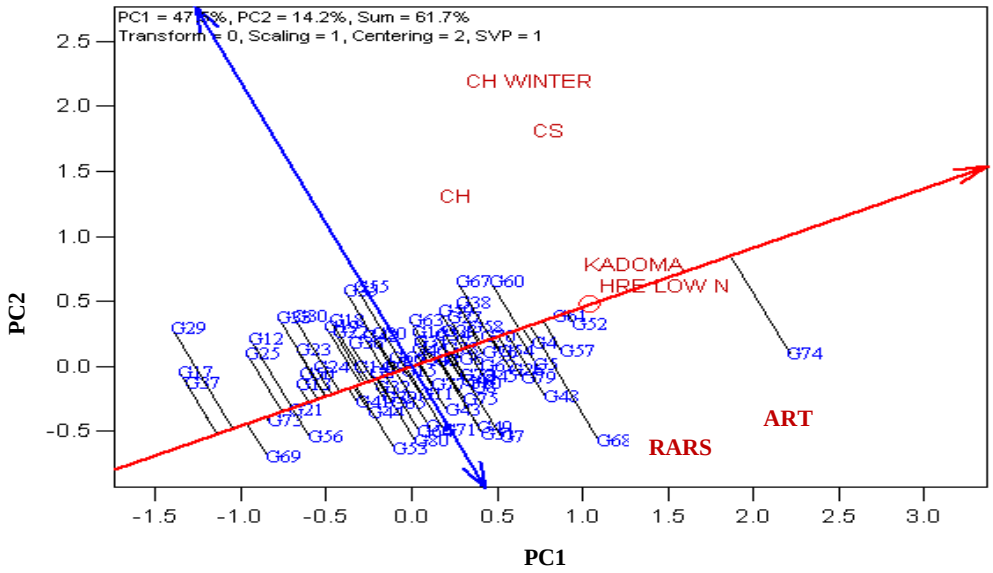

Figure 4.5 Yield stability and performance of genotypes for seven environments and two seasons.

CH WINTER=Chiredzi winter; CH=Chiredzi Summer; CS=Chisumbanje; HRE Low N=Harare low nitrogen; RARS=Rattray Arnold Research Station; ART=Agricultural Research Trust farm.

Graphical presentation of "which-won-where" was used to assess which genotypes performed well in which environments. The polygon view of a GGE biplot clearly displayed the "which-won-where" pattern; henceforth it is a clear summary of the G x E pattern of a multi-environment yield trial (MEYT) data set. The polygon was formed from genotype markers G74, G68, G69, G37, G29, G55 and G60 (Figure 4.6). Nine perpendicular lines were drawn starting from the origin and extended beyond the polygon such that the biplot was divided into nine sectors and environments fell into two of them. Five environments namely Rattray Arnold, Agricultural Research Trust farm, Kadoma, Harare low N and Chisumbanje fell into sector 1 delineated by rays 1 and 2 and the vertex genotypes were G60, G74 and G68. Two environments Chiredzi summer and Chiredzi winter fell into sector 9.

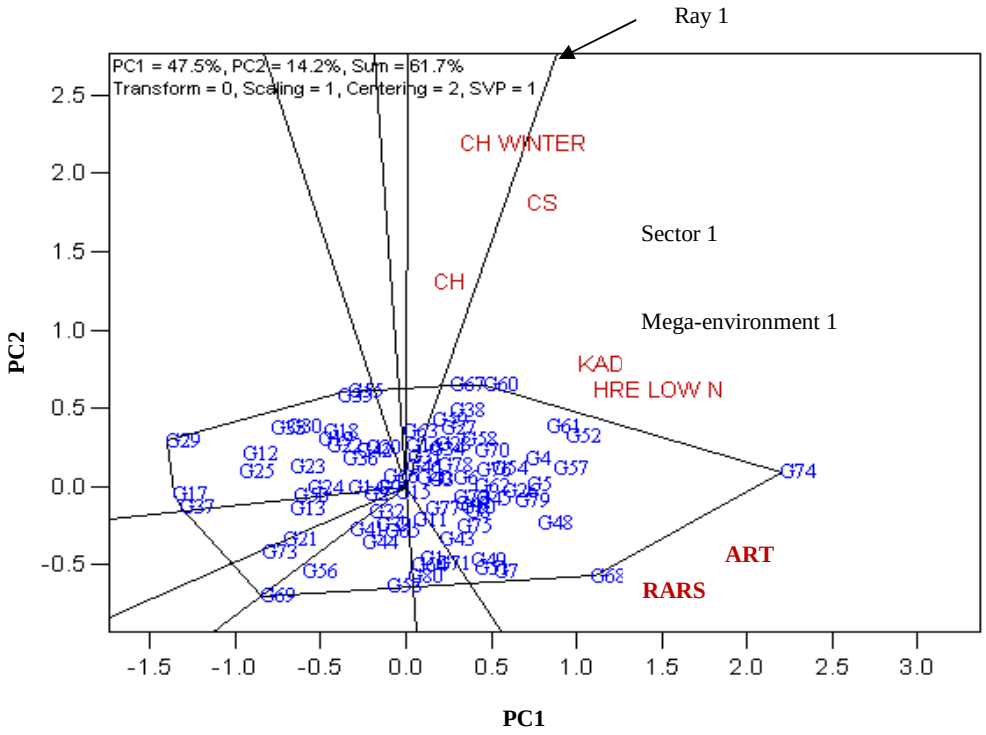

Figure 4.6 Polygon view of the genotype and genotype by environment interaction biplot based on symmetrical scaling for the "which-won-where" pattern for genotypes and environments.

CH WINTER=chiredzi winter; CH=chiredzi summer; CS=Chisumbanje; HRE Low N=Harare low nitrogen; Rattray Arnold Research Station; ART=Agricultural Research Trust farm.

A GGE biplot was drawn to show which environment discriminated genotypes better than the other environments. Environments PC1 had only positive scores, whilst PC2 had both positive and negative scores (Figure 4.7). Environments Rattray Arnold Research Station, Agricultural Research Trust farm and Harare Low N had high PC1 scores and near zero PC2 scores. Chiredzi summer, Chiredzi winter and Chisumbanje also had high PC2 scores. PC1 scores correlated with environment yield scores ($r=0.569$; $P \leq 0.05$). Correlation coefficients among test environments are presented in Table 4.6. All environments were positively correlated because all angles among them were smaller than 90°. Harare low N and Agricultural Research Trust farm had the largest highly significant positive correlation ($r=0.929$) while the lowest correlation was between Chisumbanje and Chiredzi summer.

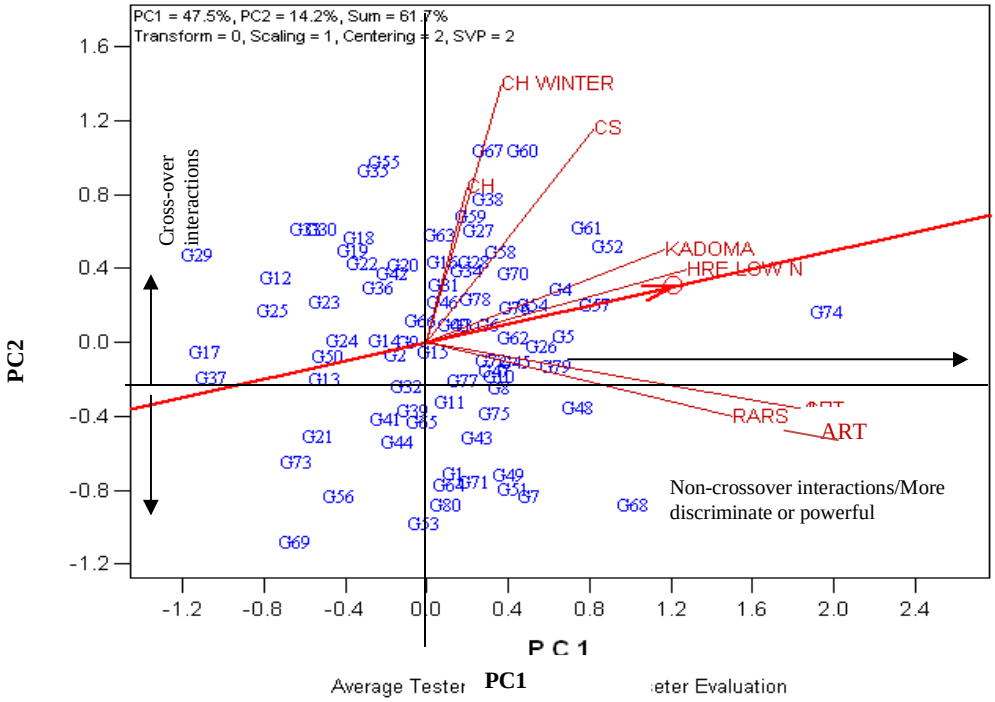

Figure 4.7 Genotype and genotype by environment interaction biplot based on environment-focused scaling for environments.

CH WINTER=Chiredzi winter; CH=Chiredzi summer; CS=Chisumbanje; HRE Low N=Harare low nitrogen; RARS=Rattray Arnold Research Station; ART=Agricultural Research Trust farm.

Table 4.6 Correlation coefficients among test environments

	ART	HRE Low N	Kadoma	CH winter	RARS	CH
HRE Low N	0.929**					
Kadoma	0.463**	0.626**				
CH winter	0.291**	0.163**	0.183**			
RARS	0.230**	0.460**	0.775**	0.127**		
CH	0.455	0.082	0.569**	0.279*	0.137	
CS	0.178	0.272	0.380	0.049	0.261	0.89**

****P≤0.001; *P≤0.05; CH WINTER=Chiredzi winter; CH=Chiredzi summer; CS=Chisumbanje; HRE Low N=Harare low nitrogen; RARS=Rattray Arnold Research Station; ART=Agricultural Research Trust farm.**

Rattray Arnold Research Station and Agricultural ResearchTrust farm were also highly and significantly correlated (r=0.230).

A hierarchical cluster analysis was also done for environments (Figure 4.8). Harare low N was closely related to Chiredzi winter and the two sites comprised of managed stress. Chiredzi summer and Chisumbanje clustered together and these environments share a similar geographical location. Chiredzi summer and Chisumbanje also have similar rainfall patterns and were both used as random drought sites. Rattray Arnold Research Station and Kadoma clustered together and they have similar rainfall patterns. Agricultural Research Trust farm did not cluster with any environment and is usually characterised by high rainfall figures that can sometimes be above 1 000 mm.

4.3.4 Genotype and genotype by environment interaction biplot analysis for 20 best performing hybrids

An across site analysis was done using Fieldbook software (Banziger and Vivek, 2007) embedded in an Excel spread sheet and the 20 best performing hybrids were selected. The yield performance data of these hybrids across seven environments is presented in Table 4.7. The bold and underlined mean yields are for those hybrids that were the best performers in each environment. The variability of the best performing hybrid from one environment to the other shows the existence of possible crossover G x E interaction. G74 (SC727) and G79 (CML395/CML444) were the check varieties and were amongst the best performing hybrids. G74 was the overall best performing hybrid as well as being the best performer in environments Agricultural Research Trust farm, Harare low N and Rattray Arnold Research Station. G61 was the best performing hybrid in Kadoma and G52 was the best performing genotype in Chiredzi winter. The genotype G57 was the best performer in Chiredzi summer and G63 in Chisumbanje. A GGE biplot based on genotype-focused scaling was shown in order to perceive the positioning of the genotypes (Figure 4.9). Genotypes with PC1 scores >0 were identified as higher yielding and adapted and those that had PC1 scores <0 were identified as lower yielding. Genotypes G74, G68 and G48 were the highest yielding with PC1 scores greater than zero, whilst G60, G61 and G63 were lower yielding with PC1 scores less than zero. G7 and G79 were high yielding and stable with PC2 scores closer to zero. G39 was low yielding but very stable with a PC2 score of zero. Yield stability and performance of the 20 best performing hybrids is presented in Figure 4.10.

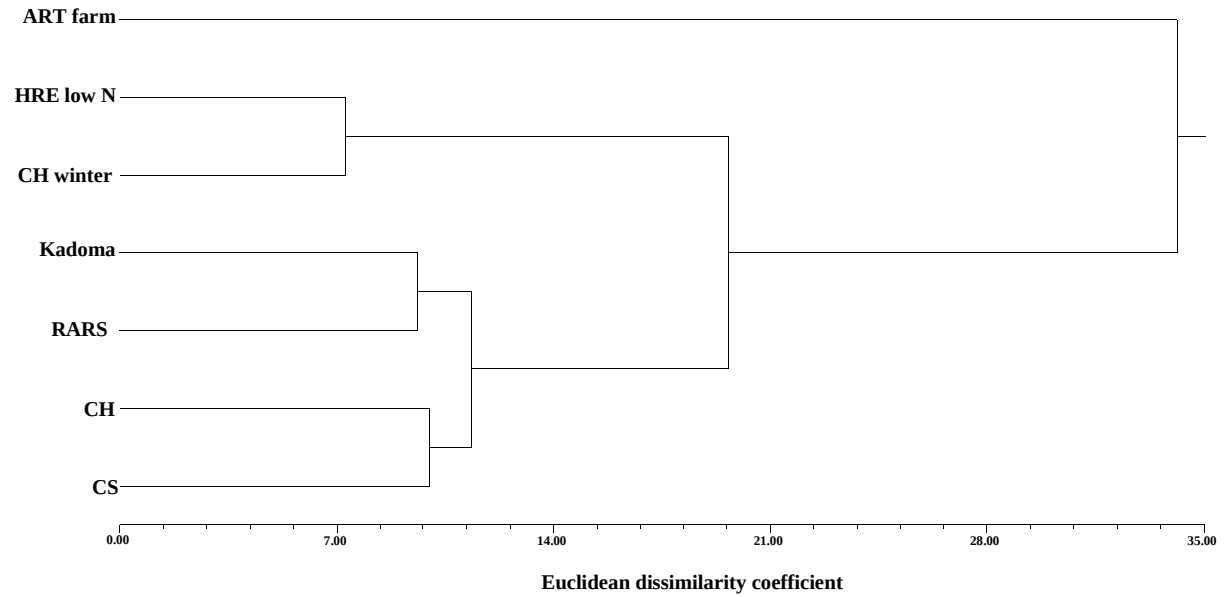

Figure 4.8 Hierarchical cluster analysis of the seven environments.

ART=Agricultural Research Trust farm; HRE low N=Harare low nitrogen; CH winter=Chiredzi winter; RARS=Rattray Arnold Research Station; CH=Chiredzi summer; CS= Chisumbanje.

Table 4.7 Mean grain yield (t ha^{-1}) for 20 genotypes across seven environments in two seasons

Genotype	E1	E2	E3	E4	E5	E6	E7	Mean t ha^{-1}
G74	**10.43**	**4.08**	5.29	2.50	**6.16**	2.50	4.85	**5.12**
G61	8.34	2.31	**6.68**	2.79	3.65	3.65	5.76	**4.74**
G57	8.20	2.44	6.42	1.78	4.29	**4.26**	5.19	**4.66**
G54	7.72	1.54	4.26	2.33	4.90	3.63	6.12	**4.36**
G48	8.36	1.63	4.60	1.99	5.39	3.23	4.61	**4.26**
G63	6.88	1.30	5.40	2.62	3.23	4.08	**6.51**	**4.29**
G52	8.91	2.35	5.04	**3.26**	4.04	3.06	3.26	**4.27**
G79	8.39	1.28	5.51	2.49	4.51	3.82	3.56	**4.22**
G45	7.52	1.97	6.03	1.92	4.21	3.00	4.59	**4.18**
G68	9.99	1.70	4.82	1.40	4.22	3.77	3.72	**4.23**
G10	7.49	1.37	5.00	2.41	4.69	3.31	4.70	**4.14**
G72	7.46	1.50	5.66	2.31	4.18	3.93	4.16	**4.17**
G34	7.08	1.68	5.98	2.64	3.60	3.65	4.30	**4.13**
G38	6.99	1.88	5.82	3.01	3.98	4.16	3.89	**4.25**
G60	6.69	2.83	6.37	2.51	4.18	3.91	4.31	**4.40**
G7	7.82	1.66	5.24	1.71	5.23	2.73	3.31	**3.96**
G58	7.22	1.96	5.51	2.43	3.89	3.93	5.02	**4.28**
G59	6.78	2.08	5.00	2.84	3.89	3.35	5.15	**4.16**
G70	8.02	1.03	4.62	2.38	4.05	3.80	4.90	**4.11**
G8	7.25	2.01	5.60	1.71	4.51	3.15	4.31	**4.08**
Mean (t ha^{-1})	**6.83**	**1.62**	**4.50**	**2.09**	**3.96**	**3.17**	**3.92**	

E1=Agricultural Research Trust farm; E2=Harare low nitrogen; E3=Kadoma; E4=Chiredzi winter; E5=Rattray Arnold Research Station; E6=Chiredzi summer; E7=Chisumbanje; Underlined and bold=highest yielder in the given environment.

G74 was again the highest yielding (biggest PC1 score) but unstable in different environments (large PC2 score). G74 was the highest yielding since it was the best performer in many testing environments with high mean yields (Table 4.7). G63 was the poorest performing genotype (low PC1 score) with low yields in different environments but highly stable (near zero PC2 score). G52 and G57 were also relatively high yielding and very stable with PC2 scores close to zero. G79 and G7 were also relatively stable, whilst G68, G48, G60 and G61 were unstable in different environments. G68 and G74 were associated with Rattray Arnold Research Station and Agricultural Research Trust farm, whilst G60 and G61 were associated with Chiredzi winter. Chiredzi summer was associated with G70 and G54.

Agricultural Research Trust farm had the longest vector followed by Harare low N, Kadoma and Rattray Arnold Research Station, whilst Chisumbanje had the shortest vector (Figure

4.11). Environments Harare low N, Agricultural Research Trust farm, Rattray Arnold Research Station and Chiredzi summer had positive PC1 scores, whilst Chiredzi winter, Kadoma and Chisumbanje had negative PC1 scores. G74 had the longest vector, whilst G45 and G22 had the shortest vectors (Figures 4.11 and 4.12). The acute angles between G60, G61, G57, G52 and G74 indicate that those genotypes performed similarly across environme nts.

Genotypes G61, G60, G57 and G52 had angles less than 90° between their vectors and environment vectors for Chiredzi winter, Kadoma and Harare low N. G79, G7, G68 and G48 had acute angles between them and environments Chiredzi summer, Rattray Arnold Research Station and Agricultural Research Trust farm. G74 and G52 were located nearer to the biplot origin. The angle between G70 and G74 was more than 90°. The environments Harare low N, Kadoma and Chiredzi winter had less than 90° angles between them. The angle between Harare low N and Chiredzi summer was more than 90° as well as between Chiredzi summer and Chiredzi winter.

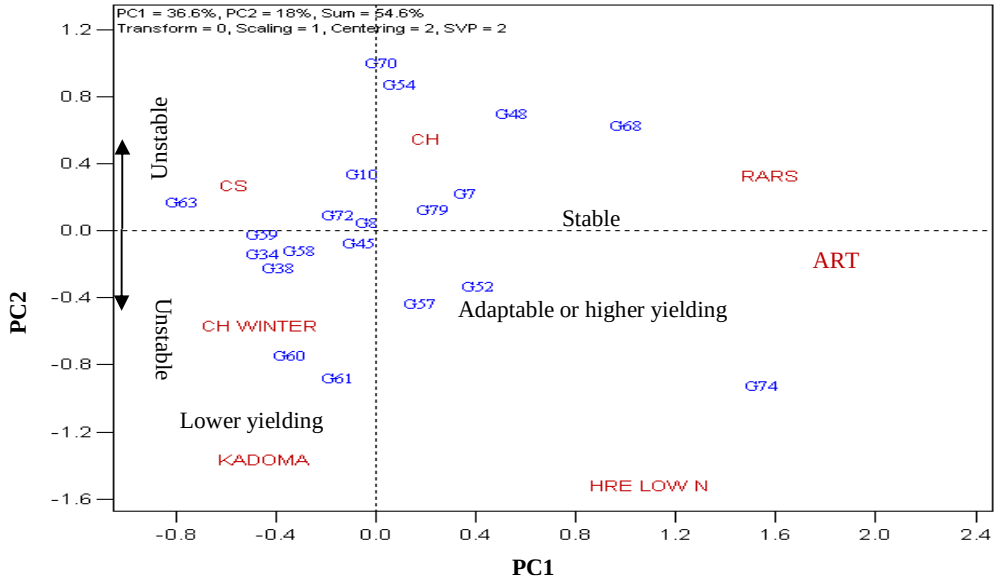

Figure 4.9 Genotype and genotype by environment interaction biplot based on genotype-focused scaling for the top 20 yielding genotypes.

PC=principal component; G=genotype; CS=Chisumbanje; CH=Chiredzi summer; CH WINTER=Chiredzi winter; RARS=Rattray Arnold Research Station; ART=Agricultural Research Trust farm; HRE Low N=Harare low nitrogen.

A six-sided polygon was formed from genotype markers G68, G74, G61, G60, G63 and G70 (Figure 4.13). The polygon is produced by linking markers of varieties that are the furthest away from the biplot origin such that all other genotypes are contained within the polygon. Six perpendicular lines, starting from the origin were drawn extending beyond the polygon such that the biplot was divided into six sectors. All six sectors had locations within them. Three environments Agricultural Research Trust farm, Rattray Arnold Research Station and Harare low N fell within sector 1 outlined by rays 1 and 2 and the vertex genotypes for this sector were G74 and G68. Sector 1 therefore formed mega-environment 1. Sector 2 and 3 comprising of environments Kadoma and Chiredzi winter formed mega-environment 2 and the vertex genotypes were G60 and G61. Chisumbanje and Chiredzi summer formed the third mega-environment in sectors 4 and 5 and the vertex genotypes were G63 and G70.

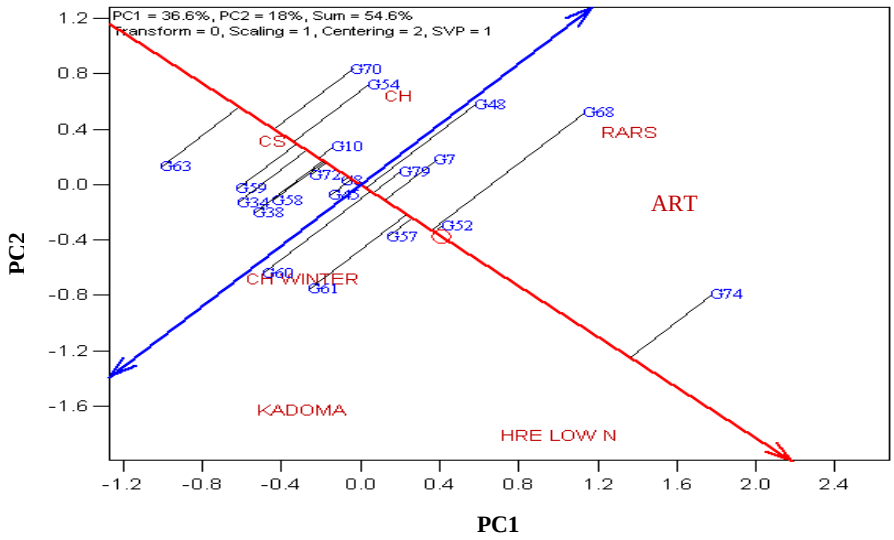

Figure 4.10 Grain yield stability and performance of the 20 top yielding genotypes in seven environments across two seasons.

PC=principal component, G=genotype, CS=Chisumbanje, CH=Chiredzi, CH winter=Chiredzi winter, ART=Agricultural Research Trust farm; RARS=Rattray Arnold Research Station, HRE Low N=Harare low nitrogen.

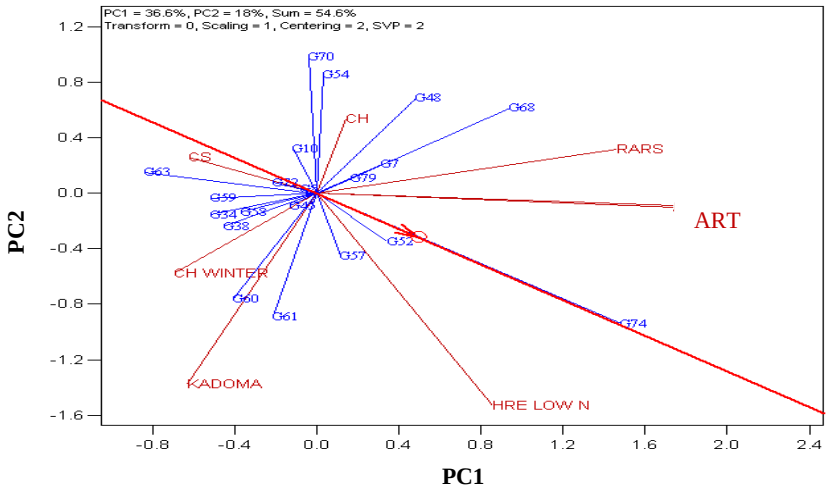

Figure 4.11 Relationship amongst testing environments and genotype by testing environments for the 20 top yielding genotypes.

PC=principal component; G=genotype; CS=Chisumbanje; CH=Chiredzi summer; ART=Agricultural Research Trust farm; CH Winter=Chiredzi winter; HRE Low N=Harare low nitrogen.

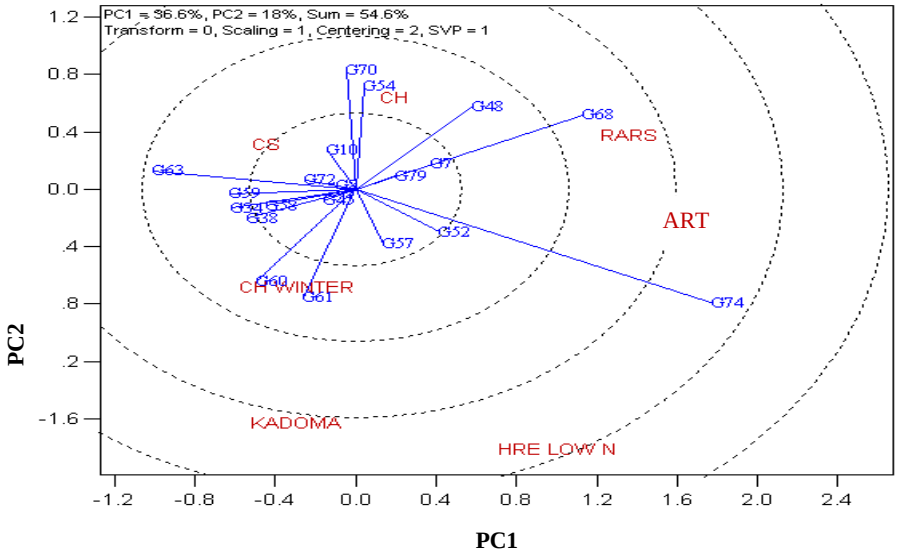

Figure 4.12 Genotype and genotype by environment interaction biplot based on genotype and environment focused scaling for comparison of genotypes and environments for top 20 yielding genotypes.

PC=principal component; G=genotype; CS=Chisumbanje; CH=Chiredzi summer; ART=Agricultural Research Trust farm; CH WINTER=Chiredzi winter; HRE Low N=Harare low nitrogen.

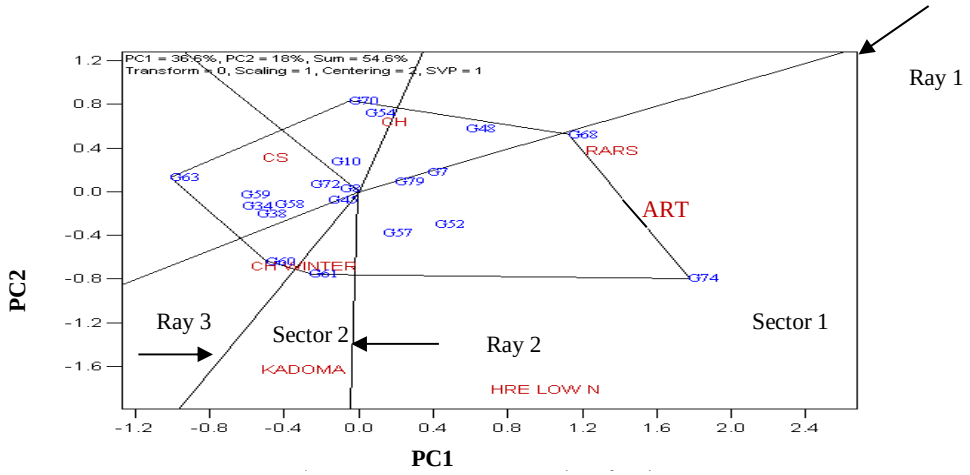

Figure 4.13 Polygon views of the genotype and genotype by environment interaction biplot based on symmetrical scaling for the "which-won-where" pattern for genotypes and environments for the 20 top yielding genotypes.

PC=principal component; G=genotype; CS=Chisumbanje; CH=Chiredzi summer; ART=Agricultural Research Trust farm; CH Winter=Chiredzi winter; HRE Low N=Harare low nitrogen.

4.4 Discussion

In this study the environment explained 85% of the total G + E + G x E variation, whilst genotypes explained 7.0% and G x E interaction 13.1%. These results are consistent with findings by other researchers (Yan *et al*., 2000; Kaya *et al*., 2006; Muungani *et al*., 2007; Jalata, 2011). Gauch and Zobel (1997) reported that in normal multi-location yield experiments location accounted for about 80% of the total variation, whilst genotype and G x E interaction each accounted for about 10%. As a percentage of the total sum of squares the environment accounted for 40.7%, genotype 3.6% and G x E interaction for 6.7% of the variation. These results are also consistent with findings from other investigators (Sabaghnia *et al*., 2008; Ramburan *et al*., 2011). However in this study the contribution by G x E interaction as a percentage of total sum of squares was lower than what has been reported by other two research groups. Environment and environment by year accounted for 60% of the total variability in this study. Muungani *et al*. (2007) reported environment and environment x year contributions of 70.44%. This result is an indication that variation amongst environments within and across years justifies the need for multi-environment yield trials. The huge yield disparity due to location is pertinent to genotype assessment and mega

location analysis (Fox and Rosielle, 1982; Gauch and Zobel, 1996) and again the large G x E interaction compared to genotype contribution suggests the probable existence of different mega locations.

In this study G74 (SC727) was the best performing genotype in environments Agricultural Research Trust farm, Harare low N and Rattray Arnold Research Station and this was an indication of a non-crossover G x E interaction. Results in this study also indicated the presence of a crossover G x E interaction as described by Baker (1988), Yan and Hunt (2001), Kaya *et al*. (2006) and Jalata (2011). The G x E interaction was further analysed with the aid of the AMMI model for grain yield stability. The ANOVA indicated highly significant contribution of environments, genotypes and G x E interaction to variation and results are in agreement with findings from other studies (Sabaghnia *et al*., 2008; Ramburan *et al*., 2011; Thangavel *et al*., 2011). There is a declining impact of the G x E interaction sum of squares with an increasing number of IPCA axes. In this study IPCA1 accounted for 48.27% of the G x E sum of squares interaction and IPCA2 accounted for 27.82% and in total they accounted for 76.10%. Findings are in line with Yan (2002) and Thangavel *et al*. (2011) who reported that most of the interaction occurred in the first few axes. AMMI analysis appears to be able to extract a large portion of G x E interaction and thus is efficient in analysing G x E interaction as demonstrated by Zobel *et al*. (1988).

A GGE biplot based on genotype-focused scaling was illustrated in order to identify the positioning of genotypes. The GGE biplot analysis of yield for the 80 genotypes across seven environments explained 61.7% of genotype main effects and G x E interaction with the primary (PC1) and secondary (PC2) scores contributing 47.5% and 14.2% respectively. On the other hand the GGE biplot for the 20 genotypes explained 54.6% of the genotype main effect and G x E interaction. Similar findings have been recorded by other investigators (Kaya *et al*., 2006; Muungani *et al*., 2007; Jalata, 2011). PC1 scores >0 successfully detected genotypes that are high yielding and PC1 scores <0 discriminated the low yielding ones. On the other hand the PC2 scores showed the genotypic stability of the genotypes. Genotypes of interest were divided into two groups, where group 1 consisted of the stable and high

yielding genotypes (G52, G57, G79 and G7) and group 2 consisted of unstable but high yielding genotypes (G74, G68, G48, G61 and G60).

Genotypes with above average means were G48 to G74 and genotypes with below average means were G45 to G63. The longer the environmental vector, the more significant is the genotype main effect and the more significant the selection based on mean performance (Jalata, 2011). Thus genotypes with above average mean performance can be selected for future breeding. In this study the average environment vector was long enough for the selection of genotypes to be done based on yield mean performances. An ultimate genotype must demonstrate both high average yield performance and high stability across locations (Kaya *et al*., 2006; Yan and Tinker, 2006; Jalata, 2011). The ideal genotypes in this study were identified as G52 (CML548/2N3d-B) and G57 (CML444-BB/RS61P-B).

In this study the vertex genotypes for the mega environment 1 were G74 and G68 and these genotypes were the winning genotypes in the specified environments i.e. Rattray Arnold Research Station, Agricultural Research Trust farm and Harare low N, whilst G60 and G61 were the winning genotypes in Kadoma and Chiredzi Winter. Since there was a high correlation between genotype PC1 scores and genotype main effects and as the GGE biplot sufficiently explained the GGE variation, it can be statistically demonstrated that locations in the same sector share the same winning genotypes (Yan *et al*., 2000).

A GGE biplot which hinges on environment-focused scaling was depicted to assess the pattern of environments. Environment PC1 had only positive scores and similar results were reported by previous scientists (Yan *et al*., 2000; Yan and Hunt, 2001; Kaya *et al*., 2006; Yan and Tinker, 2006). This consequently proposes that the PC1 represents comparative genotype yield differences across environments, which leads to a non-crossover G x E interaction. Genotypes with large PC1 scores can be easily recognised in environments with larger PC1 scores (Yan *et al*., 2000). The environment PC2 scores had both positive and negative scores and this was an indication of crossover G x E interaction. This leads to disproportionate genotype yield differences across environments. In circumstances where resources are limiting and there is a need to carry out multi-environment yield trials, Kadoma, Harare low

N, Agricultural Research Trust farm and Rattray Arnold Research Station may be the better test environments since they had near zero PC2 scores and large PC1 scores. Agricultural Research Trust farm was the most discriminating environment (largest PC1 score) and had the longest environment vector. Chiredzi summer, Chiredzi winter and Chisumbanje had large PC2 scores which would therefore mean that the cultivar differences observed at these sites may not exactly reflect cultivar differences in average yield over all sites.

All environments in the study were positively correlated because the angles among them were less than 90°. Kaya *et al.* (2006) reported similar results. The angle between Rattray Arnold Research Station and Agricultural Research Trust farm was very small, hence the significant correlation (r=0.230). However, there were inconsistencies, since the largest correlation would have been expected to be between Chiredzi summer and Chiredzi winter but instead it was between Harare low N and Agricultural Research Trust farm which had a much larger angle between them than between Chiredzi summer and Chiredzi winter. The biplot did not explain 100% of the GGE variation so some discrepancies were expected (Yan, 2002).

In a situation where the same trait is measured on the same genotypes in different environments indirect selection can be applied. The significant correlation coefficients between test environments suggest that indirect selection for grain yield can be applied across the test environments. For instance the higher yielding genotypes at Agricultural Research Trust farm may also show similar responses at Harare low N, Kadoma, Chiredzi winter and Rattray Arnold Research Station. The existence of significant correlation between environments showed that the information obtained was similar so that testing environments can be reduced to minimise the cost without significantly affecting the validity of the data.

An environment that is more representative of other test environments is the one with a smaller angle with the average environment axis (Yan and Tinker, 2006). Agricultural Research Trust farm was the most representative, followed by Harare low N, whilst Rattray Arnold Research Station and Chiredzi summer were the least representative. Environments that are both discriminating and representative such as Agricultural Research Trust farm are

ideal test environments for selecting generally adapted genotypes and the discriminating and non-representative such as Rattray Arnold Research Station and Chiredzi winter are useful for selecting specifically adapted genotypes if the target environments can be divided into mega-environments. Again the discriminating and non-representative environments can be used for culling unstable genotypes if the target environment is a single mega environment.

In this study hybrids had above average performance in all environments as indicated by angles that were <90°. The genotype and environment means biplot using AMMI analysis clustered environments into four groups: Group 1 included Agricultural Research Trust farm and Rattray Arnold Research Station, group 2 consisted of Chisumbanje, group 3 consisted of Harare low N, Chiredzi winter and Chiredzi summer and lastly group 4 consisted of Kadoma. Among all location-year testing environments, environment Agricultural Research Trust farm interacted with genotypes the same way as environment Rattray Arnold Research Station. The clustering of environments can be explained by similar weather conditions and similar growing conditions. Agricultural Research Trust farm and Rattray Arnold Research Station were optimum environments located in the Highveld and also with annual average rainfall of ±800 mm. Environments Harare low N, Chiredzi winter and Chiredzi summer were mainly associated with stress management. Harare was a low N site, Chiredzi winter a managed drought site and Chiredzi summer was a random drought site, associated with severe mid-season droughts. Chisumbanje clustered on its own because it has distinct soils and is located in the south with low altitude. Hierarchical cluster analysis based on hybrid grain yield clustered environments based on geographical location and stress conditions and similar results were reported by Gissa (2008). Harare low N and Chiredzi winter are distant geographical locations but clustered together as a result of prevailing stress conditions in both environments. When resources are limiting one can select one environment where more than one environment exist in the cluster.

4.5 Conclusions

The level of G x E interaction of the single cross hybrids in this study was larger than that of genotype main effects but smaller than that of environment main effects. Genotypes demonstrated both crossover and non-crossover types of G x E interactions. The former led

to substantially different genotype rankings across test environments, therefore making selection difficult. The stable and high yielding genotypes were identified as well as the discriminating and representative test environments. Genotypes G52 (CML548/2N3d-B) and G57 (CML444-BB/RS61P-B) were identified as the most ideal genotypes due to high grain yield performance and stability. Agricultural Research Trust farm was identified as the most discriminating and representative test environment. The optimum environments discriminated the genotypes differently from the stress environments. In some cases environments sharing the same location but with different stress levels discriminated the genotypes similarly and this showed the possibility of developing genotypes under both stress and optimal environments.

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CHAPTER 5

Genetic variation among CIMMYT and Zimbabwean maize inbred lines

Abstract

Genetic characterisation of breeding lines is of great importance as it enables breeders to maximise on heterosis in hybrid combinations as well as maintain genetic diversity in the breeding material. The Zimbabwe National Breeding Programme (DR&SS) acquires germplasm from CIMMYT, IITA and other breeding programmes to enhance genetic diversity of its breeding materials. The objective of this study was to analyse the genetic and morphological diversity and heterotic relationships amongst and between DR&SS and CIMMYT lines in order to facilitate selection of parents for drought tolerant hybrid crosses. A set of 23 inbred lines (10 from DR&SS and 13 from CIMMYT) were evaluated for 14 morphological traits across seven sites in Zimbabwe in the 2009/10 and 2010/11 seasons. The morphological data revealed that there was variability amongst inbred lines that could be manipulated through selection and hybridisation. The variability was further substantiated using PCA, where the overall diversity could not be explained by a few eigenvectors. All traits accounted for the variability; however traits such as grain yield, texture, ear aspect, common rust, GLS and anthesis days were the major contributors. Lines clustered into two major clusters and four sub-groups using the Euclidean dissimilarity coefficient and in some cases lines related by pedigree were tightly clustered. Lines were also fingerprinted using 1 129 SNP markers. Molecular analysis yielded a total of 2 258 alleles, with an average of two alleles per locus. Cluster analysis based on Rogers' dissimilarity coefficient revealed two major clusters and five sub-groups among the lines. Moderate genetic diversity was observed with an average dissimilarity of 0.32 and average polymorphic information content (PIC) value of 0.258. The clustering was largely not consistent with the available pedigree information of the lines. Information generated in this study will, however, aid breeders to decide on which hybrids to constitute and evaluate.

5.1 Introduction

Maize is the staple food crop and main source of carbohydrates in the majority of the Zimbabwe populace, with a per capita consumption of 150 kg annum^{-1}. There are different players in the seed industry who are making an effort to provide a wide range of varieties for selection by farmers and the Zimbabwe National Breeding Programme (DR&SS) is one of them. The programme acquires germplasm from CIMMYT, IITA and other breeding programmes to enhance genetic diversity of its breeding materials through initiation of new breeding projects. Hybrids and populations developed in a maize breeding programme become important sources for inbred line extraction.

Genetic diversity evaluation is used by maize breeders as an alternative method for germplasm selection (Devi and Singh, 2011). Genetic progress depends on the existence of genetic variability. Thus, genetic characterisation of the available germplasm is critical for efficient development of new varieties (Amorim *et al*., 2003). Genetic characterisation of maize germplasm can be used to group different genotypes into heterotic groups whereby lines in the same group are similar compared to lines in different groups (Lu *et al*., 2009). Information on the relationship between maize inbred lines is critical in designing a breeding programme especially when it comes to selecting parents to be used in hybrid development. Maize hybrids are generally constituted from inbred lines from complimentary and opposite heterotic groups in order to exploit hybrid vigour. Hybrid development in maize has generally become the backbone of the formal seed sector across continents as a result of high yielding and adapted varieties that have led to infrastructure development and improvement of the seed value chain as a whole (Devi and Singh, 2011). Hybrid development is therefore dependant on the availability of diverse homozygous inbred lines and evaluating their performance in different combinations across different locations (Bauman, 1981; Smith, 1986; Hallauer, 1990; Troyer, 2004; Koutsika-Sotiriou and Karagounis, 2005).

Inbred line development and selection in maize breeding programmes as well as evaluating hybrid performance in multi-location trials are easy but expensive and require a lot of time. A small number of inbred lines can be used to produce a large number of hybrids, of which evaluating all of them usually poses a challenge (Hallauer, 1990). Therefore making use of

genetic markers in assessing diversity amongst inbred lines has been suggested as a solution towards overcoming bottlenecks, thereby allowing the performance of single cross hybrids to be predicted (Lanza *et al*., 1997). Genetic diversity and the levels of genetic variation in maize can be estimated using both molecular and morphological markers. Earlier studies have shown that molecular markers are not influenced by the environment like morphological traits (Williams *et al*., 1990; Smith and Smith, 1992). Recent studies have further shown remarkable evidence of reasonable environmental influence on plant development (Molinier *et al*., 2006; Li *et al*., 2009). Hence combining morphological and molecular analysis should form a reliable basis for germplasm improvement. Molecular markers that have been found in abundance in all genomes that have been studied are SNP markers.

It is obvious that SNP markers would become a marker of choice considering their abundance in different genomes as well as their ability to be processed automatically. SNP markers have been incorporated in different applications such as identifying cultivars, genetic map construction, positional cloning of targeted loci, genetic diversity assessment, determining ancestry, association mapping as well as marker-assisted breeding (Gupta *et al*., 2001; Rafalski, 2002; Lijavetzky *et al*., 2007). Maize is considered highly polymorphic amongst different crop species with an average SNP frequency of 1% (Tenaillon *et al*., 2001), followed by rice with a SNP frequency of 0.5-0.78% (IRGSP, 2005). Ravel *et al*. (2006) found that wheat had a SNP frequency of 0.5%, whilst Zhu *et al*. (2003) found that soybean had a SNP frequency of 0.36%. Rapeseed was found to have the lowest SNP frequency (0.16%) (Farman *et al*., 2002). There are a large number of SNP markers available for use in maize, of which many were developed using DNA sequences of known genes. According to Tenaillon *et al*. (2001) the SNP frequency for chromosome 1 of maize has been estimated to be between 1 in 104 bp for two randomly paired landraces and 1 in 124 bp in 36 inbred lines. The objective of this study was to analyse morphological and genetic diversity and heterotic relationships among 10 DR&SS elite inbred lines and 13 CIMMYT inbred lines. The genetic analysis results would then assist in selecting parents for drought tolerant hybrid crosses. The lines under evaluation in this study are potential candidates for the national drought breeding programme. Once the heterotic relationships amongst the lines

are determined, hybrid development would be done in such a way that inbred lines from divergent groups are crossed in order to maximise hybrid vigour.

5.2 Materials and methods

5.2.1 Germplasm selection

A total of 23 inbred lines were chosen for characterisation and of these lines, 10 were from DR&SS and 13 from CIMMYT. Elite lines were chosen from both programmes with CIMMYT lines being chosen specifically for their biotic stress resistance or abiotic stress tolerance. All maize lines were the white kernel types. The DR&SS lines included the parental genotypes of popular and widely grown commercial hybrids but being susceptible to both biotic and abiotic stresses such as drought and MSV disease. Details of the lines are given in section 3.2.1. Lines were planted at seven sites in 2009/2010 and again at the same sites in the 2010/2011 season, to facilitate capturing of morphological data but two random drought sites were excluded from the analysis.

5.2.2 Site selection

Details of sites used are given in sections 3.2.2 and 4.2.2.

5.2.3 Experimental design and morphological traits

The experimental design is as given in section 3.2.4. Data was recorded as described in Table 3.2. Ear rots were measured as a percentage of rotten ears, whilst disease scores for GLS, northern leaf blight, common rust (*Puccinia sorghi* Schw.) and MSV were measured on a scale of 1-5 where 1 is clean of infection and 5 is severely diseased. Husk cover was measured as a percentage of plants with ears that were not completely covered by husks.

5.2.4 Deoxyribonucleic acid extraction

Seedlings for all 23 lines were raised in plastic seed trays for about 2-3 weeks (until they reached the 3-4 leaf stage). Equal amounts of leaf tissue were harvested from 10 plants per inbred line. The leaf tissue was bulked, cut into pieces with scissors and transferred into 1.2 ml strip tubes that contained two 4 mm stainless steel grinding balls. The tissue was freeze-

dried (lyophilised) for three days using a Labconco freeze-dryer (Labconco, Kansas City, MO, USA) as described in the user's manual. The lyophilised leaf samples were ground into fine powder using a GenoGrinder (SPEX Certi Prep, Metuchen, NJ, USA) at 500 strokes per min for 4 min. Genomic DNA was extracted using a modified version of the high throughput mini-prep Cetyltrimethylammonium bromide (CTAB) method (Mace *et al.*, 2003). A modified CTAB extraction buffer was used (200 mM tris (hydroxymethyl) aminomethane (Tris), pH 7.5; 50 mM ethylenediaminetetra acetate (EDTA), pH 8.0; 2 M NaCl; 2% (w/v) CTAB; 1% (v/v) beta-mercaptoethanol). A total of 700 µl of the extraction buffer was added to each sample. Grinding was done for 30 seconds in order to mix the powder with the extraction buffer. Samples were then incubated at 65°C in a water bath for 30 min with continuous gentle rocking. Tubes were gently inverted every 10 min to thoroughly mix the tissue with the extraction buffer. Tubes were removed from the water bath and allowed to cool for 10 min in a fume hood. Samples were then gently mixed and centrifuged at 3 500 revolutions per min (rpm) for 10 min. A total of 500 µl of the aqueous phase was transferred into new tubes and 600 µl chloroform:isoamylalcohol (24:1) was added to the sides of the tubes. This was followed by gentle mixing through rocking for 5 min and centrifuging at 3 500 rpm for 10 min. The upper aqueous layer was transferred into fresh strip tubes and the chloroform:isoamylalcohol wash repeated. A total of 400 µl of the upper aqueous phase was transferred into fresh strip tubes and 0.66 volumes of 100% cold isopropanol was added and mixing was gently done for 5 min in order to precipitate the nucleic acid. Centrifuging at 3 500 rpm was done for 20 min to form a pellet at the bottom of the tube. Pellets were then washed with 70% (v/v) ethanol (600 µl) followed of centrifugation for 10 min after which the ethanol was discarded through decantation. The wash step was done twice. Pellets were allowed to air-dry in the fume hood until the ethanol had completely evaporated. The air-dried pellets were suspended in 200 µl TE buffer (Tris/EDTA) water bath for 45-90 min at 45°C with gentle mixing every 10 min. The quality of the isolated DNA was checked after running aliquots of DNA samples on a 0.8% (w/v) agarose gel that contained 15 ng μl^{-1} GelRed. DNA concentration was measured using a NanoDrop ND-1000 spectrophotometer (Nanodrop Products, Wilmington, NC, USA).

5.2.5 Single nucleotide polymorphism genotyping

DNA samples were sent to KBiosciences laboratory Hoddesdon, Herts, UK, for SNP genotyping. The assays were validated using KBioscience competitive allele-specific PCR (KASPar) genotyping chemistry. A total of 1 242 SNPs were used. The SNPs were selected out of the available 1 250 SNPs, of which eight were not able to return data, which would pass the in-house quality control checks at KBiosciences. The SNP markers were developed at Cornell University and were converted in partnership with CIMMYT (http://www.intergratedbreeding.net/snp-marker-conversion). The analysis was carried out in a 96-well plate format. The system comprised of two components, the assay mix (three unlabelled primers, the SNP specific component of the system) and the reaction mix (all other components required including the universal fluorescent reporting system). Two forward primers, one for each SNP allele and one common reverse primer were used. There was a forward primer for each SNP allele and each forward primer had a different SNP sequence at the 3′-end and a unique unlabelled tail sequence at the 5′-end and this would bind to the fluorescently labelled molecular beacon. The genotyping process basically involved nine steps. The first step involved the assay design using the Primer Picker software (http://ww.kbioscience.co.uk/primer-picker/). This was followed by sample arraying in a microtitre 96-well PCR plate. PCR reactions were set up in a total volume of 8 µl containing 20 ng μl^{-1} template DNA, 2 µl of reaction mix (mix information proprietary), 0.11 µl of assay mix (mix information proprietary), 0.026 µl of KTaq polymerase, 0.064 µl $MgCl_2$ and 1.8 µl of H_2O. The concentration of $MgCl_2$ in the reaction mix was 2.2 mM. The combined assay mix and reaction mix were dispensed over the DNA samples using a liquid dispenser. A fusion laser welding system was used to seal the plates. The PCR cycling was done in a KBioscience "Duncan" water bath cycler. A hot start activation that lasted for 15 min was done at 94°C. Two cycling steps followed where the first consisted of 20 cycles at 94°C for 10 sec, 57°C for 5 sec and 72°C for 10 sec. The second cycle consisted of 18 cycles at 94°C for 10 sec, 57°C for 20 sec and 72°C for 40 sec. The plates were read using a fluorescence resonance energy transfer (FRET) plate reader. The fluorophores 6-carboxyfluorescein (FAM) and 2′chloro-7′-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC) were used for distinguishing between genotypes and the reference dye, 6-Carboxy-X-Rhodamine,

succinimidyl ester (ROX) was used as a passive reference. The FAM and VIC data were plotted on the X- and Y-axes respectively.

5.2.6 Statistical analysis

ANOVA for morphological traits was done using AGROBASE (2005). Pearson correlation coefficients were computed using SPSS version 15.0 for Windows (2006). The genotypic (σ^2_g) and phenotypic (σ^2_p) variances were calculated from expected mean squares of ANOVA using the formula suggested by Hallauer and Miranda (1988):

Genotypic variance:

$\sigma^2_g = (MS_g - MS_e)/r$

Where: MS_g = mean square of genotypes

MS_e = mean square error

r = number of replications

Phenotypic variance:

$\sigma^2_p = \sigma^2_e + \sigma^2_g$

Where: σ^2_e = error variance

σ^2_g = genotypic variance

Genotypic coefficient of variation:

$GCV = (\sqrt{\sigma^2_g} / X)\ 100$

Where: σ^2_g = genotypic variance

X = mean of the trait

Phenotypic coefficient of variation:

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

Where: X = mean of the trait

Broad sense heritability was calculated using the following formulae:

$H^2_B = \sigma^2_g / \sigma^2_p$

Where h^2_B = broad sense heritability

σ^2_g = genotypic variance

σ^2_p = phenotypic variance

Genetic advance:

$GA = k\sigma_p H^2$

Where: k = the standardised selection differential at 5% selection (2.063)

σ_p = phenotypic standard deviation of the character

H^2 = heritability estimate

The importance of different traits in explaining multivariate variation was assessed using PCA in GenStat 14th Edition (2011). KBiosciences SNP viewer version 1.99 was used to view the SNP data. Powermaker version 3.25 (Liu and Muse, 2005) was used for computing summary statistics of the genetic data such as number of alleles, number of genotypes, allele and genotype frequencies, PIC, heterozygosity and GDs from allele frequencies. GDs based on morphological data were calculated using the Euclidean dissimilarity coefficient (Kaufman and Rousseeuw, 1990) using the following formula:

$$d_E = \sqrt{\sum_{i=1}^{m} \sum_{j=1}^{ni} (p_{ij}-q_{ij})^2}$$

Where p_{ij} and q_{ij} = allele frequencies of the j^{th} allele at the i^{th} locus in two individuals under consideration

ni = number of alleles at the i^{th} locus

m = number of loci

GDs based on SNP data were calculated using the Rogers' dissimilarity coefficient using the following formula:

$$d_R = 1/m \sum_{i=1}^{m} \sqrt{\tfrac{1}{2} \sum_{j=1}^{ni} (p_{ij}-q_{ij})^2}$$

Where p_{ij} and q_{ij} = allele frequencies of the j^{th} allele at the i^{th} locus in two individuals under consideration

ni = number of alleles at the i^{th} locus

m = number of loci

Euclidean (Kaufman and Rousseeuw, 1990) dissimilarity coefficients calculated in SPSS version 15.0 for Windows (2006) using morphological data were subjected to cluster analysis using the unweighted pair-group method with arithmetic average algorithm (UPGMA) using sequential agglomerative hierarchical nested cluster analysis (SAHN) programmes in NTSYSpc 2.21n software (Rohlf, 1993). On the other hand Rogers' (Rogers, 1972) dissimilarity coefficients calculated in Powermaker version 3.25 (Liu and Muse, 2005) based on SNP data were subjected to cluster analysis using the weighted method with neighbour-joining (Saitou and Nei, 1987) in NTSYSpc 2.21n. Relationships among inbred lines were visualised in dendrograms. The goodness of fit of the clustering matrices was calculated using the COPH and MXCOMP programmes in NTSYSpc. To further visualise the relationship among the inbred lines PCA was done using DARwin version 5.0.158 software (Perrier and Jacquemould-Collet, 2006).

5.3 Results

5.3.1 Performance of inbred lines as measured using morphological traits

Combined ANOVAs were done within years and across years (Tables 5.1-5.3). Lines were significantly different for all measured traits in 2009/10 and 2010/11 and across the two years except for stem lodging. Environment x line was significant for all other traits in the 2009/10 season except stem lodging, whereas in the 2010/11 season it was only significant for anthesis days, plant height, ear height, root lodging, ears per plant and ear rot. Environments were significantly different for all traits within years and across years. Environment x line was significant for all traits except stem lodging (Table 5.3). There was considerable variability amongst lines as indicated by differences between the minimum and maximum values for all traits (Table 5.4). Lines generally showed high prolificacy (ears per plant) of close to 1 and CML539 had the highest value of 1.36 (Figure 5.1) but this did not translate to very high yield. The CIMMYT line CML548 had the highest yield of 2.91 t ha^{-1} and the lowest yielder was SC5522 with 0.51 t ha^{-1} (Table 5.4).

Table 5.1 Mean squares for grain yield and other morphological traits across five sites in the 2009/10 season

Source	DF	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER
Site	4	50.257***	7759.176***	570.010***	72396.516***	15361.314****	0.294***	56289.468***	4156.685***	3.538***	1331.052***
Rep within site	5	0.291	411.872	32.024	1254.612*	141.240	1.06	849.766	42.049	0.106	70.853
Line	24	5.284***	1640.426***	179.675***	3571.819***	1134.714***	0.046***	664.169*	94.286	0.211***	575.432***
Site x Line	96	1.007***	1105.274***	173.252***	823.974*	255.795**	0.014***	558.545*	92.486	0.101***	545.878***
Residual	120	0.389	356.464	15.132	526.962	161.315	0.006	384.174	72.826	0.049	195.883
LSD (0.05)		0.553	16.718	3.444	20.326	11.246	0.068	17.355	7.556	0.196	12.392
SED		0.279	8.444	1.740	10.266	5.680	0.034	8.766	3.816	0.099	6.259

*****P≤0.001; **P≤0.01; *P≤0.05; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; RL=root lodging; SL=stem lodging; EPP=ears per plant; ER=ear rot; LSD=least significant difference; SED=standard error difference; DF=degrees of freedom; Rep=replication.**

Table 5.2 Mean squares for grain yield and other morphological traits across five sites in the 2010/11 season

Source	DF	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER
Site	4	14.983***	13306.760***	219.586***	49869.996***	18165.796***	0.092***	8235.236***	6740.890***	2.958***	1821.460***
Rep within site	5	3.261	54.064***	2.988	513.856*	175.668	0.004	807.542***	33.644	0.156***	67.915
Line	24	5.310***	186.000***	40.119***	2750.149***	1219.998***	0.022***	806.932***	93.100	0.299***	416.727***
Site x Line	96	2.198	13.652***	17.642	536.634***	276.671***	0.006	312.844***	83.304	0.100***	172.149*
Residual	120	1.975	5.314	13.780	200.973	114.393	0.004	152.593	86.389	0.034	119.062
LSD (0.05)		1.245	2.041	3.287	12.553	9.470	0.059	10.938	8.230	0.163	9.662
SED		0.629	1.031	1.660	6.340	4.783	0.030	5.524	4.157	0.082	4.880

*****P≤0.001; **P≤0.01; *P≤0.05; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; RL=root lodging; SL=stem lodging; EPP=ears per plant; ER=ear rot; LSD=least significant difference; SED=standard error difference; DF=degrees of freedom; Rep=replication.**

Table 5.3 Mean squares for grain yield and other traits in the 2009/10 and 2010/11 seasons

Source	DF	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER
Site	4	22.562***	20507.048***	698.203***	65054.837***	21803.078***	0.340***	17132.156***	4920.383***	4.470***	2085.520***
Line	24	6.569***	973.506***	122.220***	5141.323***	1733.709***	0.037***	1105.932***	136.260*	0.446***	753.513***
Rep within site x year	4	4.441**	582.420*	43.765*	2210.585***	396.135*	0.013*	2071.635***	94.616	0.327***	173.459
Year	1	1.004	3931.208***	208.658***	19158.050***	13302.482***	0.173***	20446.734***	155.57	0.354**	1699.799**
Site x Line	96	1.899**	528.737***	92.558***	684.587***	301.929***	0.009***	439.824***	88.993	0.106***	307.768***
Site x Year	4	42.678***	558.888*	91.393***	57211.675***	11724.032***	0.046***	47392.547***	5977.193***	2.026***	1066.992***
Line x Year	24	4.025***	852.921***	97.575***	1180.646***	621.003***	0.030***	365.169	51.127	0.064*	238.646*
Site x Line x Year	96	1.306	590.189***	98.336***	676.021***	230.537**	0.010***	431.566**	86.798	0.095***	410.259***
LSD (0.05)		0.669	8.274	2.340	11.737	7.223	0.044	10.078	5.489	0.125	7.720
SED		0.340	4.201	1.188	5.958	3.667	0.022	5.117	2.787	0.064	3.919

*****P≤0.001; **P≤0.01; *P≤0.05; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; RL=root lodging; SL=stem lodging; EPP=ears per plant; ER=ear rot; LSD=least significant difference; SED=standard error difference; Rep=replication.**

Table 5.4 Mean performance of maize inbred lines for 14 traits evaluated in the 2009/10 and 2010/11 seasons

Line	GYD	AD	ASI	PH	EH	EPO	RL	EPP	ER	GLS	RUST	SEN	TEX	EA
2Kba	1.17	75.7	2.1	151.2	95.7	0.42	31.9	0.75	13.8	2.4	1.9	0.6	5.2	5.0
2N3d	1.98	77.9	3.0	192.3	82.2	0.43	39.3	0.79	27.5	2.6	1.8	3.8	3.9	4.4
CML312	1.68	78.1	2.6	164.6	73.7	0.32	30.0	0.57	13.4	2.9	1.6	3.8	2.8	3.1
CML545	2.58	69.6	-1.2	162.5	83.1	0.47	23.4	0.91	7.3	2.6	1.6	3.9	3.9	3.8
CML395	2.39	78.9	1.7	204.9	98.9	0.49	16.2	0.85	22.3	2.0	1.5	3.2	3.7	4.0
CML544	1.95	75.1	-0.1	141.1	68.6	0.43	17.3	0.98	6.5	2.1	1.3	3.7	3.9	4.1
CML442	2.27	78.8	3.8	164.9	71.3	0.40	19.3	1.02	13.8	2.0	2.0	3.4	4.7	4.9
CML444	2.58	81.6	-0.3	171.2	97.9	0.51	24.4	0.84	2.8	3.3	1.7	3.2	3.0	3.4
CZL03007	1.91	75.5	-0.9	150.1	70.7	0.40	45.7	0.95	5.5	2.0	1.7	3.0	2.2	2.9
CML536	1.81	82.8	0.6	185.3	89.2	0.39	20.6	0.85	4.3	2.2	2.6	2.8	2.2	2.8
CML537	2.32	77.9	1.4	171.7	74.9	0.39	17.7	0.71	8.2	3.0	1.5	3.1	2.8	3.2
CML538	2.37	74.3	-0.6	168.9	79.6	0.42	14.9	0.88	11.7	2.5	1.3	3.5	3.4	4.0
CML539	1.99	71.3	-0.1	134.3	68.7	0.43	29.9	1.36	2.4	2.3	1.5	3.5	3.4	3.8
K64r	1.37	75.7	0.6	129.2	61.9	0.45	35.9	0.82	30.6	2.1	1.9	4.4	4.4	4.7
N3.2.3.3	1.52	79.0	2.3	180.7	90.6	0.51	44.2	0.59	8.5	3.4	1.8	4.0	4.4	4.7
NAW5885	1.30	78.3	6.6	165.9	83.3	0.41	39.3	0.63	21.0	2.6	1.4	3.1	3.2	3.2
RA214P	1.75	80.6	3.9	194.1	92.4	0.45	20.1	0.60	30.8	2.1	1.3	2.9	3.2	3.8
RS61P	2.53	74.8	1.5	146.0	79.0	0.47	27.6	0.86	8.5	2.5	1.3	2.9	3.3	3.5
SC5522	0.51	80.7	3.6	194.7	95.7	0.44	45.2	0.62	22.1	2.3	1.6	4.6	2.6	2.8
SV1P	1.05	62.9	-0.1	133.3	71.9	0.43	32.5	0.91	1.9	2.7	3.1	4.0	3.4	4.1
WCOBY	1.19	75.0	1.5	142.9	71.5	0.48	28.0	0.70	19.8	2.9	1.9	3.3	3.1	3.6
CML548	2.91	76.5	2.0	159.7	74.5	0.42	22.4	0.90	16.1	2.6	1.7	3.4	2.5	3.1
CZL052	1.52	73.0	-0.1	164.1	78.2	0.44	45.5	0.76	3.8	2.4	1.4	3.5	3.1	4.1
Mean	**1.88**	**76.3**	**1.5**	**163.6**	**80.4**	**0.43**	**28.8**	**0.83**	**12.4**	**2.5**	**1.8**	**3.4**	**3.4**	**3.8**
Min	**0.51**	**62.9**	**-1.2**	**129.2**	**61.9**	**0.32**	**14.9**	**0.57**	**1.9**	**2.0**	**1.3**	**0.6**	**2.2**	**2.8**
Max	**2.91**	**82.8**	**6.6**	**204.9**	**98.9**	**0.51**	**45.7**	**1.36**	**30.8**	**3.4**	**3.1**	**4.6**	**5.2**	**5.0**
LSD (0.05)	**0.49**	**1.8**	**1.7**	**13.4**	**14.2**	**0.05**	**11.6**	**0.17**	**15.9**	**0.8**	**0.5**	**0.8**	**0.9**	**0.9**
MSE	**0.34**	**4.3**	**3.5**	**210.7**	**94.1**	**0.00**	**189.2**	**0.04**	**177.0**	**0.2**	**0.1**	**0.3**	**0.7**	**0.7**

GYD=grain yield (t ha^{-1}); AD=anthesis days; ASI=anthesis silking interval (days); PH=plant height (cm); EH=ear height (cm); EPO=ear position (0-1); RL=root lodging (%); EPP=ears per plant (#); ER=ear rot (%); GLS=grey leaf spot (1-5); RUST=common rust (1-5); SEN=senescence (1-10); TEX=texture (1-5); EA=ear aspect (1-5); LSD=least significant difference; MSE=mean square error; Min=minimum; Max=maximum.

In terms of maturity SV1P was the earliest (62.9 days) and the latest was CML536 (82.8 days). All lines exhibited good anthesis silking interval values except NAW5885, which had an anthesis silking interval value of 6.6. Lines generally had foliar disease scores of less than 3 except CML444 and N3.2.3.3, which had GLS scores of 3.3 and 3.4 respectively. The rust scores were lower than the GLS scores (Figure 5.2), however SV1P was the only line with a high rust score of 3.1. Lines showed high ear rot percentages and this resulted in poor ear aspect scores (Table 5.4). K64r (30.6) and RA214P (30.8) had the highest ear rot scores. The texture of the lines ranged from semi-flint to dent.

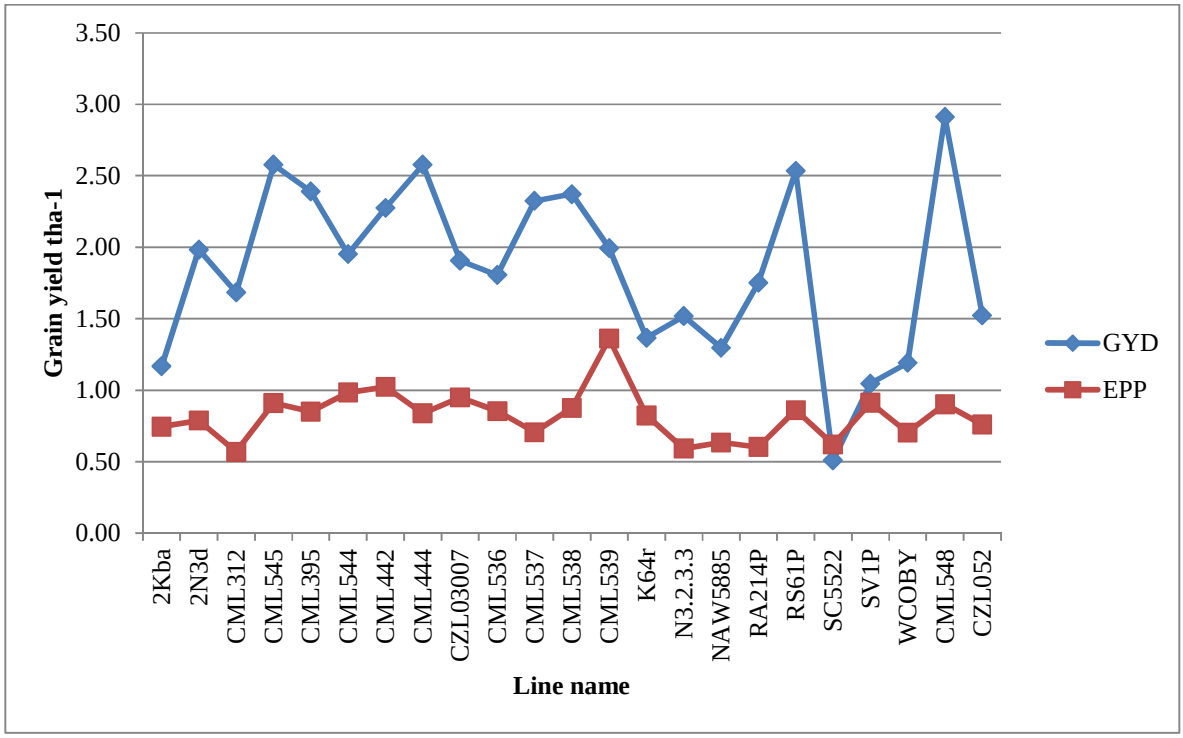

Figure 5.1 Grain yield performance and ears per plant for the lines across seasons.

GYD=grain yield; EPP=ears per plant

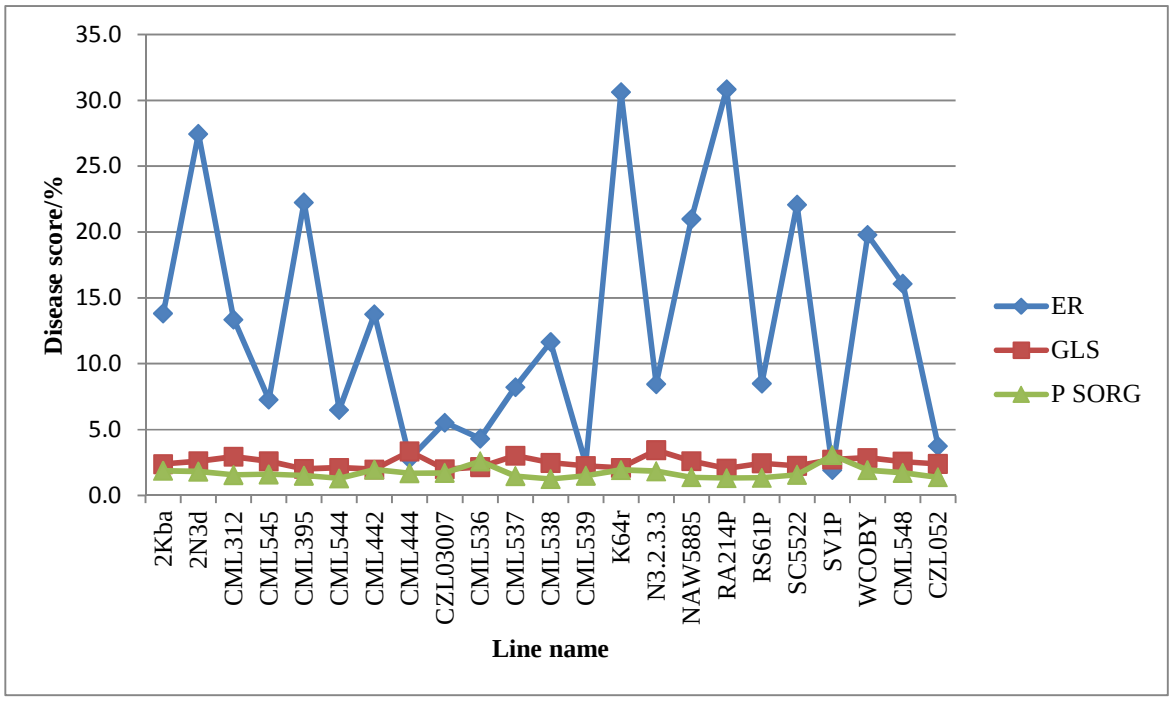

Figure 5.2 Response of lines to ear rot and foliar diseases across seasons.

ER=*Exserohilum turcicum*; GLS=grey leaf spot; P SORG=*Puccinia sorghi.*

The genetic and phenotypic variances as well as heritability estimates for the lines are presented in Table 5.5. Broad sense heritability estimates calculated showed that half of the traits were highly heritable with more than 50% heritability. Grain yield had the highest heritability estimate of 0.81 and stem lodging had the lowest heritability estimate of 0.02. Other traits with high heritability estimates (>0.50) were plant height, ear height, ear position and ears per plant (Table 5.5). Plant height had the largest genetic variance (274.8) whilst ear position had the smallest genetic variance (0.003). Phenotypic variances were higher than genetic variances for anthesis days, anthesis silking interval, plant and ear height, root and stem lodging, ears per plant and ear rot. Traits with high genotypic coefficients of variation included grain yield, anthesis days, anthesis silking interval, plant height, ear height, root lodging and ear rot (Table 5.6). Anthesis silking interval had the highest phenotypic coefficient of variation (34.6) whilst ear position had the lowest value (10.3). Plant (16.30) and ear (9.14) height had high genetic advance values compared to other traits. Grain yield had the highest genetic advance (25.0%) as a percentage of the mean, whilst ear position had the lowest value (1.1%).

PCA computed using the correlation matrix grouped the 14 traits into 14 components, which accounted for 100% of the variability present among the lines evaluated. The first nine PCs explained 94% of the total variation, whilst the five eigenvectors with eigenvalues greater than one accounted for 74.1% of the entire variability available among the inbred lines (Table 5.7). The first PC that explained 26.2% of the variation among genotypes was mainly attributed to variation in anthesis days, anthesis silking interval, plant and ear height, stem lodging, ears per plant and ear rot. The second PC that accounted for 17.7% of the variation was dominated by grain yield, anthesis silking interval and root and stem lodging.

In the third PC (12.1%) GLS, texture, ear position, ear height, anthesis silking interval, root lodging, ear rot and common rust were the most important traits. Ear position, ear aspect, ear rot and GLS were important delineating traits associated with the fourth PC, which accounted for 9.7% of the total variation. The fifth PC which explained 8.4% of the total variation was associated with variation due to senescence, common rust, ear position, ear height, ear rot and grain yield. Each trait was found to be an important source of variation in the PCA.

Table 5.5 Genetic and phenotypic variances and heritability estimates

Trait	Genetic variance	Phenotypic variance	Heritability
GYD	0.428	0.528	0.81
AD	53.515	164.043	0.33
ASI	0.642	17.968	0.04
PH	274.785	357.189	0.77
EH	87.892	113.471	0.78
EPO	0.003	0.005	0.71
RL	10.562	66.417	0.16
SL	0.180	9.429	0.02
EPP	0.011	0.021	0.52
ER	2.955	57.543	0.05

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; RL=root lodging; SL=stem lodging; EPP=ears per plant; ER=ear rot.

Table 5.6 Estimates of genotypic and phenotypic coefficients of variation and genetic advance of the maize inbred lines across all environments in the 2009/10 and 2010/11 seasons

Trait	GCV (%)	PCV(%)	GA	GA (% of mean)
GYD	47.7	53.0	0.47	25.0
AD	83.8	14.7	5.75	7.5
ASI	65.4	34.6	0.14	9.3
PH	12.9	14.8	16.30	10.0
EH	10.5	11.9	9.14	11.4
EPO	8.8	10.3	0.05	1.1
RL	60.6	15.2	2.89	10.0
EPP	11.5	15.9	0.11	13.3
SL	12.7	91.8	0.16	1.4
ER	48.9	21.5	0.65	5.2

GCV=genotypic coefficient of variation; PCV=phenotypic coefficient of variation; GA=genetic advance; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; RL=root lodging; SL=stem lodging; EPP=ears per plant; ER=ear rot.

5.3.2 Correlation coefficients among morphological traits

Pearson coefficient correlations for the traits are presented in Table 5.8. Significant correlation coefficients are highlighted in bold. Grain yield was negatively and significantly ($P \leq 0.01$) correlated with root lodging ($r=-0.613$) and positively correlated ($P \leq 0.05$) with ears per plant ($r=0.415$). Correlation of grain yield with anthesis silking interval, ear height, stem lodging, ear rot, rust, senescence, texture and ear aspect was negative and non-significant.

Table 5.7 Eigenvectors, eigenvalues and individual and cumulative percentage of variation explained by first nine principal components for 14 morphological traits of maize inbred lines

	Eigenvectors								
Variables	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**
Grain yield	0.02	0.54	-0.12	-0.04	0.21	-0.03	0.01	0.41	0.11
Anthesis days	-0.41	0.02	-0.18	-0.10	0.00	0.23	-0.02	0.49	-0.05
Anthesis silking interval	-0.33	-0.29	-0.23	0.03	-0.05	-0.23	0.02	0.33	-0.01
Plant height	-0.44	0.07	0.01	-0.03	0.08	0.30	0.25	-0.08	0.45
Ear height	-0.40	0.10	0.25	0.17	-0.22	0.37	-0.03	-0.13	0.08
Ear position	-0.06	0.10	0.46	0.45	0.33	0.26	-0.10	0.07	-0.47
Root lodging	0.03	-0.49	0.23	-0.08	0.05	0.22	-0.42	0.04	0.30
Stem lodging	0.30	-0.40	-0.14	0.02	0.03	0.32	-0.10	0.40	-0.04
Ears per plant	0.38	0.24	-0.19	0.16	0.05	0.34	0.01	0.23	0.10
Ear rot	-0.27	-0.26	-0.31	0.29	0.29	-0.17	0.17	-0.04	-0.36
Grey leaf spot	-0.09	0.04	0.59	-0.26	0.02	-0.42	-0.05	0.42	-0.01
Common rust	0.17	-0.18	0.23	0.03	-0.42	0.13	0.74	0.18	-0.12
Senescence	0.10	-0.17	0.12	-0.23	0.72	0.05	0.41	-0.09	0.21
Ear aspect	0.09	-0.06	0.07	0.72	-0.01	-0.33	0.04	0.14	0.52
Grain texture	0.06	0.21	-0.61	0.04	-0.01	0.12	-0.01	0.14	-0.18
Eigenvalue	3.67	2.47	1.69	1.36	1.18	0.20	0.82	0.55	0.44
Individual percentage variation explained	26.2	17.7	12.1	9.7	8.4	6.8	5.9	4.0	3.2
Cumulative percentage variation explained	26.2	43.9	56.0	65.7	74.1	80.9	86.8	90.8	94.0

PC= principal component.

Anthesis days were positively and significantly correlated with anthesis silking interval (r=0.489) and plant (r=0.662) and ear (r=0.499) height, whilst on the other hand it was significantly and negatively correlated with ears per plant (r=-0.430). The largest significant and positive correlation was between grain texture and ear aspect (r=0.938). Anthesis silking interval was significantly and negatively correlated with ears per plant (r=-0.527) as well as positively and significantly correlated with ear rot (0.617). Correlation between plant height and ear height was significant and positive (r=0.753). Stem lodging and ears per plant were significantly and negatively correlated with plant and ear height. Root and stem lodging were significantly and positively correlated with each other. Ears per plant were significantly and negatively correlated with ear rot. GLS, rust, ear position and senescence were not significantly correlated with any of the morphological traits.

Table 5.8 Pearson coefficient correlations for grain yield and other morphological traits measured from the inbred lines in the 2009/10 and 2010/11 seasons

Trait	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER	GLS	RUST	SEN	TEX
AD	0.073													
ASI	-0.307	**0.489***												
PH	0.089	**0.662****	0.410											
EH	-0.046	**0.499***	0.266	**0.753****										
EPO	0.086	-0.024	-0.173	0.099	0.390									
RL	**-0.613****	-0.087	0.161	-0.083	-0.017	0.047								
SL	-0.386	-0.259	-0.046	**-0.493***	**-0.479***	-0.168	**0.546****							
EPP	**0.415***	**-0.430***	**-0.527****	**-0.468***	**-0.421***	0.007	-0.269	0.354						
ER	-0.234	0.0377	**0.617****	0.344	0.129	0.060	0.050	-0.004	**-0.450***					
GLS	0.039	0.003	0.003	0.024	0.164	0.231	0.127	-0.274	-0.388	-0.263				
RUST	-0.321	-0.283	-0.139	-0.191	-0.088	-0.104	0.090	0.300	0.093	-0.213	0.072			
SEN	-0.090	-0.150	-0.086	0.020	-0.330	0.108	0.206	0.218	0.006	0.105	0.104	0.028		
TEX	-0.085	-0.170	0.110	-0.170	0.023	0.276	-0.037	0.074	0.086	0.203	-0.064	0.025	-0.185	
EA	-0.082	-0.245	-0.008	-0.182	-0.070	0.291	0.001	0.070	0.133	0.165	-0.087	0.104	-0.117	**0.938****

****P≤0.01; *P≤0.05; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; Ear height; EPO=ear position; RL=root lodging; SL=stem lodging; EPP=ears per plant; ER=ear rot; GLS=grey leaf spot; RUST=common rust; SEN=senescence; TEX=texture; EA=ear aspect.**

5.3.3 Genetic distances and heterotic grouping among lines based on morphological data

Estimates of genetic distances based on morphological data expressed as Euclidean distances for all 253 pairwise comparisons averaged 0.37 and ranged from 0.109 between CML538 and CML537 to 0.911 between K64r and CML395 (Table 5.9). Low genetic distances were observed amongst the inbred lines CML538 and CML537 (0.109), CML442 and CML312 (0.126), RS61P and WCOBY (0.146), CML537 and CML442 (0.160), CML312 and CML545 (0.169), CML442 and CML548 (0.175), CML545 and CML537 (0.179), RS61P and CML548 (0.180), CML538 and CML545 (0.182), SV1P and CML539 (0.183), CML312 and CML537 (0.188) and WCOBY and CML548 (0.185). Most of these lines are related by pedigree and they belong to the same heterotic groups. High genetic distances were observed between K64r and CML395 (0.911), CML395 and SV1P (0.846), CML395 and CML539 (0.852), RA214P and K64r (0.808), RA214P and CML539 (0.790), RA214P and SV1P (0.762), SC5522 and K64r (0.756), SC5522 and SV1P (0.726), CML395 and CML544 (0.725), CML395 and CZL03007 (0.724), CML539 and SC5522 (0.721), SC5522 and CML544 (0.715) and K64r and CML536 (0.719).

The dendrogram obtained from UPGMA cluster analysis using the Euclidean dissimilarity coefficient classified the inbred lines into five main clusters (Figure 5.3). The dendrogram exhibited a poor goodness of fit with a matrix correlation of r=0.70. The total detected morphological variation between the 23 inbred lines was a dissimilarity of 53%. Generally grouping of lines was not in line with the known CIMMYT A and B and DR&SS SC and N3 heterotic grouping. In main groups I and II lines basically did not group according to already known heterotic groupings. Group I clustered separately from the other groups, indicating that the four lines in this group were the most distantly related from the other lines, based on morphological data. Group II also clustered separately indicating that the three lines differed significantly from the other lines.

Table 5.9 Estimates of genetic distances based on Euclidean distances and morphological data for all pair-wise comparisons of 23 inbred lines

LINE		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
2Kba	**1**	0.462	0.264	0.226	0.578	0.349	0.317	0.300	0.319	0.396	0.359	0.356	0.397	0.486	0.361	0.276	0.535	0.256	0.507	0.397	0.335	0.328	0.356
2N3d	**2**		0.336	0.409	0.331	0.619	0.387	0.423	0.506	0.329	0.384	0.421	0.686	0.695	0.277	0.311	0.316	0.541	0.250	0.697	0.558	0.441	0.434
CML312	**3**			0.169	0.510	0.294	0.126	0.315	0.255	0.303	0.188	0.246	0.390	0.464	0.303	0.209	0.455	0.256	0.453	0.408	0.293	0.213	0.281
CML545	**4**				0.493	0.273	0.200	0.235	0.321	0.287	0.179	0.182	0.404	0.537	0.325	0.272	0.457	0.246	0.496	0.382	0.325	0.238	0.305
CML395	**5**					0.725	0.510	0.404	0.724	0.308	0.438	0.436	0.852	0.911	0.422	0.497	0.213	0.671	0.384	0.846	0.714	0.555	0.611
CML544	**6**						0.279	0.438	0.336	0.503	0.319	0.320	0.295	0.420	0.540	0.412	0.649	0.223	0.715	0.279	0.243	0.286	0.421
CML442	**7**							0.349	0.323	0.297	0.160	0.228	0.384	0.466	0.370	0.273	0.456	0.257	0.491	0.419	0.287	0.175	0.340
CML444	**8**								0.462	0.223	0.257	0.241	0.575	0.705	0.279	0.319	0.383	0.383	0.449	0.543	0.471	0.356	0.371
CZL03007	**9**									0.482	0.392	0.441	0.252	0.369	0.387	0.305	0.661	0.231	0.552	0.279	0.278	0.317	0.227
CML536	**10**										0.226	0.277	0.594	0.719	0.255	0.339	0.336	0.431	0.350	0.605	0.509	0.347	0.382
CML537	**11**											0.109	0.479	0.587	0.331	0.284	0.379	0.309	0.478	0.467	0.355	0.203	0.332
CML538	**12**												0.515	0.610	0.368	0.303	0.357	0.326	0.514	0.475	0.361	0.228	0.371
CML539	**13**													0.304	0.573	0.466	0.790	0.227	0.721	0.183	0.272	0.365	0.401
K64r	**14**														0.663	0.488	0.808	0.367	0.756	0.367	0.280	0.430	0.516
N3.2.3.3	**15**															0.219	0.385	0.408	0.234	0.556	0.474	0.360	0.238
NAW5885	**16**																0.388	0.282	0.348	0.442	0.296	0.224	0.224
RA214P	**17**																	0.588	0.368	0.762	0.597	0.450	0.534
RS61P	**18**																		0.568	0.208	0.146	0.180	0.267
SC5522	**19**																			0.726	0.607	0.485	0.418
SV1P	**20**																				0.243	0.349	0.358
WCOBY	**21**																					0.185	0.327
CML548	**22**																						0.272

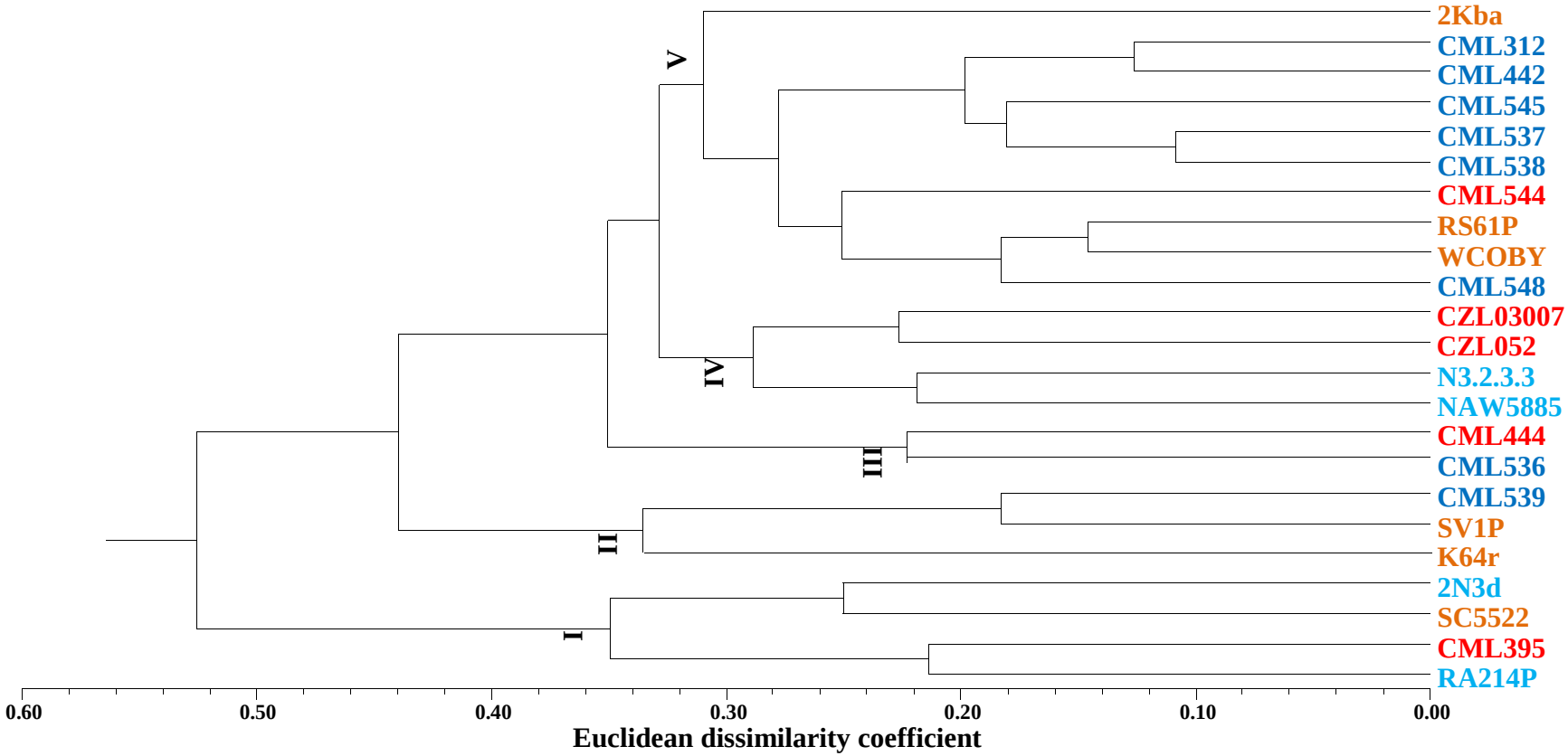

Figure 5.3 Unweighted pair-group method with arithmetic average algorithm cluster analysis of 23 maize inbred lines based on morphological data combined over two seasons and seven locations.

Dark blue=A group; Light blue=N3 group; Red=B group; Orange=SC group

Most of the lines (16) clustered together in groups III, IV and V. Lines clustered mainly according to heterotic groups in the subgroups of groups IV and V. Although group IV consisted of four lines belonging to two different heterotic groups, lines paired according to B and N3 groups in the two subgroups. Group V was the biggest group with 10 lines and contained subgroups in which the lines grouped according to the known heterotic groupings. However CML548 belonging to heterotic group B, clustered with three lines belonging to heterotic groups B and SC. Five CIMMYT lines belonging to heterotic group A (CML312, CML442, CML545, CML537 and CML538) clustered together in the same subgroup and were also closely related based on pedigree data. Morphologically the most similar lines were CML537 and CML538 [MAS(CML206/CML312)-23-2-1-1-B and ZM621A-10-1-1-1-2-B] with 89% similarity followed by CML312 and CML442 [S89500-F2-2-2-1-1-B and (M37W/ZM607-#-B-F37SR-2-3SR-6-2-X)-8-2-X-1-B] with a similarity of 87% and RS61P and WCOBY (share a similar parent SC5522) with a similarity of 85%.

On the other hand grouping in terms of morphological traits followed a certain trend. The four lines in group I (RA214P, CML395, SC5522 and 2N3d) all had mean plant heights of above 190 cm, with high ear placement. These lines were also late maturing with a mean number of days to anthesis of above 75 days and high ear rot percentages. Group II (K64r, SV1P and CML539) was generally characterised by early to medium maturing and shorter plants of semi flint to semi dent texture. Lines in group III were tall, late maturing and had a similar number of ears per plant. The sub-groups within group IV consisted of lines with similar anthesis silking interval values, where NAW5885 and N3.2.3.3 had positive anthesis silking interval values whilst CZL052 and CZL03007 had negative anthesis silking interval values. Finally in group V CML537 and CML538 had similar grain yield means, whilst CML442 and CML312 had similar days to anthesis, plant heights and ear rot scores. RS61P and WCOBY had similar GLS scores and their anthesis silking interval values were the same (1.5). The outlier lines did not pair with other lines because of the unique morphological characteristics that they exhibited. CML548 was the highest yielder (2.91 t ha^{-1}) amongst the lines, whilst K64r had the second highest ear rot score (30.6). CML545 had the best anthesis silking interval value (-1.2) and 2Kba had a poor ear aspect score (5.0).

5.3.4 Single nucleotide polymorphism performance and quality

The data for all individuals tested was presented as a graph using the SNP viewer and the graph indicated the colour of fluorescence and the identified genotype as shown in Figure 5.4, where three clearly defined groups can be seen namely homozygotes G:G (red), homozygotes C:C (blue), heterozygotes G:C (green) while the genotypes indicated in pink were outliers that did not cluster clearly with one of the identified groups. All SNP data (23 lines by 1 242 SNPs) were initially scored. SNP markers that were monomorphic (26 markers) or had more than 20% missing data points (87 markers) were removed from further analyses. As a result, a total of 1 129 SNPs (91%) were included in the analysis of which 297 SNPs had 10-20% missing data whilst 832 had less than 10% missing data.

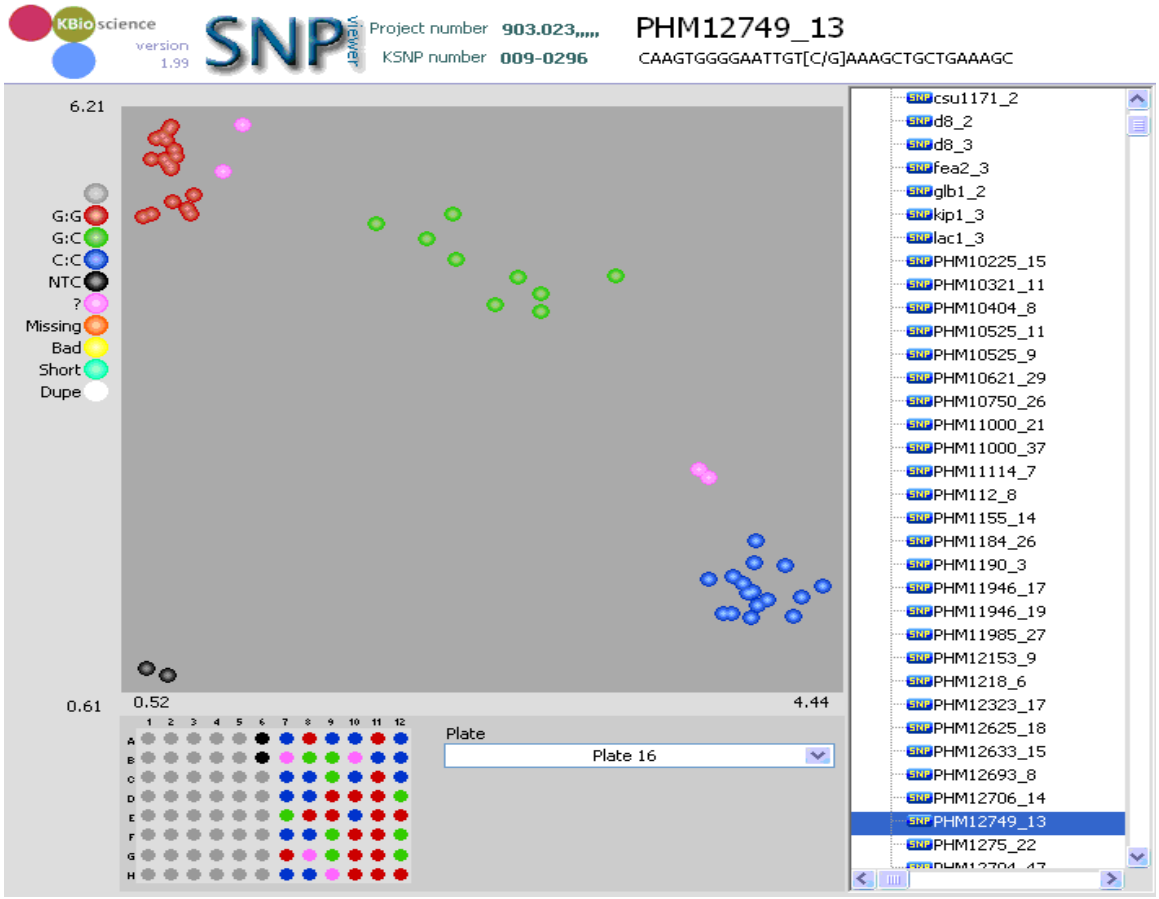

Figure 5.4 Example of information extracted from each single nucleotide polymorphism marker using the single nucleotide polymorphism viewer. Data presented is for single nucleotide polymorphism marker PHM12749_13, which detects a C/G single nucleotide polymorphism in the maize genome.

In the 23 inbred lines, 2 258 alleles were detected at 1 129 loci with two alleles per locus. An even distribution of minor allelic frequency was observed with continued classes from 0.01 to 0.50 (Figure 5.5 and Appendix 9). Only 9.5% (107/1 129) of the SNPs had a minor allele frequency of less than 0.05, whilst approximately 53.3% (602/1 129) of the markers had a minor allele frequency of more than 0.20. In addition 123, (10.9%) of the markers had a minor allele frequency close to 0.50 thereby showing almost equal allele frequencies for the two alternative alleles. The average PIC was 0.252 and ranged from 0.024 to 0.375 with a peak distribution between 0.311 and 0.375 (Figure 5.6 and Appendix 10). About 41.7% of the markers had PIC values greater than 0.30. The estimated gene diversity ranged from 0.025 to 0.500 with an average of 0.312 (data not shown). SNPs were well dispersed across the 10 chromosomes and the coverage ranged from 65 on chromosome 7 to 206 on chromosome 1 (Table 5.10). The inbred lines exhibited heterozygosity at 0.09% of the genetic loci. The number of heterozygous loci per inbred line was determined. There were four inbred lines with a large number of heterozygous loci and these were 2N3d (427), CML537 (403), WCOBY (358) and CZL052 (312) (Table 5.11). There was no line with 100% homozygous loci, however CML442 had 99.29% homozygous loci.

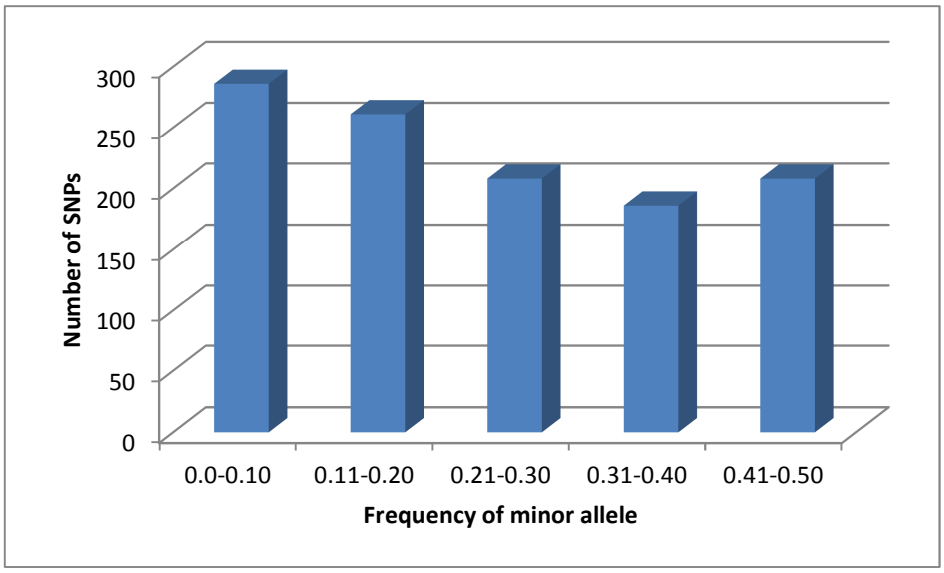

Figure 5.5 Frequency distribution of minor alleles among 23 inbred lines based on 1 129 single nucleotide polymorphism (SNP) markers.

5.3.5 Genetic distances and heterotic grouping of lines based on single nucleotide polymorphism markers

The Rogers dissimilarity coefficient based on SNP data revealed that low GDs were recorded amongst lines. Distances ranged from 0.11 to 0.38 with a mean of 0.32 (Table 5.12). Lines with the lowest genetic distances were SC5522 and CML548 (0.108), SV1P and WCOBY (0.143), 2N3d and WCOBY (0.172), 2Kba and N3.2.3.3 (0.176), CML537 and WCOBY (0.192) and RA214P and CML544 (0.195).

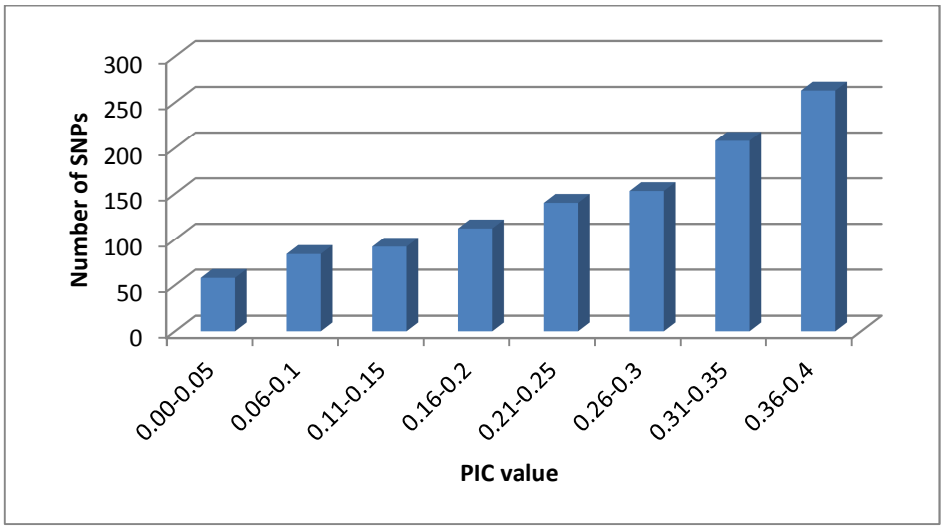

Figure 5.6 Polymorphic information content (PIC) among 23 inbred lines based on 1 129 single nucleotide polymorphism (SNP) markers.

Table 5.10 Distribution of single nucleotide polymorphism markers over the 10 maize chromosomes

Chromosome number	Number of SNPs
1	206
2	121
3	139
4	120
5	130
6	89
7	65
8	102
9	83
10	74
Total	1 129

SNP=single nucleotide polymorphism

Table 5.11 Number of heterozygous loci and percentage homozygosity of maize inbred lines

Name of inbred line	Origin	Number of heterozygous loci	% homozygosity
2Kba	DR&SS	29	97.43
2N3d	DR&SS	427	62.18
CML312	CIMMYT	28	97.52
CML545	CIMMYT	72	93.62
CML395	CIMMYT	32	97.17
CML544	CIMMYT	31	97.25
CML442	CIMMYT	8	99.29
CML444	CIMMYT	91	91.94
CZL03007	CIMMYT	17	98.49
CML536	CIMMYT	15	98.67
CML537	CIMMYT	403	64.30
CML538	CIMMYT	27	97.61
CML539	CIMMYT	14	98.76
K64r	DR&SS	13	98.85
N3.2.3.3	DR&SS	65	94.24
NAW5885	DR&SS	27	97.61
RA214P	DR&SS	20	98.23
RS61P	DR&SS	19	98.32
SC5522	DR&SS	12	98.94
SV1P	DR&SS	55	95.13
WCOBY	DR&SS	358	68.29
CML548	CIMMYT	14	98.76
CZL052	CIMMYT	312	72.36

The highest recorded distances were between CML536 and 2N3d (0.384), CZL03007 and CML536 (0.381), CML395 and CML536 (0.378), CML544 and CML536 (0.376), CML539 and CML536 (0.376), CML536 and CML442 (0.375) and CML538 and CML536 (0.374).

Neighbour-joining cluster analysis in NTSYS grouped the 23 lines into two major groups (Figure 5.7). The dendrogram showed a good goodness of fit (r=0.87). There was no clear separation of lines according to their origin (CIMMYT and DR&SS). The major groups did not show grouping according to the known heterotic groups. However, within some of the subgroups of the main groups there was consistency with the known heterotic groups. Group I consisted of two lines that both belong to similar heterotic groups (SC and B). Group II was divided into two subgroups A and B.

Table 5.12 Estimates of genetic distances based on single nucleotide polymorphism data and Rogers' distances for all pairwise comparisons

Line		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
2N3d	**2**	0.298																					
CML312	**3**	0.302	0.289																				
CML545	**4**	0.360	0.328	0.286																			
CML395	**5**	0.339	0.341	0.319	0.352																		
CML544	**6**	0.320	0.335	0.288	0.338	0.340																	
CML442	**7**	0.351	0.357	0.302	0.348	0.272	0.300																
CML444	**8**	0.329	0.295	0.265	0.216	0.321	0.297	0.296															
CZL03007	**9**	0.340	0.333	0.289	0.331	0.306	0.308	0.310	0.266														
CML536	**10**	0.358	0.384	0.326	0.237	0.378	0.376	0.375	0.332	0.381													
CML537	**11**	0.349	0.204	0.266	0.326	0.330	0.351	0.345	0.320	0.338	0.364												
CML538	**12**	0.341	0.332	0.296	0.334	0.313	0.253	0.222	0.288	0.306	0.374	0.337											
CML539	**13**	0.350	0.341	0.317	0.339	0.314	0.337	0.331	0.292	0.287	0.376	0.348	0.318										
K64r	**14**	0.275	0.357	0.316	0.361	0.232	0.340	0.270	0.331	0.325	0.366	0.315	0.308	0.332									
N3.2.3.3	**15**	0.176	0.259	0.245	0.327	0.346	0.323	0.364	0.328	0.316	0.350	0.349	0.343	0.361	0.333								
NAW5885	**16**	0.334	0.343	0.299	0.318	0.314	0.301	0.319	0.292	0.290	0.344	0.340	0.308	0.289	0.330	0.339							
RA214P	**17**	0.332	0.350	0.297	0.331	0.326	0.195	0.285	0.283	0.295	0.368	0.337	0.262	0.320	0.335	0.339	0.290						
RS61P	**18**	0.335	0.335	0.283	0.322	0.332	0.309	0.299	0.273	0.275	0.363	0.345	0.293	0.299	0.335	0.339	0.295	0.266					
SC5522	**19**	0.344	0.353	0.312	0.240	0.341	0.326	0.332	0.281	0.306	0.363	0.341	0.306	0.316	0.342	0.338	0.332	0.328	0.304				
SV1P	**20**	0.277	0.280	0.243	0.259	0.290	0.285	0.304	0.276	0.270	0.256	0.272	0.292	0.299	0.279	0.295	0.260	0.285	0.277	0.272			
WCOBY	**21**	0.294	0.172	0.275	0.279	0.342	0.333	0.348	0.246	0.320	0.285	0.192	0.326	0.349	0.327	0.288	0.321	0.328	0.321	0.334	0.143		
CML548	**22**	0.352	0.351	0.303	0.276	0.334	0.321	0.329	0.251	0.306	0.369	0.354	0.310	0.298	0.332	0.357	0.316	0.318	0.291	0.108	0.266	0.338	
CZL052	**23**	0.334	0.239	0.294	0.329	0.325	0.216	0.243	0.269	0.282	0.360	0.276	0.223	0.319	0.332	0.330	0.307	0.203	0.297	0.329	0.292	0.244	0.324

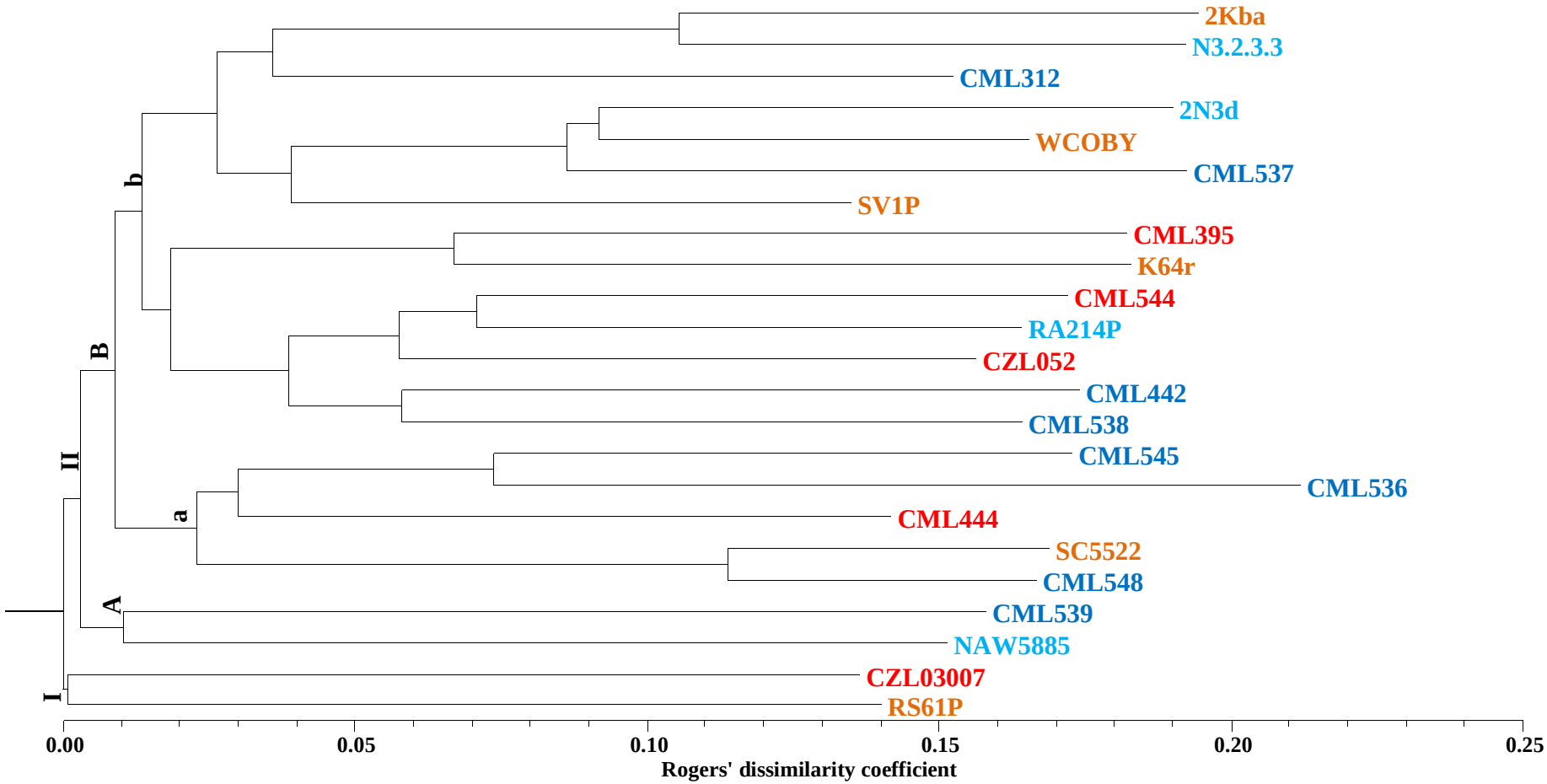

Figure 5.7 Neighbour-joining cluster analysis for the 23 maize inbred lines based on Rogers' dissimilarity coefficient using single nucleotide polymorphism data.

Dark blue=A group; Light blue=N3 group; Red=B group; Orange=SC group.

Group A comprised of two lines belonging to similar groups namely NAW5885 (N3) and CML539 (A). Group B was further divided into subgroups and later into sub-subgroups. In subgroup a CML536 and CML545 clustered together and these two lines are related by pedigree since they share a common parent. Furthermore SC5522 and CML548 in subgroup a clustered together but these lines belong to opposite groups according to the known classification. CML442 and CML538, clustering together in subgroup b, were derived from similar populations. CML395 and K64r clustered together and these lines belong to related groups B and SC respectively. 2N3d has N3.2.3.3 as one of its parents and the two lines clustered in the same subgroup and a similar scenario was noted with CML312 and CML537, where CML537 has CML312 as one of its parents and the two again clustered in the same subgroup. The rest of the lines did not group according to known heterotic grouping and/or pedigree. It was noted that there were four lines with a high number of missing data namely CML545 (33.92%), SV1P (29.05%), WCOBY (28.79%) and 2N3d (27.90%) and since missing data can skew the data these lines were later excluded from the analysis and a dendogram consisting of 19 lines was constructed (Figure 5.8). This was done to ascertain if the grouping was going to be improved without the four lines. The dendrogram also showed a good goodness of fit (r=0.81). Lines were still divided into two major groups and similar groupings were maintained. One of the exceptions was for RS61P and CZL03007. In the initial grouping these lines grouped in the same subgroup but after removal of the four lines, these lines were still in the same group but did not cluster together in the same subgroup. Furthermore CML536 did not show any relationship with any of the lines where it previously grouped together with CML545.

A PCA biplot was further drawn for the 23 inbred lines using the SNP data in order to assess the grouping pattern of lines (Figure 5.9). Lines were classified into five main groups. There was still a mixture of different heterotic groupings within each group. However, lines known to be in the same group appeared closer together within the biplot. Group I consisted of WCOBY, RS61P, SV1P (SC group) and CML544 (B group). Group II consisted of five lines namely 2Kba, CML312, CML548, CML442 and CML545.

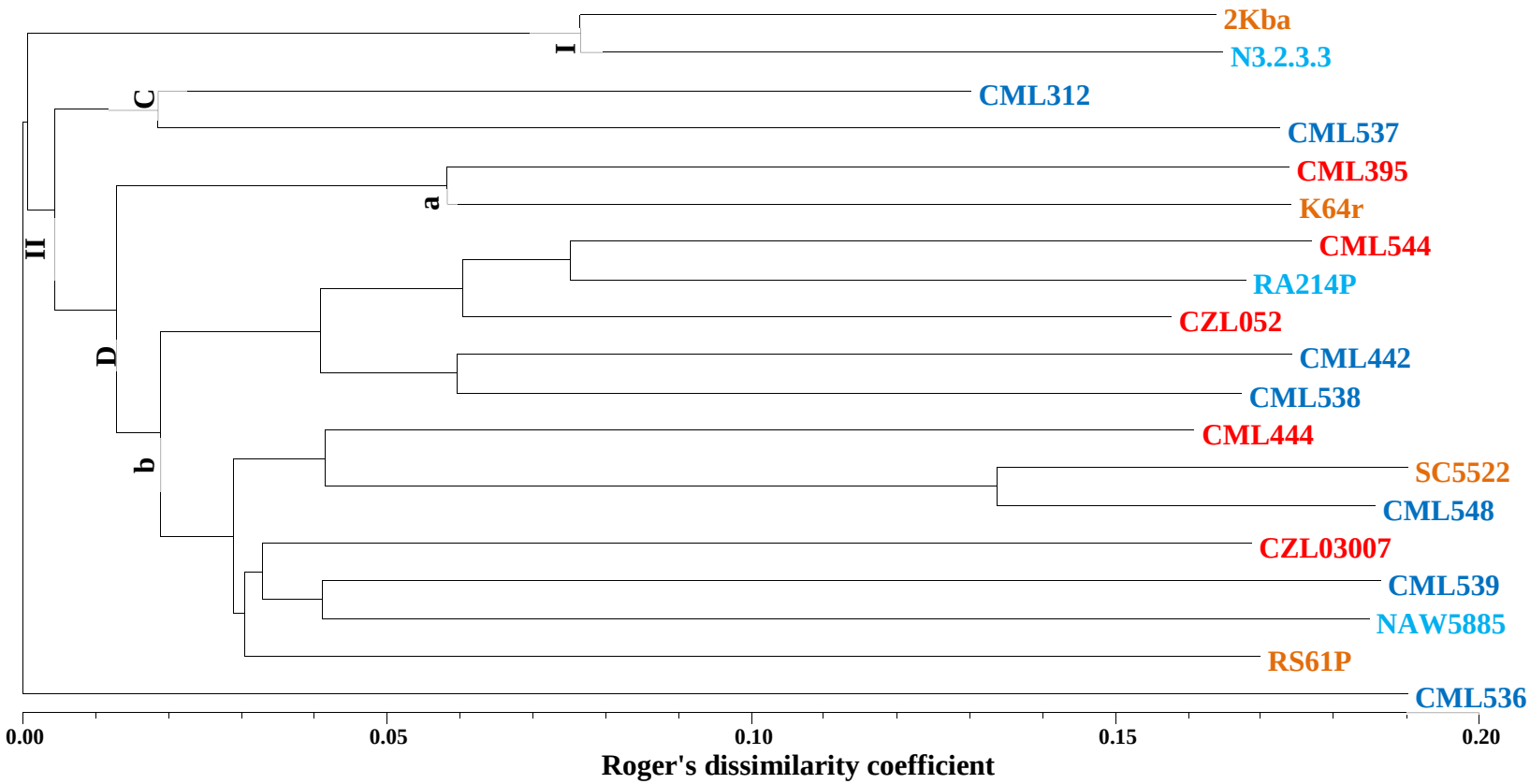

Figure 5.8 Neighbour-joining cluster analysis for the 19 inbred maize inbred lines based on Rogers' dissimilarity coefficient using single nucleotide polymorphism data. (Lines with a high percentage missing data excluded from the analysis.)

Dark blue=A group; Light blue=B group; Red=B group; Orange=SC.

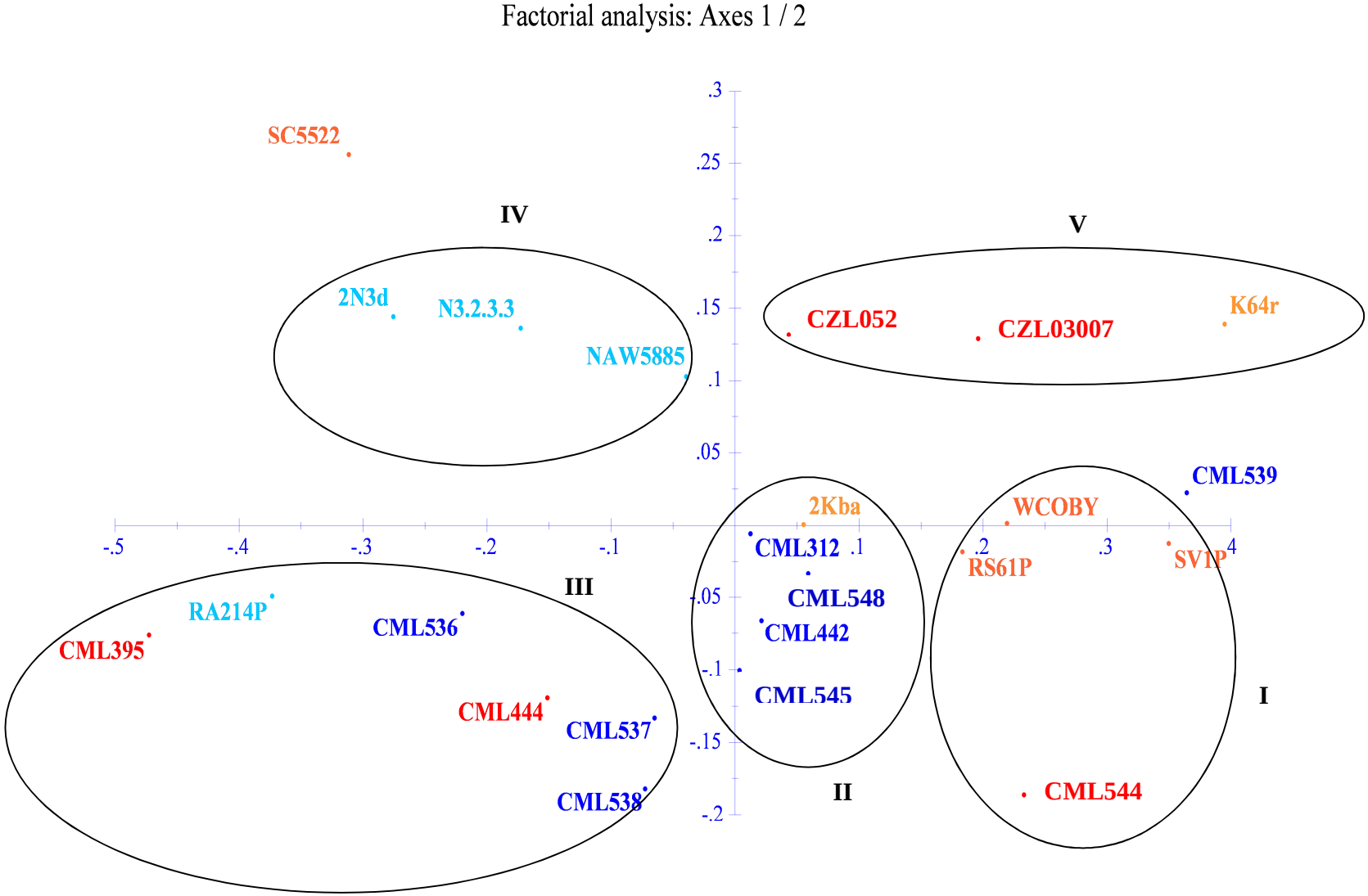

Figure 5.9 Principal component analysis for the 23 maize inbred lines based on single nucleotide polymorphism data.
Dark blue=A group; Light blue=N3 group; Red=B group; Orange=SC group.

This group was mainly dominated by lines from group A (CML312, CML548, CML442 and CML545) and these lines are related by pedigree. The lines that constituted group III were CML537, CML538, CML536 (group A), CML444 (group B), RA214P (group N3) and CML395 (group B).

Lines CML537 and CML538 closely grouped together in the biplot and are also in the same heterotic grouping (A), whilst CML536, also in group A, appeared a little further from the other two lines. Group IV comprised of N3.2.3.3, 2N3d and NAW5885. Lines 2N3d and N3.2.3.3 appeared closer to one another on the biplot and these lines are closely related as 2N3d is a direct derivative from N3.2.3.3. These three lines belong to the N3 heterotic group. Group V comprised of CZL052, CZL03007 (both from group B) and K64r (group SC).

5.3.6 Comparison of dendrograms based on morphological and single nucleotide polymorphism data

The two dendrograms showed different groupings of lines and no clear grouping according to known heterotic groups was observed for both dendrograms. However, the morphological dendrogram showed more consistency based on known heterotic groups compared to the SNP dendrogram. This may be mainly because lines were initially grouped using testers and the grouping was mainly based on field data. The grouping in the morphological dendrogram corresponded with the morphological data of the lines and in some cases lines related by pedigree grouped together. In the morphological dendrogram lines CML312, CML442, CML545, CML537 and CML538 clustered in the same group and these lines belong to group A, whereas in the SNP dendrogram these lines were split into different groups although some of them still appeared together within those groups. The grouping in the SNP dendrogram showed that it was mostly consistent with the pedigree relationships amongst the lines as revealed by close clustering of CML312 and CML537. CML537 has CML312 as one of its parents hence its close relationship with the line. Lines CML442 and CML538 clustered together in the SNP dendrogram and these two lines were derived from similar populations. The grouping in the SNP dendrogram did not seem to have any relationship with the morphological traits of the lines.

5.4 Discussion

Existence of genetic variability in any given germplasm is important as it facilitates the process of selection and superior germplasm can be easily identified. The significant morphological differences between lines for the tested traits were an indication that there existed variability amongst the 23 tested lines. However the most ideal sample for the best estimate of genetic variability is at least more than 30 lines. Morphological trait variations amongst maize inbred lines were also reported by Bar-Hen *et al*. (1995) and Gissa (2008). Prasanna *et al*. (2001) reported that genetic variability for most traits in maize is high and amenable to enhancements. However, Karanja *et al*. (2009) reported similarities in most of the morphological traits in maize inbred lines and they concluded that this could have been due to lines being able to balance environmental response differences. Again Karanja *et al*. (2009) only used 10 lines which were perhaps from a similar background The variation detected in the current study can therefore be exploited by breeders through selection, hybridisation and recombination of desirable genotypes.

Environments were significantly different in 2009/10 and 2010/11 as shown in the combined analysis and this was due to differences in the growing conditions under which lines were evaluated. Lines were evaluated under optimum, drought and low N conditions. Significant environment x line interaction for most traits was undesirable as it indicated different ranks of lines in performance over the five locations. These results are contrary to the findings by Gissa (2008) where non-significant environment x line interaction was reported for most traits. Gissa (2008) evaluated maize inbred lines over two locations, where one site was in Harare, Zimbabwe and the other site in Bako, Ethiopia. The significant environment x line interaction detected in the current study makes selection decisions difficult. The DR&SS lines were lower yielding than CIMMYT lines for all environments, indicating that more breeding efforts should be devoted at improving the yield performance of these lines, especially under stress environments. Nevertheless the most critical issue is the performance of the lines in hybrid combinations.

Knowledge of the relationship amongst different morphological traits in maize is critical in designing effective selection programmes for crop improvement (Gissa, 2008). Positive and

significant correlation of grain yield with ears per plant shows that the higher the number of ears per plant the higher the grain yield. On the other hand, negative and significant correlation of grain yield with root lodging is an indication that an increase in root lodging results in reduced grain yield. Positive and non-significant correlation of grain yield with plant height might be an indication that taller plants tended to have higher yield compared to shorter ones and this might have been so in a few cases. Bolanos and Edmeades (1996) reported that correlations between grain yield and anthesis silking interval were weak under optimal conditions and strong under stress conditions. However in this study grain yield was negatively but non-significantly correlated with anthesis-silking interval across all environments. The results show that under non-stress environments anthesis-silking interval does not have much influence on grain yield compared to stress environments.

According to Johnson *et al*. (1955) heritability by itself does not give an insight as to the amount of genetic progress that would be expected through selection of superior genotypes, but should always be considered simultaneously with GA. In this study five traits namely grain yield, plant and ear height, ear position and ears per plant had broad sense heritability estimates >0.50. Results therefore suggest that further selection for these traits would be effective for the set of lines used in this study. Singh (2005) reported that selection for a character is fairly easy if its heritability is high, but selection may be difficult or impractical for a character with low heritability. Lines exhibited both high GCVs and heritability estimates for grain yield, plant height and ear height. Genetic coefficients in combination with heritability estimates are expected to give a better picture of the GA that a breeder can expect from selection (Assefa *et al*., 1999). Therefore the traits that showed a high GCV, heritability and GA as percentage of the mean in the current study, namely grain yield, plant and ear height and ears per plant would be useful as a base for selection. High genetic gain indicates that there is a better scope for selection of traits for genetic improvement of the crop.

PCA further substantiated the existence of broad morphological variation among genotypes. Results indicated that the overall diversity could not be explained by a few eigenvectors and these findings are in agreement with findings reported by Gissa (2008). In that study the first

four PCs explained 65.7% of the variation contrary to the findings by Yoseph *et al*. (2005) who reported that 71.8% of the total variation in 62 traditional Ethiopian highland maize accessions was explained by the first four PCs. In the current study variations in all traits were dissected into 14 PCs which accounted for 100% of the variability present among lines. However, traits such as grain yield, texture, ear aspect, common rust, GLS and anthesis days were the major contributors.

A total of 14 morphological traits were used in the study and these traits successfully revealed the existence of considerable variability among the inbred lines. These findings are in agreement with findings by other researchers. Karanja *et al*. (2009) used 28 morphological traits to group 10 maize inbred lines, whilst Lucchini *et al*. (2003) used 32 morpho-phenological and agronomic traits to group 20 Italian flint maize landraces. There were cases in the current study where lines closely related by pedigree tightly clustered together. Therefore the dendrogram showed the resolution power of morphological traits for grouping maize inbred lines. However the dendrogram had a poor goodness of fit (r=0.70) and this could be explained by the small number of morphological traits that were used in this study. Groupings were also sometimes in agreement with known heterotic groupings. Gerdes and Tracy (1994) successfully grouped closely related inbred lines using morphological data in agreement with pedigree data. However, reports consistently indicate that morphological markers have shortcomings in that they are subject to prevailing environmental conditions (Bernardo, 1992; Yoseph *et al.*, 2005). Lines related by pedigree also grouped together in some cases. CML548 and CML538 clustered together with CML312 and the two lines have CML312 as one of their parents. CML442 and CM537 clustered together and the two lines share a common parent. Gissa (2008) also reported maize inbred lines closely related by pedigree clustering together using morphological traits. The grouping was also in some cases in agreement with existing heterotic grouping, for example RS61P and WCOBY both belong to SC group and N3.2.3.3 and NAW5885 to the N3 group and these lines clustered together in the dendrogram. Five CIMMYT lines, CML312, CML442, CML545, CML537 and CML538 belonging to group A, also clustered in the same group.

Clustering of lines using morphological traits in this study also followed a certain pattern. Lines clustered according to grain yield, anthesis days, anthesis silking interval, plant height, ear rot, GLS and ears per plant. Other authors have also reported lines clustering according to morphological traits for example Yoseph *et al*. (2005) reported lines clustering together according to grain yield and plant and ear height, whilst Gissa (2008) reported lines clustering according to plant and ear height, ear diameter, number of rows per ear and leaf area.

The 23 inbred lines were furthermore characterised using SNP markers. Markers with ≤20% missing values were used in this study and this constituted 91% of the tested SNP markers. Lu *et al*. (2009) eliminated SNPs with more than 20% missing data from the analysis, which therefore suggests that SNPs with up to 20% missing data can be safely used to produce good results. In the 23 inbred lines 2 258 alleles were detected at 1 129 loci with two alleles as expected due to the bi-allelic nature of SNP markers. In this study an average PIC value of 0.258 was reported and Lu *et al.* (2009) reported similar results. Kota *et al*. (2008) reported that generally the SNPs are biallelic and possess the maximum PIC value of 0.50, findings from this study are in agreement with these findings. SNP markers generally show low PIC, hence the need to increase marker density (Lu *et al*., 2011). Gene diversity shows the likelihood that two randomly selected alleles from the test sample are different. Mean gene diversity reported in the current study was 0.31 and similar results on SNP markers in maize were reported by Lu *et al*. (2009) and Kassa *et al*. (2012). Lu *et al*. (2009) reported mean gene diversity of 0.32, whilst Kassa *et al*. (2012) reported mean gene diversity of 0.25. Literature has revealed that SNP markers generally show low gene diversity (Liu *et al*., 2009; Kassa *et al*., 2012). Nonetheless, SNP markers are gaining popularity over SSR markers in genetic diversity studies especially in maize because of their abundance and they have been automated leading to significantly reduced costs.

Approximately 9.5% of markers had a minor allele frequency of less than 0.05 in the current study, whilst Lu *et al*. (2009) reported 8.8% of SNP markers with less than 0.05 minor allele frequency and Yan *et al*. (2009) reported 16.3% of markers with below 0.010 minor allele frequency. Kassa *et al*. (2012) reported 37.7% of SNP markers with minor allele frequencies

between 0.051 and 0.200 and in the current study 53.3% of the markers had a minor allele frequency of more than 0.200 with 10.9% having minor allele frequency close to 0.500. On the other hand Lu *et al.* (2009) reported 18.7% of the SNP markers with minor allele frequency close to 0.500. Markers exhibiting higher minor allele frequency values are considered important for screening maize germplasm from diverse sources. Results from the current study therefore suggest that SNP markers used were appropriate since more than half of the markers had high minor allele frequency and the sources of the germplasm used were diverse. The tested lines generally exhibited more than 90% homozygosity except for the four lines 2N3d, WCOBY, CML537 and ZM523B. Results suggest that these lines have not reached the required level of homozygosity therefore there exists a need to continue selfing these lines until they reach the desired homozygosity before they can be used in the breeding programme. The other reason for the high levels of heterozygosity could be the purity of the seed that was used. Low genetic purity of some of the lines might have resulted in reduced effectiveness of SNPs in discriminating lines according to genetic distances as a result clustering was generally not in agreement with known heterotic groupings.

The dendrogram obtained from the neighbour-joining clustering algorithm based on SNP markers showed that all inbreds could be distinguished from each other. Two dendrograms were presented, one with all 23 lines and another with 19 lines after removal of the four lines that had high percentages of missing data. The dendrograms showed a good fit (r=0.87 and r=0.80 respectively). It was noted that removal of the four lines did not improve clustering of the lines, since a similar pattern was observed. The average GD amongst the inbred lines based on Rogers' dissimilarity coefficient was 0.32, indicating low levels of polymorphism in the inbreds. The GDs in the current study ranged from 0.108 to 0.384 and results are similar to findings reported by other researchers (Lu *et al*., 2009; Kassa *et al*., 2012). Lu *et al.* (2009) working on CIMMYT, Chinese and Brazilian maize germplasm reported genetic distances ranging from 0.140 to 0.349 using SNP markers, whist Kassa *et al.* (2012) working on CIMMYT germplasm from eastern and southern Africa reported genetic distances ranging from 0.003 to 0.450, again using SNP markers. This can be explained by the fact that the National Breeding Programme has over the years used CIMMYT germplasm in the development of its new maize inbred lines. On the other hand the National Breeding

Programme's heterotic grouping is based on N3 and SC and the two lines N3.2.3.3 and SC5522 are part of CIMMYT's heterotic groups A and B, respectively (Mickelson *et al*., 2001). Low genetic distances could also be explained by the fact that CIMMYT has been mixing germplasm during the time they were emphasising on population breeding at the expense of hybrid breeding.

The SNP data was also subjected to PCA in order to ascertain if a better grouping of lines be obtained. Both neighbour-joining cluster analysis and PCA managed to cluster lines related by pedigree in some cases. In the current study the pattern of grouping from PCA was more reliable than neighbour-joining clustering and similar results were reported by Kassa *et al*. (2012). Although PCA grouped lines better than neighbour-joining cluster analysis, clear differentiation according to known heterotic groups was not evident. The CIMMYT lines used in the study could not be divided into known heterotic groups based on SNP data. All previous SNP and SSR data available for CIMMYT germplasm also did not show clear separation/grouping based on heterotic groups (Kassa, personal communication, 2011). Kassa *et al*. (2012) reported that SNP data did not show clear genetic differentiation of inbred lines in group A and B as defined by maize breeders at CIMMYT. This is not entirely unexpected because CIMMYT inbred lines are generally drawn from a pool, population, or mixture of pools and populations (Warburton *et al*., 2001). Likewise the DR&SS lines did not cluster into known heterotic groups. The 1 250 SNP markers used in the study were the SNPs available at K-Biosciences and they were not necessarily the required number for studying genetic distances and this could also explain the failure of the markers to clearly cluster the lines. In other studies more SNP markers have been used for example Lu *et al*. (2009) used 1 536 SNPs, Lu *et al*. (2011) used 1 936 SNPs whilst Kassa *et al*. (2012) used 1 536 SNPs.

The information generated in this study can be used for selecting single cross hybrids to be used as testers in the National Breeding Programme. According to Warburton *et al*. (2005) clusters with more than five lines may be considered to form a good potential heterotic group. Lines used in the current study namely RS61P, NAW5885, CML444, ZM523A, CML442, CML537 and CML539 are potential parents for the drought breeding programme

at DR&SS. Therefore the information that has been generated will assist breeders in selecting parents for producing hybrids with good hybrid vigour. This will be done by crossing lines from divergent groups. On the other hand good single cross testers will be identified from lines within the same group and these will then be used as female parents and they will be crossed with male parents from another group. This will help in speeding up the process of producing and releasing drought tolerant hybrids for commercial production. Lines in similar groups can also be used to develop F_2 populations that can be used in recombinant inbred line production.

As with all marker classifications, the classification obtained in this study is subject to change with addition of new lines and use of different markers. Random sampling of lines should be done so that there is no biasness towards lines with similar pedigrees or ancestry as this is bound to affect the effectiveness of clustering. Again genetic contamination of the lines used should be minimised as seen in the current study that low genetic purity might have contributed towards reduced effectiveness of SNP markers in clustering lines. SSR markers have been compared with SNP markers in genetic diversity studies in maize and Jones *et al*. (2007) found that SNPs had a clear advantage over SSRs in repeatability of genotyping results and proportion of missing data. However Hamblin *et al*. (2007) suggested that more SNP markers were required in genetic diversity studies compared to SSR markers. Therefore the clustering observed in the study cannot be considered to be static. However, the data generated should assist breeders in decision making when it comes to which hybrids to constitute and evaluate, also considering currently available information and experience. Information from DNA-based markers can help in improving the efficiency of field trials conducted for the purpose of identifying promising heterotic patterns thereby resulting in efficient maize breeding programmes.

5.5 Conclusions

Characterisation of maize inbred lines with the aim of exploring genetic diversity is of great importance and helps breeders in selecting superior germplasm to be used as parents in a breeding programme. However, DR&SS lines performed poorer than the CIMMYT lines, especially under stress environments. Hence the National Breeding Programme's breeding

efforts should be devoted towards increasing grain yield and other traits such as anthesis silking interval, ears per plant, tolerance to foliar diseases, ear rot and standability traits (plant height, root and stem lodging). Lines displayed a substantial amount of variability for the studied morphological traits. The existing genetic variability can therefore be exploited in developing single cross hybrids to be used as testers, three-way cross hybrids and synthetics. The selected lines can also be recombined to develop recycled inbred lines. Based on the morphological traits, some inbred lines related by pedigree grouped together, indicating that the agronomic traits can be used for primary characterisation of the maize inbred lines. In the 23 inbred lines 2 258 alleles were detected at 1 129 loci each with two alleles per locus as expected. The SNP based genetic distances were low with an average of 0.32 suggesting minimal genetic variation among the CIMMYT and DR&SS inbred lines and the low GDs might have been aggravated by genetic contamination. The UPGMA clustering algorithm grouped the inbred lines into two major groups, which generally did not agree with their pedigree records but within subgroups some lines grouped according to pedigree and in some cases lines from the same heterotic group clustered together. Results of this study revealed that SNP markers did not consistently cluster lines according to known heterotic groups. However, there were cases where lines related by pedigree or in the same heterotic group clustered together. Both neighbour-joining cluster analysis and PCA managed to cluster together lines related by pedigree or lines within the same heterotic group, however PCA proved to be more efficient than neighbour-joining. Information generated using both morphological data and SNP markers can be used to better understand the genetic relationships amongst CIMMYT and DR&SS lines which should lead to more effective utilisation of the inbred lines in the breeding programme.

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CHAPTER 6

Relationships between heterosis, genetic distances and combining ability data in maize hybrids

Abstract

Hybrid vigour (heterosis) is critical for grain yield and is one of the crucial factors determining growth of the hybrid seed sector in maize. Success of a maize hybrid breeding programme that handles many crosses and field evaluations can be improved greatly with the use of superior hybrid value predictors. The current study was done to estimate heterosis effects among CIMMYT and DR&SS elite maize inbred lines and to determine correlations between GD, heterosis and SCA under optimum, drought and low N conditions. Seventy-two hybrids generated using a NCDII from 10 DR&SS and 13 CIMMYT elite inbred lines were evaluated across seven environments in the 2009/10 and 2010/11 seasons under optimum, low N and drought conditions. The 23 lines used in the crosses were genotyped using SNP markers. An average of 112.29% MPH and 76.40% HPH were realised across environments and this was an indication of the potential of the inbred lines for hybrid development. The negative heterosis (-5.1% MPH and -7.4% HPH) detected for days to anthesis indicated earlier anthesis of hybrids compared to their parental inbred lines. Correlations of GDs determined using either morphological or SNP data with heterosis under drought conditions were too low to be of predictive value. Significant positive correlations and regressions were recorded for SCA with MPH, HPH and *per se* performance of hybrids. The HPH and MPH also showed significant positive associations and linear regressions along with high coefficients of determination with *per se* performance of hybrids especially under drought conditions. Results indicated that CIMMYT lines CML444, CZL03007, CML536, CML539 and CML548 can contribute to the DR&SS maize stress breeding programme. Therefore the genetic and morphological distances were of little importance in predicting performance, heterosis and SCA effects of hybrids.

6.1 Introduction

Knowledge of heterotic groups and heterotic patterns is helpful in plant breeding as it helps breeders to utilise their germplasm in a more efficient and consistent manner through utilisation of complementary lines for maximising the outcomes of a hybrid breeding programme. The choice of parents is extremely important in the production of hybrids and hybridisation itself does not result in hybrid vigour, hence heterosis depends on the genetic background of parents. Heterosis is defined as the improved performance of hybrids and shows the advantage of hybrid performance compared with the parental lines from which they were constituted (Hallauer *et al*., 2010). Precise information on the relationship between maize inbred lines is critical for their identification and manipulation of heterotic patterns in germplasm pools (Livini *et al*., 1992). The idea of heterotic grouping was initially established by maize researchers who observed that inbred lines from different populations had a tendency to produce greater performing hybrids when crossed with inbred lines from different groups (Hallauer *et al*., 2010).

Hallauer *et al*. (2010) defined a heterotic group as a group of interrelated or unrelated inbred lines from a similar or diverse population, which shows a comparable combining ability when hybridised with inbred lines from another population. The Zimbabwe national maize breeding programme uses two heterotic groups namely SC and N3, whilst CIMMYT uses heterotic groups A and B. The SC grouping is equivalent to the B grouping, whilst the N3 grouping is equivalent to the A grouping. Genotypes within a group will usually show no or little heterosis when crossed to each other because they are generally genetically closely related but there are exceptions to this rule. High heterosis from germplasm derived from within a heterotic group has been observed in some experiments (Vasal *et al*., 1999). Heterotic groups thus generally represent broad sources of germplasm, which exhibit optimum heterosis when crosses are made between groups. Heterosis estimation is thus important in maize breeding in order to identify the best combinations of progenitors to form potential hybrids and to study gene action.

Breeding approaches in maize have been established to benefit from expression of hybrid vigour in inbred line crosses. Hybrid production exploits the phenomenon of heterosis. The

search for high yielding varieties in Zimbabwe maize breeding programmes saw a major shift from OPV to hybrid development in the early 1930s. Zimbabwe maize breeding has been a success story over the years with hybrids being developed for both small scale and commercial production. According to Mashingaidze (2006) commercial adoption of hybrids in terms of area planted increased from 22% (1949/50) to 88% (1960/61) and 93% (1966/67). Adoption was, however, slower in communal lands before independence. The Mangwende communal area for instance had a hybrid adoption of 42% in 1975 but by 1985 it stood at 99% (Mashingaidze, 2006). Currently 90% of the total production area (in both the commercial and communal sector) is planted to hybrids with the remainder being planted to other products such as OPVs, synthetics and recycled seed. The National Breeding Programme uses introductions from CIMMYT and other breeding programmes to take advantage of genetic diversity. The level of genetic diversity between genotypes is usually unknown and the only alternative is to investigate it through development of crosses (Hallauer *et al*., 2010). If a cross between two parents exhibits high heterosis it is usually determined that the two genotypes are genetically more diverse than two genotypes that show slight or no heterosis in their crosses.

Since heterosis in F_1 progeny is determined by genetic diversity of the inbred parents, the usual method of selecting parents only on the basis of their performance and adaptation under different environments does not lead to significant selection results (Allard, 1960). Therefore in selecting ideal parental lines for a hybridisation programme, information on GD, combining ability and heterotic patterns is vital (Beck *et al*., 1990). Hybrid performance has been predicted using GD and the effectiveness of the prediction was better with crosses amongst inbred lines from the same heterotic group than in crosses amongst inbred lines from diverse heterotic groups (Melchinger, 1999). Several investigators have used GD to predict hybrid performance (Cheres *et al*., 2000; Betran *et al*., 2003; Dhliwayo *et al*., 2009; Devi and Singh, 2011; George *et al*., 2011). Some investigators have reported significant association between marker based GD and heterosis (Lee *et al*., 1989; Smith *et al*., 1990; Melchinger *et al*., 1992; Betran *et al*., 2003; Amorim *et al*., 2006; Srdic *et al*., 2007; George *et al*., 2011), whilst others have reported non-significant or no association (Balestre *et al*., 2008; Legesse *et al*., 2008; Dhliwayo *et al*., 2009; Devi and Singh, 2011). Therefore the

application of molecular markers in maize is not conclusive. The study was done to i) assess the validity of known heterotic groups, ii) estimate heterosis effects among CIMMYT and DR&SS maize inbred lines and iii) estimate correlations between GD, heterosis and SCA under optimum, drought and low N environments.

6.2 Materials and methods

6.2.1 Germplasm

Seventy-two single cross hybrids and 23 parental inbred lines were evaluated for agronomic performance in a total of 14 sites in 2009/10 and 2010/11 seasons. Details of parental lines are given in section 3.2.1.

6.2.2 Sites

The site details are given in sections 3.2.2 and 4.2.2. The details of the measured and derived traits are as described in Table 3.3.

6.2.3 DNA extraction and SNP genotyping

Details of DNA extraction and SNP genotyping are given in sections 5.2.4 and 5.2.5.

6.2.4 Statistical analysis

ANOVA was performed for individual and across sites using the PROC MIXED procedure from SAS (SAS Institute, 2002). Varieties were taken as fixed effects. The means were used for SCA estimation. Line x tester analysis was used to estimate SCA effects for hybrids in all environments and across environments.

Mid-parent heterosis (MPH) was calculated as:

$MPH = [(F_1 – MP)/MP] \times 100$

Where: F_1 = Mean of the F_1 hybrid performance

$MP = (P_1 + P_2)/2$ in which P_1 and P_2 are the means of the inbred parents respectively

High-parent heterosis (HPH) was calculated as:

HPH = $[(F_1\text{-HP})/HP] \times 100$

Where: HP = Mean of the better parent

GCA and SCA effects were estimated according to Singh and Chaudhary (1977) using the following formulae:

GCA effects for lines:

$$g_i = x_i\ldots/tr - x\ldots./1tr$$

GCA effects for testers:

$$g_t = x_{.j.}/lr - x\ldots./ltr$$

SCA effects:

$$s_{ij} = x_{ij.}/r - x_i.../tr - x_{.j.}/lr - x\ldots/lrt$$

Where: l = number of lines

t = number of testers

r = number of replications

S.E. $(g_i - g_j)$ line $= (2M_e/r \times t)^{½}$

S.E. $(g_i - g_j)$ tester $= (2M_r/l \times r)^{½}$

S.E. $(s_{ij} - s_{kl})$ $= (2M_e/r)^{½}$

GDs for SNP data were calculated from allele frequencies using Powermaker version 3.25 (Liu and Muse, 2005) as described in section 5.2.6. Means per environment and across environments were used to calculate Pearson correlation coefficients (r) between GD, F_1 grain yield, mid-parent (MP), high-parent (HP), MPH, HPH and SCA using SPSS version 15.0 for Windows (2006). Morphological traits were used for computing Euclidean distances in SPSS version 15.0 for Windows (2006) as described in section 5.2.6.

6.3 Results

The means presented in this chapter are for the 10 best and 10 poorest hybrids in terms of SCA effects and data for all crosses is presented in the appendices section (Appendices 11 and 12) for better presentation. Also to note is that the mean values, minimum and maximum values presented in the different tables are for the entire data set and not only for the crosses presented.

6.3.1 Grain yield, specific combining ability and mid- and high-parent heterosis across all environments

The average MPH and HPH across all environments were 112.29% and 76.40% respectively. Hybrid L5 (2N3d) x T1 (CML395) had the best SCA effects of 0.86 but it was not necessarily the best in terms of mean grain yield, MPH and HPH (Table 6.1). L3 (RS61P) x T9 (CML444) had the highest mean yield of 4.78 t ha^{-1} but did not have the highest heterosis values. The GD between these two lines was 0.27. L4 (NAW5885) produced crosses with high mean yields as well as high MPH. SC5522 (L7) produced two crosses with high positive SCA effects of 0.77 and 0.75 respectively but these crosses had mean yields of below 3.0 t ha^{-1} and MPH of 140.92% and 140.17% respectively. This line also produced a cross with CZL03007 which had the highest MPH (237.58%) (data not shown) and the GDs between the respective parental lines were above average. L6 (2Kba) x T11 (CML548), L1 (K64r) x T3 (CML539) and L4 (NAW5885) x T6 (CZL03007) had MPH and HPH values above 100%. L6 (2Kba) x L9 (CML444) had the lowest MPH of 22.20% and the lowest negative HPH of -11.19%. MPH and HPH for the 10 hybrids with best SCA effects across all environments are presented graphically in Figure 6.1. There was no significant difference between MPH and HPH values for L3 (RS61P) x T9 (CML444). L7 (SC5522) x T1 (CML395) and L7 (SC5522) x T10 (CML536) had high MPH values compared to the HPH values, whilst L6 (2Kba) x T11 (CML548), L1 (K64r) x T3 (CML539) and L4 (NAW5885) x T6 (CZL03007) had high MPH and HPH values. L5 (2N3d) x T1 (CML395) despite having the highest SCA value (Table 6.1) had the second lowest MPH amongst the 10 hybrids (Figure 6.1).

Table 6.1 Hybrid mean grain yield, specific combining ability, mid- and high-parent heterosis and genetic distance across all environments

Hybrid	GYD(t ha^{-1})	SCA	MPH (%)	HPH (%)	GD
L5/T1	2.43	0.86	88.41	74.60	0.34
L3/T9	4.78	0.79	93.18	91.31	0.27
L7/T1	2.29	0.77	140.92	27.03	0.34
L7/T10	2.34	0.75	140.17	53.92	0.36
L6/T11	4.13	0.68	166.80	136.08	0.35
L4/T4	4.22	0.51	159.25	32.09	0.32
L1/T3	3.93	0.50	175.72	132.76	0.33
L1/T7	4.06	0.50	88.07	56.05	0.36
L4/T6	4.06	0.47	195.02	147.91	0.29
L1/T5	3.86	0.36	124.83	78.79	0.31
L2/T11	3.45	-0.44	80.19	37.15	0.36
L5/T5	2.28	-0.45	88.41	74.60	0.20
L5/T7	1.99	-0.50	73.36	53.20	0.33
L4/T11	3.35	-0.50	77.91	28.69	0.32
L4/T3	3.16	-0.52	111.97	75.22	0.29
L8/T1	2.11	-0.54	87.20	62.14	0.33
L3/T4	3.64	-0.65	69.65	60.94	0.30
L8/T3	2.97	-0.76	127.62	74.99	0.32
L1/T6	2.52	-0.81	89.94	60.51	0.33
L6/T9	2.10	-0.92	22.20	-11.19	0.33
Mean	**4.00**	**0.008**	**112.29**	**76.40**	**0.31**
Min	**2.29**	**-0.92**	**22.20**	**-11.19**	**0.20**
Max	**5.31**	**0.86**	**237.58**	**181.71**	**0.37**

GYD=Grain yield; SCA=specific combining ability; MPH=mid parent heterosis; HPH=high parent heterosis; GD = genetic distance; L1= K64r; L2=N3.2.3.3; L3=RS61P; L4=NAW5885; L5=2N3d; L6=2Kba; L7=SC5522; L8=RA214P; T1=CML395; T3=CML539; T4= CML442; T5=CML537; T6=CZL03007; T7=CML545; T9=CML444; T10=CML536; T11=CML548 ; Min=minimum; Max=maximum.

The F_1 means were higher than the parental means for plant height, ear height and ears per plant across all environments (Table 6.2). Anthesis days had negative MPH and HPH values as shown by the minimum and the maximum values. The F_1 hybrids showed considerable heterosis for both plant and ear height. The minimum and maximum hybrid values for plant height and ear height were higher than the parental values. Ears per plant had mean heterosis values of less than 10% for both HPH and MPH respectively. The minimum MPH and HPH for ears per plant were both negative but the maximum values were 86.72 and 71.45 respectively.

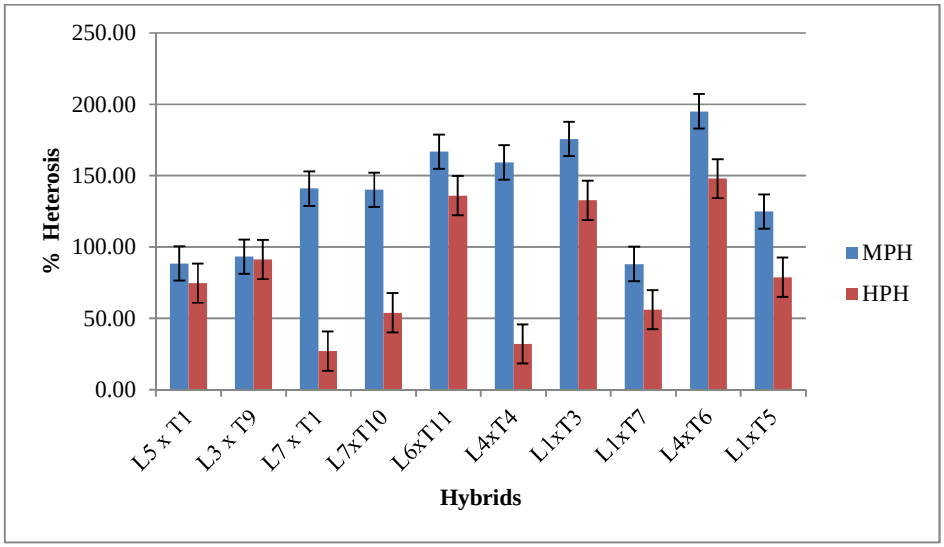

Figure 6.1 The high- and mid-parent heterosis for 10 selected hybrids across all environments.

L1=K64r; L3=RS61P; L4=NAW5885; L5=2N3d; L6=2Kba; L7=SC5522; T1=CML395; T3=CML539; T4=CML442; T5=CML537; T6=CZL03007; T7=CML548; T9=CML444; T10=CML536; MPH=mid-parent heterosis; HPH=high-parent heterosis.

Table 6.2 F_1, parental, mid- and high-parent heterosis means for anthesis days and other agronomic traits across all environments

	AD	PH	EH	EPP
F1 mean	67.5	259.9	125.4	0.53
Min	63.8	209.7	96.2	0.46
Max	72.7	291.1	156.3	0.94
Parental mean	71.2	163.3	82.1	0.48
Min	68.2	129.9	65.3	0.43
Max	75.9	195.6	97.3	0.55
MPH mean	-5.1	59.9	53.3	9.80
Min	-9.7	28.9	15.6	-4.12
Max	-1.2	80.0	58.5	86.72
HPH mean	-7.4	49.9	42.7	4.80
Min	-14.5	10.3	4.1	-12.84
Max	-2.7	76.1	71.4	71.45

MPH=mid-parent heterosis (%); HPH=high-parent heterosis (%); AD=anthesis days; PH=plant height (cm); EH=ear height (cm); EPP=ears per plant (#); Min=minimum; Max=maximum.

Under optimum conditions the parental mean was higher than the F_1 mean for anthesis days indicating that hybrids flowered earlier than the parental lines (Table 6.3). Both MPH (-5.1) and HPH (-3.5) for anthesis days were negative. The F_1 mean for plant height was higher

than the parental mean and positive MPH and HPH were observed. Ear height means for both F_1 and parental lines were similar, but positive heterosis was observed for the trait. Negative MPH and HPH means were observed for ears per plant and the minimum and maximum F_1 values were lower than the parental minimum and maximum values. However the maximum values for both MPH and HPH were positive.

The F_1 means for anthesis days were lower than the parental means under both low N and drought conditions (Tables 6.4 and 6.5). Negative MPH and HPH means were observed under both conditions, however maximum values for MPH and HPH were positive under low N and under drought conditions only HPH was positive. Positive MPH and HPH for plant and ear height were realised under both low N and drought conditions. The F_1 mean for ears per plant was higher than the parental mean under low N conditions and positive MPH and HPH means were realised. The minimum values for both MPH and HPH for ears per plant under low N conditions were negative, however high maximum values of 349.3 and 311.2 respectively were observed. Under drought conditions F_1 mean for ears per plant was lower than the parental mean, but the maximum F_1 value was higher than the parental maximum value. A positive MPH mean (1.80) was realised and the HPH mean (-7.90) was negative. The minimum values for both MPH and HPH were negative but high maximum values were realised.

6.3.2 Mean grain yield, specific combining ability, mid- and high-parent heterosis under optimum conditions

The minimum grain yield was 2.91 t ha^{-1} and the maximum was 6.79 t ha^{-1}. L5 (2N3d) x T1 (CML395) and L3 (RS61P) x T9 (CML444) were the best specific combinations under optimum conditions (0.99) in terms of the SCA effects (Table 6.6). However, they were not the best in terms of grain yield as well as MPH and HPH. MPH ranged from 16.91% to 253.53% whilst HPH ranged from -9.21% to 162.7% (Appendix 11).

Table 6.3 F_1, parental, mid- and high-parent heterosis means for anthesis days and other agronomic traits under optimum conditions

	AD	PH	EH	EPP
F1 mean	67.7	161.5	80.4	0.94
Min	63.8	124.9	61.9	0.66
Max	73.1	200.9	98.9	1.18
Parental mean	70.3	160.9	80.9	1.00
Min	66.7	129.9	65.3	0.84
Max	75.9	195.6	80.9	1.48
MPH mean	-5.1	59.1	57.8	-6.00
Min	-11.0	18.9	9.6	-43.10
Max	1.2	82.4	99.1	20.41
HPH mean	-3.5	57.1	56.9	-13.70
Min	-8.1	23.8	9.5	-56.60
Max	1.8	109.3	102.4	10.70

MPH=mid-parent heterosis (%); HPH=high-parent heterosis (%); AD=anthesis days; PH=plant height (cm); EH=ear height (cm); EPP=ears per plant (#); Min=minimum; Max=maximum.

Table 6.4 F_1, parental, mid- and high-parent heterosis means for anthesis days and other agronomic traits under low nitrogen conditions

	AD	PH	EH	EPP
F1 mean	72.9	217.0	77.9	0.67
Min	69.2	177.7	58.2	0.36
Max	78.5	268.8	98.3	0.94
Parental mean	77.1	106.8	40.4	0.41
Min	70.8	93.0	32.2	0.15
Max	85.6	135.1	53.6	0.80
MPH mean	-6.6	99.3	90.0	79.60
Min	-16.6	39.5	19.7	-37.70
Max	5.6	141.1	183.8	349.30
HPH mean	-6.0	93.9	79.3	49.30
Min	-18.7	26.5	-1.5	-45.80
Max	9.7	139.8	188.8	311.20

MPH=mid-parent heterosis (%); HPH=high-parent heterosis (%); AD=anthesis days; PH=plant height (cm); EH=ear height (cm); EPP=ears per plant (#); Min=minimum; Max=maximum.

Table 6.5 F_1, parental, mid- and high-parent heterosis means for anthesis days and other agronomic traits under managed drought conditions

	AD	PH	EH	EPP
F1 mean	96.4	223.7	109.3	0.77
Min	88.4	166.9	66.4	0.10
Max	104.8	260.3	148.3	1.49
Parental mean	105.5	174.9	85.6	0.81
Min	97.9	138.9	46.9	0.41
Max	117.7	227.5	129.6	1.26
MPH mean	-9.9	26.9	27.5	1.80
Min	-18.6	-10.6	-21.8	-87.20
Max	-2.1	58.9	105.9	137.40
HPH mean	-9.7	35.2	44.9	-7.90
Min	-21.7	-8.7	-19.5	-88.40
Max	9.4	81.8	186.7	163.90

MPH=mid-parent heterosis (%); HPH=high-parent heterosis (%); AD=anthesis days; PH=plant height (cm); EH=ear height (cm); EPP=ears per plant (#); Min=minimum; Max=maximum.

L7 (SC5522) x T6 (CZL03007) had the highest MPH of 253.53% and L1 (K64r) x T2 (CML312) had the highest HPH of 162.77%. L3 (RS61P x T9 (CML444), L1 (K64r) x T3 (CML539), L8 (RA214P) x T8 (CML538), L4 (NAW5885) x T6 (CZL03007), L2 (N3.2.3.3) x T9 (CML444), L8 (RA214P) x T6 (CZL03007) and L2 (N3.2.3.3) x T6 CZL03007 had high values above 100% for both MPH and HPH.

6.3.3 Mean grain yield, specific combining ability, mid- and high-parent heterosis under low nitrogen conditions

The mean grain yield, SCA, MPH, HPH and GD for the hybrids under low N conditions are presented in Table 6.7. L5 (2N3d) x T1 (CML395) was again the best specific combination under low N conditions with a HPH value of 126.62%. A number of hybrids had negative MPH and HPH values, however there are some that had high heterosis values for example L1 (K64r) x T2 (CML312) had the highest MPH of 273.74% and L6 (2Kba) x T5 (CML537) had the highest HPH value of 155.50% (Appendix 12).

6.3.4 Mean grain yield, mid- and high-parent heterosis and specific combining ability under drought conditions

The MPH and HPH minimum values were negative but maximum values of 180.61% and 162.07% respectively were recorded for the hybrid L5 (2N3d) x T11 CML548 (Table 6.8).

The hybrid also had the highest grain yield of 3.26 t ha^{-1}. L5 (2N3d) x T9 (CML444) was the best specific combination but had low MPH and a negative HPH. L8 (RA214P) x T3 (CML539) was the poorest yielder with 0.59 t ha^{-1} accompanied by lowest MPH and HPH values of -65.23% and -74.83% respectively. L4 (NAW5885) x T6 (CZL03007) was the poorest specific combiner with an SCA value of -1.07.

Table 6.6 F_1 mean grain yield (t ha^{-1}), specific combining ability, mid- and high-parent heterosis and genetic distance under optimum conditions

Hybrid	F_1 GYD	SCA	MPH	HPH	GD
L5/T1	3.66	0.99	76.68	61.49	0.34
L3/T9	6.42	0.99	124.14	155.18	0.27
L1/T3	5.56	0.94	173.19	131.78	0.33
L7/T10	2.95	0.84	136.19	49.48	0.36
L7/T1	3.10	0.83	134.68	40.44	0.34
L8/T8	6.16	0.76	156.75	127.89	0.26
L6/T12	3.72	0.74	133.05	89.06	0.33
L6/T6	5.28	0.72	123.93	81.66	0.34
L4/T6	5.85	0.70	175.41	130.06	0.29
L2/T9	6.05	0.67	187.98	151.43	0.33
L8/T6	6.09	-0.54	153.83	144.65	0.29
L5/T11	3.79	-0.55	97.91	66.71	0.35
L2/T6	4.84	-0.55	143.84	116.91	0.32
L4/T8	4.81	-0.57	105.21	60.11	0.31
L4/T9	4.47	-0.68	125.11	84.85	0.29
L5/T7	3.24	-0.71	85.74	73.83	0.33
L6/T9	3.23	-0.86	49.51	19.32	0.33
L1/T6	3.45	-0.97	72.36	47.44	0.33
L8/T3	4.32	-1.05	94.20	85.41	0.32
L3/T4	4.29	-1.10	48.63	59.10	0.30
Mean	**5.19**	**0.03**	**117.39**	**85.29**	**0.31**
Min	**2.91**	**-1.10**	**16.91**	**-9.21**	**0.20**
Max	**6.79**	**0.99**	**253.53**	**162.77**	**0.37**

GYD=grain yield (t ha^{-1}); SCA=specific combining ability; MPH=mid-parent heterosis (%); HPH=high-parent heterosis; GD=genetic distance; Min=minimum; Max=maximum; L1=K64r; L2=N3.2.3.3; L3=RS61P; L4=NAW5885; L5=2N3d; L6=2Kba; L7=SC5522; L8= RA214P; T1=CML395; T3=CML539; T4=CML442; T6=CZL03007; T7=CML545; T8=CML538; T9=CML444; T10=CML536; T11=CML548.

6.3.5 Heterotic grouping in relation to field heterosis

Mean MPH and HPH for across all environments, known heterotic groupings and grouping according to SNP markers are presented in Table 6.9. The DR&SS N3 grouping is equivalent to CIMMYT A grouping, whilst SC is equivalent to CIMMYT B grouping. The grouping according to SNP markers was in some cases consistent with the known heterotic grouping,

e.g. L5 (2N3d) and T5 (CML537) are in a similar known heterotic grouping (N3 and A) and the SNP markers grouped them into the same group (Figure 5.7). The hybrid had moderate MPH (88.41) and HPH (74.60) and its mean yield across environments was 4.05 t ha^{-1}. L6 (2Kba) and T11 (CML548) are in different known heterotic groups (SC and A respectively) and the grouping was consistent even based on SNP markers. The cross between these two lines exhibited high MPH and HPH of 166.8% and 136.08% respectively (Table 6.9).

Table 6.7 F_1 mean grain yield (t ha^{-1}), specific combining ability, mid- and high-parent heterosis and genetic distance under low nitrogen conditions

Hybrid	F_1GYD	SCA	MPH	HPH	GD
L5/T1	4.53	0.74	66.63	126.62	0.36
L1/T4	1.60	0.73	142.85	55.71	0.27
L7/T1	2.97	0.67	95.24	48.38	0.34
L3/T3	5.35	0.66	90.32	92.37	0.30
L3/T9	4.54	0.49	52.87	46.45	0.27
L7/T10	2.69	0.47	132.01	110.26	0.36
L4/T6	3.10	0.45	170.81	126.34	0.29
L2/T5	3.76	0.43	46.20	106.45	0.35
L8/T8	3.16	0.43	-18.45	2.07	0.26
L6/T5	3.83	0.41	148.86	155.50	0.35
L1/T6	2.02	-0.39	15.00	-12.28	0.33
L5/T3	2.57	-0.41	-17.48	-7.68	0.34
L3/T5	3.68	-0.41	69.78	145.61	0.34
L3/T6	2.39	-0.45	13.51	68.26	0.27
L2/T1	2.68	-0.50	-4.93	34.04	0.35
L6/T9	0.94	-0.53	-59.76	-69.63	0.33
L3/T4	3.27	-0.55	21.25	28.15	0.30
L4/T8	1.89	-0.56	-6.15	-39.15	0.31
L4/T5	1.95	-0.57	61.15	30.07	0.34
L8/T3	1.76	-0.64	-52.81	-36.86	0.32
Mean	**2.90**	**0.01**	**33.09**	**25.73**	**0.31**
Min	**0.94**	**-0.64**	**-66.25**	**-71.92**	**0.20**
Max	**5.35**	**0.74**	**273.74**	**155.50**	**0.37**

GYD=grain yield (t ha^{-1}); SCA=specific combining ability; MPH=mid-parent heterosis (%); HPH=high-parent heterosis (%); GD=genetic distance; Min=minimum; Max=maximum; L1=K64r; L2=N3.2.3.3; L3=RS61P; L4=NAW5885; L5=2N3d; L6=2Kba; L7= SC5522; L8=RA214P; T1=CML395; T3=CML539; T4=CML442; T5=CML537; T6=CZL03007 T8=CML538; T9=CML444; T10=CML536.

L1 (K64r) and T3 (CML539) clustered into separate groups using SNP markers and their cross exhibited high heterosis even though they were in similar (N3 and A respectively) heterotic groups. L4 (NAW5885) x T6 (CZL03007) and L6 (2Kba) x T11 (CML548) grouped both into separate heterotic groups and SNP clusters had crosses with high heterosis.

Table 6.8 Hybrid F_1 grain yield (t ha^{-1}), mid- and high-parent heterosis, specific combining ability and genetic distance under drought conditions

Hybrid	GYD	MPH	HPH	SCA	GD
L5/T9	1.99	7.33	-19.19	1.16	0.29
L4/T5	2.28	66.61	22.08	0.79	0.34
L5/T11	3.26	180.61	162.07	0.79	0.35
L3/T3	2.51	13.43	7.54	0.78	0.30
L4/T4	3.01	64.31	60.83	0.66	0.32
L6/T7	2.15	7.02	6.58	0.64	0.36
L2/T7	2.41	28.52	20.33	0.62	0.33
L8/T5	2.03	111.66	94.05	0.58	0.34
L4/T8	2.04	6.15	3.43	0.53	0.31
L1/T7	2.79	35.48	31.75	0.50	0.36
L1/T12	2.28	18.71	7.48	-0.56	0.33
L4/T7	1.91	-1.10	-4.33	-0.59	0.32
L2/T8	1.71	-7.88	-13.10	-0.60	0.34
L6/T9	1.40	-37.63	-43.27	-0.69	0.33
L3/T4	2.65	36.75	27.00	-0.74	0.30
L8/T1	1.36	1.14	-17.45	-0.76	0.33
L8/T3	0.59	-65.23	-74.83	-0.77	0.32
L5/T4	1.95	28.30	8.75	-0.80	0.34
L2/T5	1.98	51.50	13.25	-0.87	0.35
L4/T6	1.92	6.62	2.65	-1.07	0.29
Mean	**2.09**	**26.08**	**10.34**	**0.00**	**0.31**
Min	**0.59**	**-65.23**	**-74.83**	**-1.07**	**0.20**
Max	**3.26**	**180.61**	**162.07**	**1.16**	**0.37**

GYD=grain yield (t ha^{-1}); MPH=mid-parent heterosis (%); HPH=high-parent heterosis (%); SCA=specific combining ability; GD=genetic distance; Min=minimum; Max=maximum; L1=K64r; L2=N3.2.3.3; L3=RS61P; L4=NAW5885; L5=2N3d; L6=2Kba; L8=RA214P;T1=CML395;T3=CML539;T4=CML442;T5=CML537;T6=CZL03007; T7=CML545; T8=CML538; T9 =CML444; T11=CML548; T12=CZL052.

Two crosses L1 (K64r) x T5 (CML537) and L4 (NAW5885) x T3 (CML539) exhibited high MPH and yet the parental lines clustered into similar groups using SNP markers and they had similar (N3 and A) heterotic groupings. A number of crosses between lines from similar known heterotic groups exhibited high MPH and these are L7 (SC5522) x T1 (CML395), L4 (NAW5885) x T4 (CML442), L1 (K64r) x T3 (CML539), L1 (K64r) x T5 (CML537), L4

(NAW5885) x T5 (CML537), L4 (NAW5885) x T3 (CML539) and L8 (RA214P) x T3 (CML539). Some crosses such as L7 (SC5522) x T10 (CML536), L6 (2Kba) x T11 (CML548) and L4 (NAW5885) x T6 CZL03007 constituting of lines from known different heterotic groups exhibited high MPH. L6 (2Kba) and T9 (CML444) are in similar known heterotic groups (SC and B) and although SNP markers grouped them into separate groups they exhibited low MPH and negative HPH.

Table 6.9 Mid- and high-parent heterosis for hybrids as well as known heterotic groupings and grouping according to single nucleotide polymorphism markers

Hybrid	Known heterotic group		Grouping according to SNP markers		Field heterosis	
	P_1	P_2	P_1	P_2	MPH	HPH
L5/T1	N3	B	II b	II b	88.41	74.60
L3/T9	SC	B	I	II a	93.18	91.31
L7/T1	SC	B	II a	II b	140.92	27.03
L7/T10	SC	A	II a	II a	140.17	53.92
L6/T11	SC	A	II b	II a	166.80	136.08
L4/T4	N3	A	IIC	II b	159.25	32.09
L1/T3	N3	A	II b	IIC	175.72	132.76
L1/T7	N3	A	II b	II a	88.07	56.05
L4/T6	N3	B	IIC	I	195.02	147.91
L1/T5	N3	A	II b	II b	124.83	78.71
L4/T5	N3	A	IIC	II b	112.72	65.96
L2/T11	N3	A	II b	II a	80.19	37.15
L5/T5	N3	A	II b	II b	88.41	74.60
L5/T7	N3	A	II b	II a	73.36	53.20
L4/T11	N3	A	IIC	II a	77.91	28.69
L4/T3	N3	A	IIC	IIC	111.97	75.22
L8/T1	N3	B	II b	II b	87.20	62.14
L3/T4	SC	A	I	II b	69.65	60.94
L8/T3	N3	A	II b	IIC	127.62	74.99
L1/T6	N3	B	II b	I	89.94	60.51
L6/T9	SC	B	II b	II a	22.20	-11.19

P_1=parent 1; P_2=parent 2; MPH=mid-parent heterosis; HPH=high-parent heterosis; L1=K64r; L2=N3.2.3.3.; L3=RS61P; L4=NAW5885; L5=2N3d; L6=2Kba; L7=SC5522; L8=RA214P; T1=CML395; T3=CML539; T4=CML442; T5=CML537; T6=CZL03007; T7=CML545; T9=CML444; T10=CML536; T11=CML548.

6.3.6 Correlation between genetic distance, specific combining ability, high- and mid-parent heterosis and F_1 grain yield

There was a non-significant correlation of GD with F_1 grain yield, SCA, MPH and HPH across all environments, under optimum and low N conditions (Table 6.10). Under drought

conditions GD was positively and significantly correlated with *per se* performance of hybrids (0.38) and HPH (0.37). Pearson's rank correlations of SCA effects with F_1 grain yield across all environments (0.31) and under drought (0.42) were significantly positive at $P \leq 0.01$ and under optimum conditions (0.24) was significantly positive at $P \leq 0.05$. SCA correlation was non-significant with F_1 grain yield under low N conditions. MPH was positive and significantly correlated with F_1 grain yield across all environments (0.36), optimum (0.46) and drought (0.72) conditions and the correlation was not significant again under low N conditions. Highly significant and positive correlations were realised between HPH and F_1 grain yield across all environments (0.57), under optimum (0.76), drought (0.77) and low N conditions (0.64). MPH and HPH were significantly correlated with SCA in all environments except under low N.

All the 72 single cross hybrids were used in construction of Figures 6.2 to 6.6. The linear regression of *per se* performance of F_1s on HPH and MPH was significant with R^2 values of 0.59 and 0.52 respectively (Figure 6.2). SCA also established significant positive associations as well as linear regressions with HPH and MPH (Figure 6.3) and with *per se* performance of hybrids (Figure 6.4). The linear regressions of GD on HPH, MPH and SCA were not significant with R^2 values of 0.02 for both HPH and MPH and 0.008 for SCA (Figures 6.5 and 6.6).

Table 6.10 Average mid- and high-parent heterosis, and correlation among F_1 grain yield, mid- and high-parent heterosis and specific combining ability for all hybrids across all environments, optimum, drought and low nitrogen environments

Environment	Average MPH	Average HPH	r(F_1, SCA)	r(F_1, MPH)	r(F_1, HPH)	r(SCA, HPH)	r(SCA, MPH)	r(SCA, GD)	r(F_1, GD)	r(HPH, GD)	r(MPH, GD)
All environments	112.29	76.73	0.31**	0.36**	0.57**	0.31**	0.48**	0.09	-0.02	0.13	0.16
Optimum	117.39	85.29	0.24*	0.46**	0.76**	0.25*	0.37**	0.08	-0.06	-0.07	0.02
Drought	26.08	10.34	0.42**	0.72**	0.77**	0.38**	0.37**	0.06	0.38**	0.37**	0.08
Low N	72.41	25.73	0.08	0.04	0.64**	-0.07	-0.07	-0.03	-0.01	-0.01	0.14

****P≤0.01; *P≤0.05; MPH=mid-parent heterosis; HPH=high-parent heterosis; SCA=specific combining ability; F_1=grain yield (t ha^{-1}) for hybrids; GD =genetic distance; r=Pearson's coefficient of correlation.**

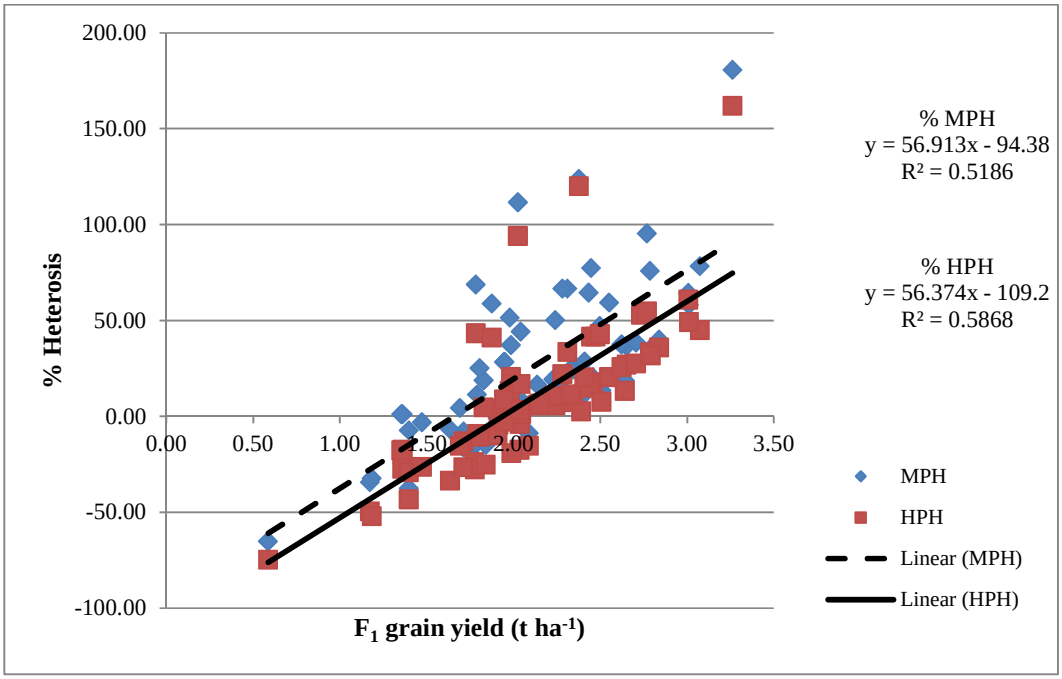

Figure 6.2 Relation of *per se* performance of hybrids with high- and mid-parent heterosis under drought conditions.

MPH=mid-parent heterosis; HPH=high-parent heterosis.

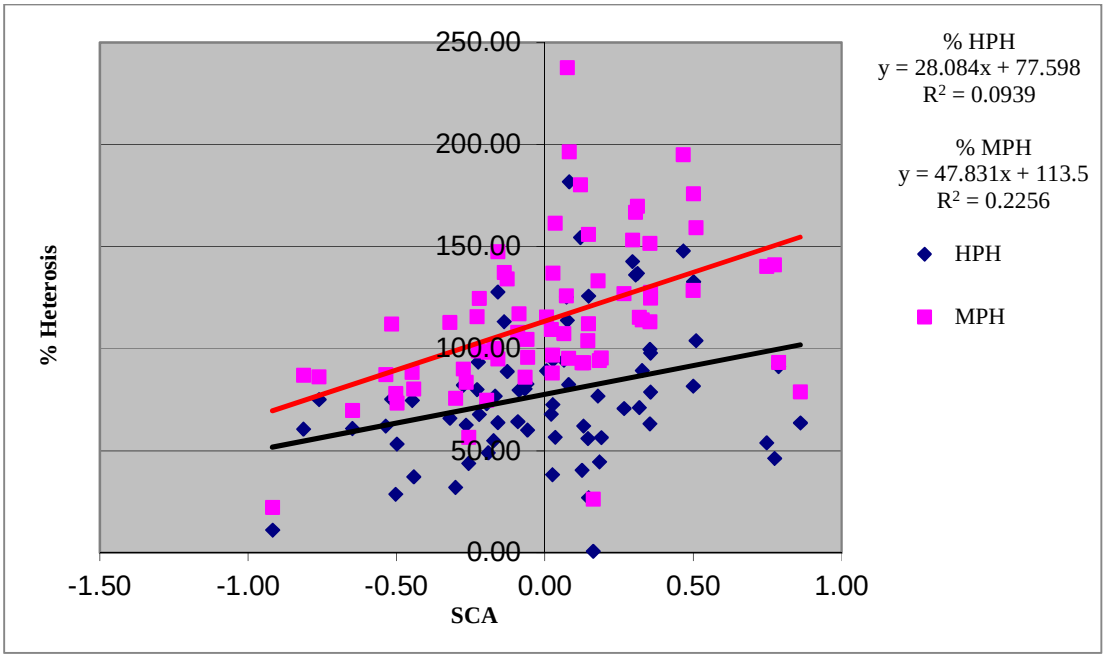

Figure 6.3 Relation of specific combining ability with high- and mid-parent heterosis across all environments.

MPH=mid-parent heterosis; HPH=high-parent heterosis; SCA=specific combining ability.

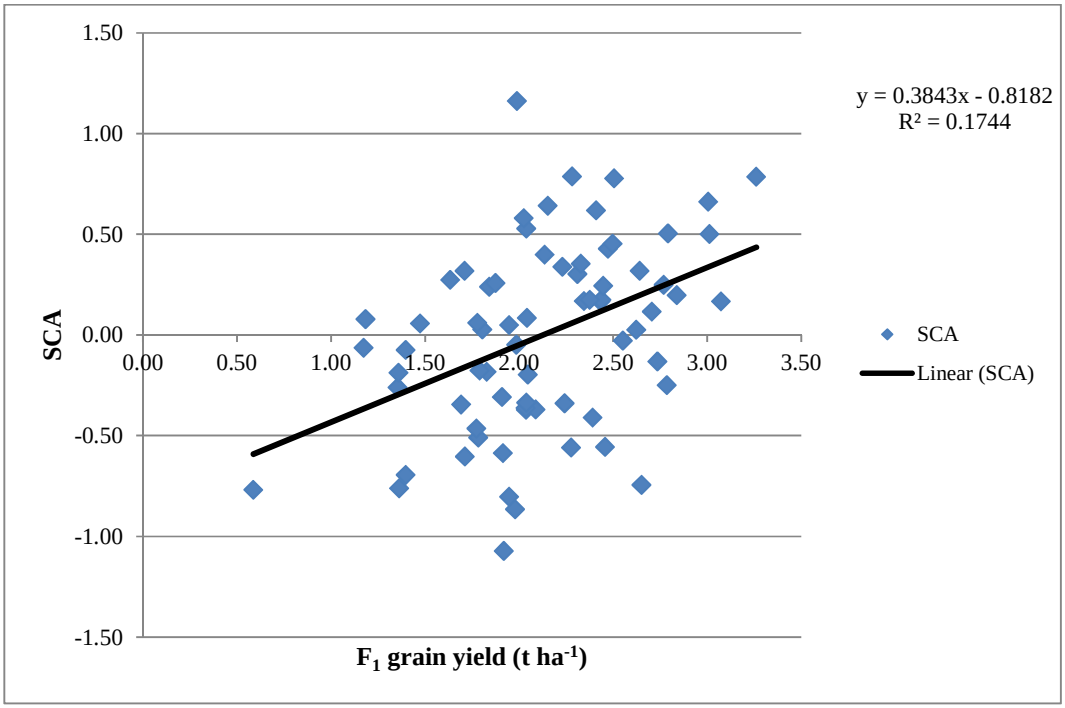

Figure 6.4 Relation of specific combining ability with *per se* performance of hybrids.
SCA=specific combining ability.

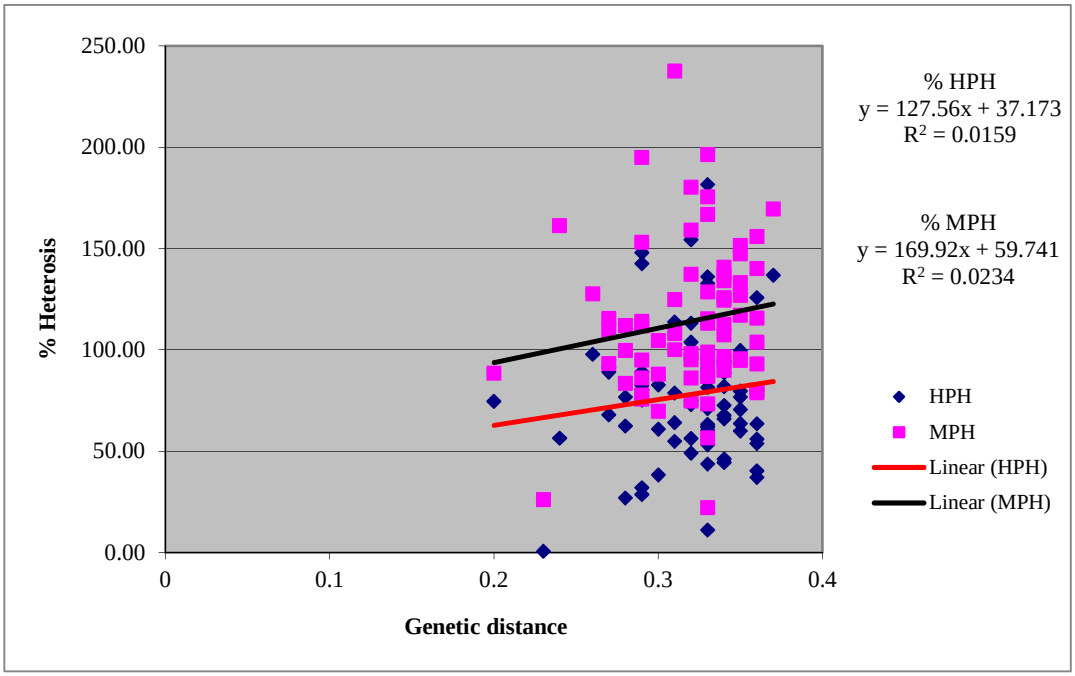

Figure 6.5 Relation of genetic distance with high- and mid-parent heterosis across all environments.

MPH=mid-parent heterosis; HPH=high-parent heterosis.

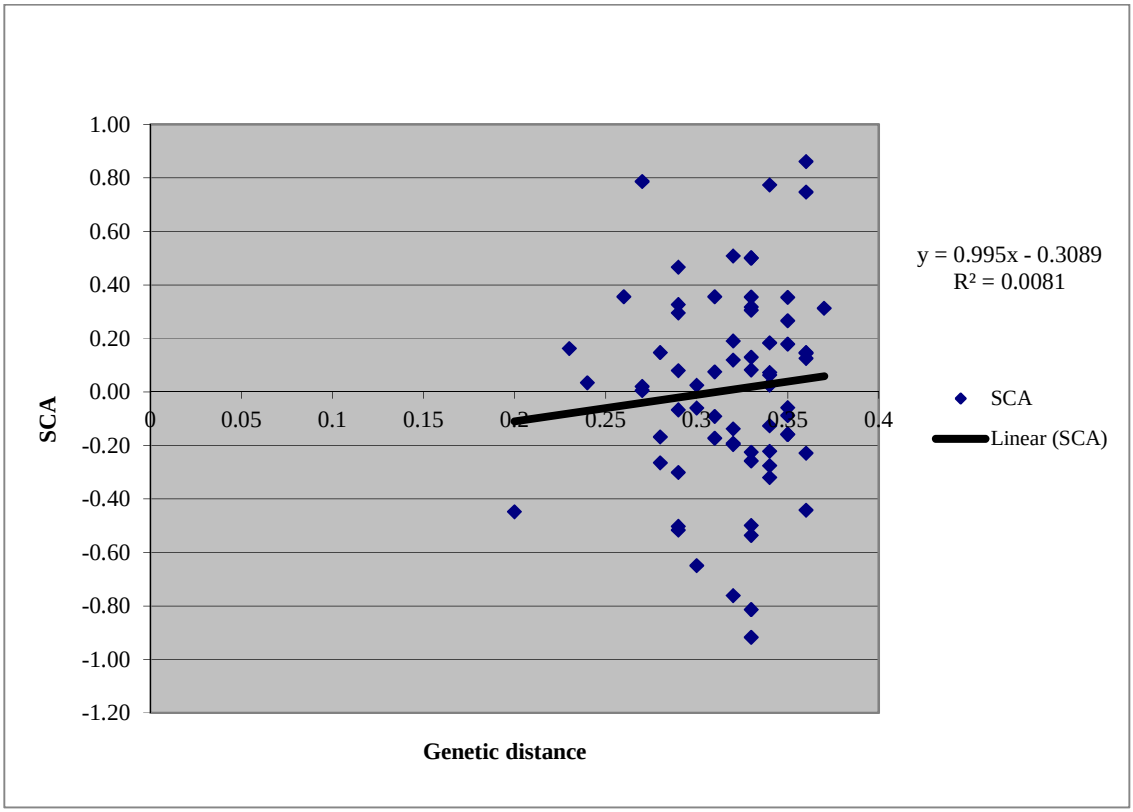

Figure 6.6 Relation of genetic distance with specific combining ability across all environments.

SCA=specific combining ability.

6.4 Discussion

Presence of a reasonable magnitude of heterosis for grain yield and related traits is critical in any hybrid breeding programme. The degree of heterosis is therefore determined by genetic diversity that exists within the germplasm being used. The average degree of MPH and HPH ranged from 26.08% and 10.34% in drought environments to 117.39% and 85.29% in optimum environments. The expression of heterosis was greater under optimum than stress conditions. This could be explained by the fact that CIMMYT inbred lines used as testers were bred for tolerance to stress conditions so they tended to perform well, thereby resulting in an increase in parental and better parent means. Results are contrary with results reported by Betran *et al*. (2003) and George *et al.* (2011) who reported higher MPH and HPH values under severe stress than under non-stress environments using tropical germplasm and CIMMYT South America maize programme germplasm, respectively. In the current study intermediate drought stress was applied whereas Betran *et al*. (2003) applied both

intermediate and severe drought stress and in addition the materials evaluated and the sample size was different. Most parents showed positive heterosis for grain yield across all environments, indicating the existence of substantial heterosis in hybrids.

When all crosses were considered across all environments for grain yield MPH averaged 112.29% and HPH 76.40%. These values are higher than those reported by Legesse *et al*. (2008) (MPH mean ranging between 0.9% and 77.2%) but lower than those reported by Betran *et al*. (2003) (MPH 171% and HPH 132%). Grain yield is expected to show higher levels of hybrid vigour compared to other traits as it has been suggested that it is a multiplicative trait that draws variation from other traits (Williams, 1959; Lippman and Zamir, 2007). Therefore it is assumed that lower values of heterosis detected for other traits may interrelate in a non-linear manner to create better heterosis levels for yield (Flint-Garcia *et al*., 2009). The levels of heterosis observed in this study indicate that there is an opportunity of using the germplasm for developing hybrid varieties suitable for stress and non-stress environments. Lines L1 (K64r), L2 (N3.2.3.3), L3 (RS61P), L4 (NAW5885) and L7 (SC5522) and testers T3 (CML539), T9 (CML444) and T6 (CZL03007) were identified as good parental lines for producing hybrids for non-stress environments. On the other hand L3 (RS61P), L4 (NAW5885), L5 (2N3d), T11 (CML548), T6 (CZL03007) and T10 (CML536) were identified as potential parental lines for producing stress tolerant hybrids.

Hybrids showed negative MPH and HPH for anthesis days with averages of -5.1% and -7.4% respectively. These results were desirable as it indicated that hybrids were earlier in anthesis than their inbred parents and similar results were reported by Gissa (2008). Due to increasing unpredictability of global weather patterns, especially amount of rainfall and its distribution, use of early maturing varieties has become a better strategy for farmers to reduce risks associated with drought. Early maturing maize provides options concerning inter-crops, relay crops, late planted crops, drought avoidance and earlier harvesting (CIMMYT-Zimbabwe, 2000). Moderately high positive MPH and HPH for plant and ear height indicate the superiority of dominance effects among the parental inbred lines for taller plant height (Gissa, 2008). In the current study mean MPH and HPH for plant height (59.9% and 49.9%) and ear height (53.3% and 42.7%) were reported and similar results were reported by Gissa

(2008) and Legesse *et al*. (2008). Gissa (2008) reported mean MPH and HPH for plant height (57.1% and 46.8%) and ear height (62.6% and 49.8%), whilst Legesse *et al*. (2008) reported MPH values for plant height ranging between 10.6% and 37.8%. Positive MPH and HPH values for ears per plant reported in this study were also ideal as it is an indication that hybrids are more prolific than their inbred parents.

Heterotic groups have been extensively used to streamline maize breeding (Tracy and Chandler, 2006). The predefined heterotic groupings of lines did not consistently predict the performance of hybrids e.g. L5 (2N3d) in the N3 group and T1 (CML395) in the B group displayed low MPH (88.41%) and HPH (74.60%) means and this was also true for L8 (RA214P) in N3 group and T1 (CML395) in B group (MPH 87.2% and HPH (62.14%). Dhliwayo *et al*. (2009) reported even lower MPH means with CIMMYT B x IITA A (4.97%) and CIMMYT B x CIMMYT A (5.84%). However, there were cases when predefined groupings resulted in corresponding high heterosis values e.g. L6 (2Kba) in the SC group and T11 in the A group showed high MPH (166.80%) and HPH (136.08%) and L4 (NAW5885) in the N3 group crossed with T6 ([CML445/ZM621B]-2-1-2-3-1-B*5) in the B group also showed high MPH (195.02%) and HPH (147.91%). In this study heterotic groupings determined by SNP markers also resulted in corresponding high MPH and HPH values but in some cases the opposite was true e.g. L4 (NAW5885) and T4 (CML442) clustered in different groups using SNP markers and showed high MPH (159.25%), whilst L7 (SC5522) and T10 (CML536) clustered in the same group but displayed high MPH (140.17%). Such phenomenon has been reported frequently in maize when genetically diverse parents produce crosses with high heterosis (Hallauer and Miranda, 1988; Hallauer *et al*., 2010). In the current study it was also noted that lines within the same heterotic group exhibited offspring with lower HPH values and similar results were reported by Flint-Garcia *et al*. (2009). Literature indicates that the use of heterotic groups and genetic distance to predict levels of heterosis have been of limited success (Moll *et al*., 1965; Melchinger, 1999). Although crosses between individuals in the same group have revealed lesser heterosis than crosses between individuals in different groups there are many exceptions, making group identity a poor predictor of heterosis (Flint-Garcia *et al*., 2009).

Research done earlier has shown that low levels of GD significantly reduced the levels of heterosis (Moll *et al*., 1965) thereby prompting other researchers to think that GD can be used to predict heterosis. Results from this study have revealed that there was significant correlation between GD and heterosis under drought conditions and the following researchers reported similar results: Betran *et al*. (2003), Amorim *et al*. (2006), Srdic *et al*. (2007) and George *et al*. (2011). However, Betran *et al*. (2003) reported increased correlations under non-stress compared to stress environments. The inbred lines used as testers in this study were specifically screened for drought tolerance and as a result their performance compared to lines used as females was different and this might explain the significant correlation of GD and heterosis. In addition GDs reported in the current study had a narrow range and this might also explain it being able to predict hybrid performance since in other studies (Melchinger, 1999; George *et al*., 2011) it has been reported that a better correlation of GD with MPH and HPH was observed among lines that are more closely related than among lines that are distantly related. Different environments furthermore influence association of GD and heterosis to different degrees, mainly because performance of parental lines and hybrids varies across environments. Previous studies have seldom detected strong association amongst heterosis and parental GD (Melchinger, 1999; Singh and Singh, 2004). Moll *et al*. (1965), Melchinger (1999) and George *et al*. (2011) have suggested that the relationship between GD and heterosis is complicated given that as the GD between the parental lines increases the level of heterosis also increases up to a certain point after which heterosis starts declining.

On the other hand there was non-significant correlation between GD and heterosis across all environments as well as under optimum and low N conditions and similar results were reported by Balestre *et al*. (2008), Dhliwayo *et al*. (2009) and Devi and Singh (2011). In the current study there was a positive and significant correlation between GD and *per se* performance of hybrids under drought conditions but generally the *per se* performance of F_1s was little influenced by GD as evidenced from non-significant negative correlations. These results are an indication that meagre variation attributable to SCA, HPH, MPH and *per se* performance of hybrids could not be explained due to SNP based GD of the parents. There were significant positive correlations between SCA, *per se* performance of hybrids, MPH and

HPH across all environments and under optimum and drought conditions but not under low N conditions. These results indicate that an improvement in selection for SCA will result in an indirect improvement of MPH and HPH for hybrids under optimum, drought and across environments. Under low N conditions the opposite is true. The non-significant correlation of SCA, HPH and MPH with *per se* performance of hybrids under low N conditions might be due to the low inbred line genetic variability caused by low yields and high error variability associated with the stress environment. Contrary to results reported by Betran *et al*. (2003) where r(F_1, SCA) across environments was double that of r(F_1, MPH) and r(F_1, HPH), in this study almost double r(F_1, HPH) was reported. Similar to the results reported by Devi and Singh (2011), MPH and HPH were also found to be key determinants of *per se* performance of hybrids in this study. HPH correlation with *per se* performance was consistent in all environments and according to Flint-Garcia *et al*. (2009) better parent heterosis is an economically relevant trait. The HPH and MPH also established positive linear regressions with *per se* performance of hybrids. As a consequence of the differential response of the inbred lines under stress conditions the correlation that involved MPH was more erratic and inconsistent than the HPH correlation.

6.5 Conclusions

The most important factor in developing adapted and high yielding hybrids is identifying parental lines that combine well and result in superior varieties. The germplasm used generally exhibited good heterosis for grain yield and other traits. Lines L1 (K64r), L2 (N3.2.3.3), L3 (RS61P), L4 (NAW5885) and L7 (SC5522) and testers T3 (CML539), T9 (CML444) and T6 (CZL03007) were identified as potential parental lines for producing hybrids for optimum environments, whilst L3 (RS61P), L4 (NAW5885), L5 (2N3d), T11 (CML548), T6 (CZL03007), T9 (CML444) and T10 (CML536) were identified as potential parents for producing hybrids for stress environments. The positive and significant association of SCA and grain yield under optimum and drought conditions confirmed SCA as a good predictor for grain yield of F_1 hybrids. MPH had poor predictive value for grain yield performance of hybrids under study, whilst HPH had a better predictive value. The GDs found in this study were small and some of the lines had low homozygosity which might have influenced the levels of heterosis realised in the study. A significant and positive SCA,

MPH and HPH association is an indicator that SCA can be used to predict MPH and HPH during selections under both optimum and drought conditions which is a desirable selection outcome. The negative MPH and HPH for days to anthesis showed that hybrids were earlier than their parental inbred lines and this is a desirable outcome as the National Breeding Programme is inclined towards breeding for early maturing hybrids. The majority of farmers targeted by the breeding programme are situated in low rainfall areas characterised by erratic mid-season droughts, so early maturing hybrids would be a better option for them. Even though the correlation of SNP distances with HPH and MPH were significant under drought conditions, the magnitudes were too low to be of high predictive value. SNP and morphological distances were found to be useful in identifying closely related and distantly related maize inbred lines but they were found to be of limited importance in predicting HPH, MHP, SCA and *per se* performance of lines. The known heterotic groups are valid since high heterosis could be observed from lines in the opposite groups, however confirming their validity with the SNP data was not conclusive maybe due to genetic contamination of some of the lines.

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CHAPTER 7

Performance of F_3 testcrosses developed from CIMMYT drought tolerant donors and Zimbabwean elite inbred lines

Abstract

Drought is one of the most devastating factors for maize production especially in sub-Saharan Africa. Therefore the urgent need to breed for drought tolerant varieties cannot be over-emphasised. In this study six DR&SS elite white maize inbred lines were crossed to two CIMMYT Zimbabwe and seven CIMMYT Mexico drought tolerant donors to initiate a segregating population after which selfing was done until the F_3 generation. A total of 196 segregating lines belonging to heterotic group A were testcrossed to group B tester CML444/CML395 and 209 segregating lines belonging to heterotic group B were testcrossed to group A tester CML539/CML442 under isolation. All testcrosses were divided into early and late maturing trials and evaluated under drought and optimum conditions using a 0.1 alpha lattice design with two replications across three environments in the 2011 winter season. The objectives of this study were to determine agronomic performance of the testcrosses under drought and well-watered conditions and to identify superior testcrosses for grain yield and yield related traits. Significant ($P \leq 0.001$) mean squares for the majority of traits were an indication of the presence of genetic variability amongst the testcrosses. The G x E interaction mean squares were highly significant ($P \leq 0.001$) for the majority of traits. Testcrosses containing the lines derived from DR&SS lines K64r, RS61P, N3.2.3.3 and CIMMYT drought tolerant donors based on DTPWC9 were generally amongst the best performing testcrosses in both trial sets under drought and well-watered conditions and across environments. There was significant correlation of grain yield with secondary traits such as anthesis days, anthesis silking interval and ears per plant under drought conditions. Genetic variances and genetic gains for plant and ear height were generally higher than those for other traits. Results therefore provide the basis for selection of segregating lines for grain yield and related traits for further generation advancement.

7.1 Introduction

Maize is the world's leading cereal crop with production of 695 million ton and per unit area yield of 4 815 kg ha^{-1} (FAOSTAT, 2008). It is grown as a staple food crop in different agro-ecological zones of Zimbabwe. Currently 80-90% of the total area under maize is attributable to communal farmers and they encounter variations in rainfall distribution from year to year, exposing their maize crop to drought. The 1981/82 drought reduced maize production by about 70% (Rukuni *et al*., 2006) and hence emphasised the need to develop drought tolerant varieties. The most elite inbred lines used by the Zimbabwe National Maize Breeding Programme under DR&SS lack tolerance to drought and there exists a need to develop drought tolerant varieties in the shortest possible time. Hence there is a need to use quick and efficient methods that will enable identification and isolation of superior drought tolerant genotypes.

The maize improvement programme involves development of lines, assessment of their performance and selection of superior ones that are later used as parental lines (Pixley *et al*., 2006). The presence of satisfactory genetic diversity and good quality genetic factors derived from parents define progress from selection for improved adaptation and desirable agronomic traits (Dreisigacker *et al*., 2004). Introgression of exotic maize into adapted breeding germplasm can widen and diversify the genetic base of adapted germplasm (Menkir *et al*., 2007). Crosses constituted from diverse genotypes maximise the number of segregating alleles, causing large genetic variance in the offspring (Cox *et al*., 1985; Messmer *et al*., 1993) thus increasing the chance for selecting genotypes that may be superior (Becelaere *et al*., 2005). Exotic maize inbred lines with higher levels of diversity can lead to concentration of alleles for increased yield potential, early maturity, short plant height, resistance to root and stem lodging, tolerance to abiotic stresses (e.g. drought) and resistance to diseases and insects (Menkir *et al*., 2007).

Estimates of genetic components of variance are useful for two aspects that are important to applied breeding programmes and these are estimates of heritability and predicted response to selection. Progress in plant breeding depends on the availability and maintenance of genetic variability. Heritability expresses the proportion of the total variance that is

attributable to the average effects of genes and determines the resemblance between relatives (Falconer, 1960). Repeatability can be defined as the proportion of the genotypic variance to the total phenotypic variance. There are certain assumptions that are made in computing repeatability and the first assumption is that the variances of different measurements are equal and have their components in the same proportions and the second assumption is that the different measurements reveal what the same character is genetically (Falconer, 1960).

As the numbers of lines to be tested at various stages of inbreeding increase over time, their evaluation in all possible hybrid combinations is not feasible. Test cross performance of experimental lines is the major selection criterion in hybrid maize breeding (Mihaljevic *et al*., 2005). Two basic systems are used namely late and early testing. Late testing involves evaluation of hybrid performance at later stages of inbreeding (S_5-S_6) and more testers are used compared to early testing. Early testing of inbreds first proposed by Jenkins (1935) has become a matter of great interest to maize breeders. In early testing, evaluation of hybrid performance is conducted in early generations of inbreeding (S_1-S_3) whereby plants are outcrossed to a tester and the resulting progeny evaluated for grain yield and general performance. Early testing procedure is of significance where yield is an important consideration or where other important factors can be evaluated easily and efficiently by a suitable tester (Allard, 1960). Therefore test crossing has been adopted extensively to evaluate the relative *per se* performance of inbred lines to aid in line advancement in pedigree breeding. Hence the objectives of this study were to determine agronomic performance of testcrosses derived from CIMMYT and DR&SS lines under drought and well-watered conditions through pedigree breeding and to identify superior lines for generation advancement.

7.2 Materials and methods

7.2.1 Germplasm

Plant selections were made at each generation and these were based on synchronisation between pollen shedding and silking, low ear placement, well filled ears and resistance to lodging and MSV. Six DR&SS elite inbred lines were crossed with nine CIMMYT drought

tolerant donors to develop a segregating population (Table 7.1). CIMMYT drought tolerant donor lines were crossed to all the DR&SS lines and 158 F1s were produced. Selfing was done and the ear to row technique was applied for generation advancement until the F_3. The F_3 segregating population was grouped according to the CIMMYT heterotic grouping, after which 196 lines belonging to group A were testcrossed to the group B tester CML444/CML395 and 205 lines belonging to group B were testcrossed to group A tester CML539/CML442. Testcrossing was done under isolation at Gwebi in Zimbabwe (17.13^0S, 31^0E, 1406 masl). Three hundred and twenty-four testcrosses were divided into maturity groups (early and late) to constitute two trial sets. The early testcross trial constituted 223 entries and seven check varieties (013WH29, 023WH31, SC513, SC403, SC411, CZH0524 and CZH0946), whilst the late testcross trial constituted of 101 entries and seven check varieties (013WH03, 013WH01, SC635, SC627, SC727, CZH0837 and CZH0616).

Table 7.1 Pedigree, source and heterotic grouping of the inbred lines used to develop the F_3 population

Inbred	Pedigree	Source	Heterotic group
1	SC5522	DR&SS	SC
2	N3.2.3.3	DR&SS	N3
3	RS61P	DR&SS	SC
4	NAW5885	DR&SS	N3
5	K64r	DR&SS	N3
6	RA214P	DR&SS	N3
7	CML442	CIMMYT-Harare	A
8	CML444	CIMMYT-Harare	B
9	DTPWC9-F16-1-1-1-1-BBB	CIMMYT-Mexico	B
10	DTPWC9-F104-5-4-1-1-BBB	CIMMYT-Mexico	B
11	DTPWC9-F92-2-1-1-1-BB	CIMMYT-Mexico	B
12	DTPWC9-F115-1-2-1-2-BBB	CIMMYT-Mexico	B
13	G16BNSeqC4-(F25x2)-4-1-4-B	CIMMYT-Mexico	A
14	G16BNSeqC4-(F20x17)-3-1-5-BB	CIMMYT-Mexico	A
15	LaPostaSeqC7-F103-1-2-1-1-BBB	CIMMYT-Mexico	B

7.2.2 Evaluation sites

Trials were evaluated under drought and optimal conditions during the winter of 2011. Three sites were used: Chiredzi Research Station (21.02°S, 31.58°E, 433 masl), Chisumbanje Research Station (20°S, 33°E, 455 masl) and Save Valley Research Station (20^0S, 32^0E, 556

masl). At each site two early and two late testcross trials were planted (one under managed drought and another under optimal conditions).

7.2.3 Management

Trials under optimal conditions were grown under irrigation and water was applied as and when it was necessary to ensure that the crop does not suffer from moisture stress. At all three sites drought was managed through irrigation at critical times only. A total of 280 mm irrigation was applied in the first eight weeks of the crop's growth for the trials under managed drought. This resulted in drought coinciding with flowering and grain filling. The stress level projected to be achieved in this trial was a yield of about 15- 20% of yields achieved under optimal conditions. This stress level delays silking and causes ear abortion in non-stress tolerant genotypes. The testcross evaluation was done using the 0.1 alpha lattice design. There were two replications in each trial, with each entry being planted in a one row plot 4 m long, with a 90 cm between row and 30 cm in-row spacing. Two seeds were planted per station and later thinned to give a plant population of 48 000 plants ha^{-1}. A total of 400 kg ha^{-1} maize fertiliser was applied as basal dressing and 350 kg ha^{-1} ammonium nitrate as topdressing split applied at four and eight weeks after crop emergence. Two applications of Dipterex 2.5% granules into the funnel of each plant were done at three and six weeks after crop emergence and the rate of application used was 4 kg ha^{-1}. Termite control was done as and when necessary using Carbaryl 85% wettable powder.

7.2.4 Data collection and analysis

Data for flowering dates (at 50% anthesis and 50% silking), plant and ear height, plant stand ability (root and stem lodging), leaf senescence, ear aspect, texture, ear rot and grain weight were recorded as described in section 3.2.4 and Table 3.2. Some derived traits such as anthesis silking interval, lodging percentage, ears per plant and yield per hectare (at 12.5% moisture adjustment) were also calculated. The individual and combined site ANOVA was done using AGROBASE (2005). In the combined ANOVA, testcrosses and locations were considered as fixed effects while replications and years were considered as random effects. The variance components were computed following Wricke and Weber (1986).

Genetic variance:

$\sigma^2_g = (M_5 + M_2 - M_3 - M_4)/rly$

Where: σ^2_g = genetic variance

$M5 = \sigma^2_e + r\sigma^2_{gly} + ry\,\sigma^2_{gl} + rl\,\sigma^2_{gy} + rly\,\sigma^2_g$

$M_4 = \sigma^2_e + r\sigma^2_{gly} + rl\,\sigma^2_{gy}$

$M_3 = \sigma^2_e + r\sigma^2_{gly} + ry\,\sigma^2_{gl}$

$M_2 = \sigma^2_e + r\sigma^2_{gly}$

rly = replication x location x year

gly = genotype x location x year

gl = genotype x location

gy = genotype x year

Genotype x year variance:

$\sigma^2_{gy} = (M_4 - M_2)/rl$

Where: rl = replication x location

Genotype x location variance:

$\sigma^2_{gl} = (M_3 - M_2)/ry$

Where: ry = replication x year

Genotype x location x year variance:

$\sigma^2_{gly} = (M_2 - M_1)/r$

Where: r = replication

Error variance:

$\sigma^2_e = M_1$

Phenotypic variance:

$$\sigma^2_P = \sigma^2_g + \frac{\sigma^2_{gl}}{L} + \frac{\sigma^2_{gy}}{Y} + \frac{\sigma^2_{gly}}{LY} + \frac{\sigma^2_e}{RLY}$$

Repeatability:

$b = \sigma^2_g/\sigma^2_P$

Where: σ^2_g = genotypic variance

σ^2_P = phenotypic variance

Genetic gain:

$\Delta_g = k\ \sigma^2 p\ b$

Where: k = selection intensity

$\sigma^2 p$ = phenotypic variance

b= repeatability

In this case repeatability is taken to be the same as broad sense heritability. Pearson correlation coefficients (r) between grain yield and secondary traits were calculated from means across environments and per environment. Statistical computations were performed with SPSS 15.0 version for Windows (2006).

7.3 Results

7.3.1 Performance of early maturing testcrosses under managed drought conditions

The means presented are for the 10 best and 10 poorest genotypes in terms of grain yield and overall grand means for each data set are presented. The early maturing testcrosses planted at Chiredzi Research Station under managed drought stress conditions were severely affected by heat scorch soon after water stress was induced at two weeks bracketing flowering, resulting in most of the entries completely failing to flower. As a result grain yield was recorded for only a few entries and the site was therefore excluded from the analysis. The ANOVA for grain yield under managed drought stress conditions for Chisumbanje and Save Valley Research Stations is presented in Table 7.2.

The two sites, genotypes and G x E interaction were significant ($P \leq 0.001$) for grain yield. Results for anthesis days and other agronomic traits across the three sites under managed drought stress are presented in Tables 7.3 and 7.4. Sites were significantly different for all traits ($P \leq 0.001$). Genotypes were significantly different at $P \leq 0.001$ for plant height, ear height, root lodging, stem lodging, ears per plant and ear aspect, significantly different at

P≤0.01 for ear rot and significantly different at P≤0.05 for senescence and texture. However genotypes were not significantly different for anthesis days and anthesis silking interval. The G x E interaction was significant for all traits except anthesis silking interval, senescence and texture.

Table 7.2 Analysis of variance for grain yield under managed drought conditions at Chisumbanje and Save Valley for early maturing testcrosses in the 2011 winter season

Source	DF	MS
Site	1	109.68***
Genotype	229	0.55***
G x E	229	0.66***
Residual	278	0.24

*****P≤0.001; DF=degrees of freedom; MS=mean square; Env=environment; G x E=genotype by environment interaction.**

Table 7.3 Analysis of variance for anthesis days and other agronomic traits under managed drought conditions across three sites for early maturing testcrosses in the 2011 winter season

Source	DF	AD	ASI	PH	EH	RL	SL	SEN
Site	2	163938.91***	1830.03***	22237.52***	5451.52***	46510.69***	83817.67***	14.948***
Genotype	229	12.86	13.51	250.72***	293.06***	171.42***	304.34***	0.027*
G x E	458	12.12**	13.30	237.76***	255.76***	175.83***	231.60***	0.025
Residual	417	9.45	12.25	3.52	105.10	57.82	87.40	0.022

*****P≤0.001; **P≤0.01; *P≤0.05; DF=degrees of freedom; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; RL=root lodging; SL=stem lodging; SEN=senescence; Env=environment; GxE=genotype by environment interaction.**

Table 7.4 Analysis of variance for ears per plant, ear aspect, texture and ear rot under managed drought conditions across three sites for early maturing test crosses in the 2011 winter season

Source	DF	EPP	EA	TEX	ER
Site	2	15.750***	51.95***	37.80***	99206.70***
Genotype	229	0.038***	1.07***	1.03*	632.77**
G x E	458	0.034***	1.01***	0.93	602.51**
Residual	417	0.005	0.71	0.82	456.72

*****P≤0.001; **P≤0.01; *P≤0.05; DF=degrees of freedom; EPP=ears per plant; EA=ear aspect; TEX=texture; ER=ear rot; Env=environment; G x E=genotype by environment interaction.**

The means for the best and poorest performing early maturing testcrosses under drought are presented in Table 7.5. The genotype G122 (DTPWC9-F104-5-6-1-1-B*4/K64r)-B-3//CML444/CML395 was the best performer in terms of grain yield under managed drought conditions with a mean yield of 2.68 t ha^{-1} followed by G25 (DTPWC9-F16-1-1-1-1-BBB/RS61P)-B-23//CML539/CML442 with a mean yield of 2.51 t ha^{-1}. The 10 best genotypes performed well above the trial grand mean of 1.53 t ha^{-1}. The poorest performer was G215 (DTPWC9-F104-5-6-1-1-B*4/NAW5885)-B-4//CML444/CML395 with an average mean yield of 0.70 t ha^{-1} and the genotype had a poor ear aspect score of 4.3 and ear rot percentage of 35.3%. Six of the ten best performing testcrosses constituted of K64r sister lines and the rest were two RS61P sister lines, one N3.2.3.3 line and one NAW5885 line, whilst the majority of testcrosses within the ten poorest performing testcrosses were NAW5885 sister lines. Most lines within the ten poorest performing lines had ear rot scores of above 20% and ear aspect scores of above 3.5. Heritability scores generally showed that genotypes played a minor role in expression of traits leaving the environment to play a major role and this was further confirmed by the lower genotypic variances compared to phenotypic variances. The R^2 values were above 0.50 for all traits measured under managed drought conditions.

Table 7.5 Performance of early maturing maize testcrosses for grain yield and other agronomic traits under managed drought

	Genotype	GYD	PH	EH	RL	SL	SEN	EPP	EA	ER
Ten best testcrosses	G122	2.68	170.8	95.0	13.7	23.1	0.58	0.41	3.4	26.0
	G25	2.51	17.8	81.7	8.3	27.0	0.55	0.62	3.8	39.9
	G131	2.44	161.7	82.5	10.7	26.2	0.55	0.53	3.6	40.8
	G14	2.44	180.8	92.5	6.3	25.1	0.55	0.53	3.5	25.4
	G51	2.36	165.0	89.2	1.5	24.8	0.53	0.46	3.1	26.8
	G46	2.36	170.8	92.5	15.0	22.1	0.55	0.55	3.7	18.6
	G128	2.28	179.2	95.0	15.2	20.7	0.55	0.57	3.3	10.5
	G209	2.28	180.0	104.2	14.7	15.9	0.58	0.51	3.3	13.7
	G190	2.28	171.7	90.8	31.9	9.0	0.52	0.60	3.3	16.1
	G114	2.27	178.3	101.7	16.3	16.4	0.53	0.48	3.9	23.6
Ten poorest testcrosses	G129	0.94	165.8	80.8	7.1	22.2	0.55	0.34	3.9	32.7
	G196	0.94	160.8	93.3	15.8	12.5	0.57	0.33	3.5	26.7
	G43	0.94	165.0	90.8	10.7	17.7	0.60	0.44	4.0	48.5
	G162	0.94	170.8	100.8	10.6	16.3	0.53	0.40	3.4	24.9
	G40	0.94	160.8	84.2	2.6	18.8	0.58	0.40	4.3	44.5
	G154	0.86	170.8	82.5	2.0	17.7	0.53	0.36	4.4	34.9
	G126	0.86	175.0	104.2	6.4	18.4	0.58	0.25	3.7	24.2
	G100	0.86	166.7	92.5	9.9	16.4	0.55	0.42	3.8	27.9
	G65	0.71	178.3	107.5	16.9	7.6	0.55	0.42	3.9	28.4
	G215	0.70	167.5	90.0	17.7	8.2	0.48	0.35	4.3	35.3
	Grand mean	1.53	170.7	95.3	11.9	17.8	0.56	0.45	3.7	28.1
	LSD	0.57	5.34	9.79	7.2	8.9	0.14	0.07	0.8	20.3
	SED	0.35	3.24	5.94	4.39	5.40	0.090	0.040	0.49	12.34
	CV%	31.84	3.29	10.80	33.5	32.6	26.36	16.56	23.0	46.0
	R^2	0.90	0.96	0.86	0.92	0.92	0.86	0.97	0.79	0.81

GYD=grain yield (t ha^{-1}); PH=plant height (cm); EH=ear height (cm); RL=root lodging (%); SL=stem lodging (%); SEN=senescence (%); EPP=ears per plant (#); EA=ear aspect (1-5); ER=ear rot (%); LSD=least significant difference;SED=standard error difference; CV=coefficient of variation; R^2=coefficient of determination.

7.3.2 Performance of early maturing testcrosses under well-watered conditions

The ANOVA for grain yield and other agronomic traits are presented in Tables 7.6 and 7.7. Sites were significantly different ($P \leq 0.001$) for all traits except anthesis silking interval. Genotypes were significantly different ($P \leq 0.001$) for grain yield, plant height, ear height, ear position, ear aspect, ear rot and texture, whilst they were not significantly different for anthesis silking interval and ears per plant. The G x E was not significant for anthesis days and anthesis silking interval. Genotype means for grain yield and other agronomic traits are presented in Table 7.8. The grain yield trial mean was 3.85 t ha^{-1} and the two best performing genotypes G82 ((NAW5885/CML442)-B-9//CML444/CML395) and G206 ((DTPWC9-F66-2-1-1-2-BBB/N3233)-B-2//CML444/CML395) performed 41.8% above the trial mean with a mean yield of 5.46 t ha^{-1}, whilst the poorest performing genotype G58 ((DTPWC9-F92-2-1-1-1-BB/K64r)-B-11//CML539/CML442) performed 57.1% below the trial mean with a mean yield of 2.20 t ha^{-1}. Testcrosses that featured within the ten best performers in terms of grain yield were mainly constituted from K64r, RS61P, NAW5885 and N3.2.3.3 with DTPWC9 donor lines. Testcrosses within the ten best performers all showed ear aspect scores of below 3.5 and the texture scores showed that genotypes ranged from semi-flint to semi-dent. The ear rot scores for all genotypes were generally below 30% with a trial mean of 17%. The R^2 values were again high and were all above 0.50. The coefficient of variation values were below 20% for all traits except ear rot.

7.3.3 Performance of early maturing testcrosses across environments

In the combined ANOVA environments were significantly different ($P \leq 0.001$) for grain yield, anthesis days, plant height, ear height, ear position, ears per plant, root and stem lodging, senescence and ear rot except anthesis silking interval (Tables 7.9 and 7.10). Genotype as well as G x E mean squares were significant for all traits. All other interactions were also highly significant. The genotype G111 ((DTPWC9-F104-5-4-1-1-BBB/K64r)-B33//CML444/CML395) was the overall best performer in terms of grain yield with a mean yield of 4.17 t ha^{-1} and this was 39.9% higher than the trial mean (Table 7.11).

Table 7.6 Analysis of variance for grain yield for early maturing testcrosses under well-watered conditions in the 2010/11 season

Source	DF	MS	P
Site	1	109.68	***
Genotype	229	0.55	***
G x E	229	0.66	***
Residual	278	0.24	

*****P≤0.001; Env=environment; G x E=genotype by environment interaction;; DF=degrees of freedom; MS=mean square; P=probability.**

Table 7.7 Analysis of variance for anthesis days and other agronomic traits under well-watered conditions across three sites for early maturing testcrosses in the 2011 winter season

Source	DF	AD	ASI	PH	EH	EPO	EPP	EA	ER	TEX
Site	2	41325.50***	9227.75	561563.97***	118377.88***	0.19***	0.95***	3.37***	6553.21***	114.46***
Genotype	229	10.30*	9501.18	374.81***	362.70***	0.07***	0.011	0.41***	114.08***	0.28***
G x E	458	9.02	9511.65	265.50***	222.37***	0.006***	0.01*	0.35***	104.53***	0.30***
Residual	417	7.93	9476.79	26.43	58.7	0.004	0.01	0.16	68.75	0.12

*****P≤0.001; *P≤0.05; DF=degrees of freedom; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; EPP=ears per plant; EA=ear aspect; ER=ear rot; TEX=texture; Env=environment; G x E=genotype by environment interaction.**

The genotype G111 also ranked fifth under well-watered conditions. It also exhibited good stand ability qualities as indicated by root and stem lodging percentages of less than 20% as well as good prolificacy (ears per plant mean) above the trial mean. Genotypes G131 ((DTP WC9-F104-5-6-1-1-B*4/K64r)-B13//CML444/CML395), G25 ((DTPWC9-F16-1-1-1-1-BBB/RS61P)-B- 23//CML539/CML442) and G209 ((DTPWC9-F104-5-4-1-1-BBB/N3233)-B-3//CML444/CML395) were within the ten best performing testcrosses both under combined analysis as well as under managed drought conditions. The poorest performing genotype under drought and well-watered conditions was G69 ((N3233/CML442)-B-12//CML444/CML395) with a mean yield of 1.84 t ha^{-1} and it was also amongst the poorest performing testcrosses under drought conditions.

Table 7.8 Performance of early maturing testcrosses for grain yield and other agronomic traits under optimum conditions

	Genotype	GYD	AD	PH	EH	EPO	EA	ER	TEX
Ten best testcrosses	G82	5.46	73.5	173.3	95.8	0.55	3.2	19.8	2.9
	G206	5.46	72.3	168.3	93.3	0.57	3.3	7.1	3.0
	G24	5.30	72.7	169.2	90.0	0.55	3.0	15.0	3.1
	G211	5.22	71.5	163.3	89.2	0.55	3.3	17.9	3.0
	G111	5.19	74.2	175.8	100.8	0.58	3.2	15.7	2.9
	G79	5.06	73	175.0	92.5	0.52	3.1	11.6	3.1
	G96	5.06	70.3	180.0	94.2	0.53	3.3	14.9	2.9
	G143	4.98	73.3	170.8	96.7	0.55	3.4	12.8	3.0
	G95	4.98	73	174.2	98.3	0.58	3.0	14.6	2.8
	G29	4.95	72.3	168.3	90.8	0.57	3.0	11.2	3.2
Ten poorest testcrosses	G179	2.67	74.5	170.0	90.0	0.53	3.9	13.9	3.3
	G50	2.67	72.7	152.5	73.3	0.48	3.7	19.4	3.6
	G64	2.60	75.2	170.0	97.5	0.57	3.8	21.2	3.3
	G176	2.59	72.3	175.0	101.7	0.58	4.2	20.3	3.5
	G38	2.59	71.3	156.7	80.0	0.53	3.7	16.5	3.3
	G154	2.59	75.3	163.3	90.8	0.57	3.9	24.1	3.6
	G167	2.44	73	161.7	93.3	0.58	3.2	11.2	3.0
	G56	2.35	73	158.3	85.8	0.57	3.8	19.1	3.5
	G62	2.28	72.3	169.2	93.3	0.57	3.6	17.6	3.5
	G58	2.20	72.7	175.0	95.0	0.55	4.0	24.6	3.4
	Grand Mean	3.85	72.8	169.3	93.3	0.55	3.4	17.0	3.2
	LSD	0.60	2.68	4.89	7.29	0.06	0.39	7.89	0.34
	CV%	13.42	3.87	3.04	8.21	11.42	11.82	48.8	11.14
	R^2	0.96	0.96	0.99	0.96	0.81	0.87	0.83	0.91
	LSD	0.57	2.68	4.89	7.29	0.06	0.39	7.89	0.34
	SED	0.35	1.63	2.97	4.42	0.037	0.23	4.79	0.20

GYD=grain yield (t ha^{-1}); AD=anthesis days; PH=plant height (cm); EH=ear height (cm); EPO=ear position (0-1); EA=ear aspect (1-5); ER=ear rot (%); TEX=texture (1-5); LSD =least significant difference; CV=coefficient of variation; R^2=coefficient of determination; LSD=least significant difference; SED=standard error deviation.

Table 7.9 Analysis of variance for grain yield across environments for early maturing testcrosses

Source	DF	MS
Site	1	48.17***
Genotype	229	1.15***
Treatment	1	3843.01***
G x E	229	1.30***
E x T	1	473.13***
G xT	229	1.16***
G x E xT	229	1.06***
Residual		0.06

*****P≤0.001; DF=degrees of freedom; MS=mean square; Env=environment; G x E=genotype by environment interaction; G x T= genotype by treatment interaction; G x E x T=genotype by environment by treatment interaction.**

Genotype G69 had ears per plant mean (0.50) that was below the trial mean (0.62). The majority of genotypes within the ten best performing genotypes had DTPWC9 donor as one of the parents.

7.3.3.1 Variance components for early maturing testcrosses

The mean grain yield under drought conditions for the early maturing testcrosses was 40% of the mean grain yield under optimum conditions (Figure 7.1). The general overview of the performance of the testcrosses under drought conditions is presented in Figure 7.2. The majority of testcrosses performed below the trial mean (1.53 t ha^{-1}). Plant height, ear height and ears per plant had repeatability values higher than 0.20. Genetic gain expected for grain yield was 10 g in grain yield per plant.

7.3.3.2 Correlation between grain yield and secondary traits for early maturing testcrosses under managed drought conditions

Grain yield was highly but negatively correlated (P≤0.01) with anthesis silking interval (Table 7.13). Correlation with anthesis days was negative but non-significant. Ears per plant were highly significant and positively correlated with grain yield. Anthesis days were significantly and negatively correlated (-0.159) with anthesis silking interval. Senescence was highly and negatively correlated with ears per plant.

Table 7.10 Analysis of variance for anthesis days and other agronomic traits under combined environments for early maturing testcrosses

Source	DF	AD	ASI	PH	EH	EPO	EPP	RL	SL	SEN	ER
Site	2	157649.36***	1423.83	234310.72***	45933.34***	0.12***	12.15***	48687.77***	96193.90***	7.47***	77730.76***
Genotype	229	12.47***	4743.49***	364.13***	438.01***	0.01***	0.02**	123.31***	153.41***	0.01***	378.27***
Treatment	1	14357.62***	8102.70	1370.66***	2700.33***	0.03***	83.82***	37945.15***	111929.83***	216.50***	85072.63***
G x E	458	12.77***	4754.42***	266.72***	255.66***	0.01***	0.02***	126.61***	158.67***	0.01***	338.66***
E xT	2	47615.05***	9633.95*	349490.77***	77896.06***	0.08***	4.54***	8094.54***	10112.51***	7.47***	28029.15***
G xT	229	7.21***	4771.21***	261.40***	217.76***	0.01***	0.03***	108.92***	125.19***	0.01***	368.58***
G x E x T	458	8.36***	4770.53***	236.53***	222.47***	0.01***	0.02***	111.41***	133.36***	0.01***	368.38***
Residual		5.26	2871.50	17.54	49.84	0.003	0.004	230.02	27.35	0.01	159.01

*****P≤0.001; **P≤0.01; *P≤0.05; DF=degrees of freedom; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; EPP=ears per plant; RL=root lodging; SL=stem lodging; SEN=senescence; ER=ear rot; Env=environment; G x E=genotype by environment interaction; G x T=genotype by treatment; G x E x T=genotype by environment by treatment.**

Table 7.11 Performance of early maturing testcrosses for grain yield and other agronomic traits under drought and optimum conditions in the 2010/11 season

	Genotype	GYD	AD	ASI	PH	EH	EPO	EPP	RL	SL	SEN	ER
Ten best testcrosses	G111	4.17	75.8	1.3	175.4	95.4	0.56	0.69	10.4	13.7	0.28	22.9
	G131	3.87	75.2	0.6	165.0	90.4	0.56	0.65	5.9	18.3	0.28	27.7
	G211	3.79	73.5	1.9	166.3	90.0	0.54	0.67	5.1	13.7	0.26	23.4
	G88	3.79	75.8	1.5	167.9	97.9	0.58	0.63	9.5	11.1	0.28	26.8
	G25	3.78	75.1	1.3	167.5	84.2	0.52	0.68	7.6	15.6	0.28	30.7
	G11	3.71	74.9	1.8	171.3	98.3	0.58	0.65	4.3	12.8	0.28	13.7
	G79	3.67	74.9	1.3	180.4	99.6	0.54	0.66	3.9	17.3	0.28	20.1
	G219	3.64	75.2	2.2	163.8	92.5	0.57	0.62	11.8	5.6	0.28	20.3
	G102	3.63	75.5	1.8	163.3	90.8	0.57	0.69	6.2	10.5	0.27	32.1
	G209	3.62	74.9	1.4	171.7	96.7	0.57	0.67	12.0	10.5	0.29	14.1
Ten poorest testcrosses	G58	2.32	75.6	2.4	178.8	100.4	0.56	0.51	5.9	8.5	0.28	20.9
	G164	2.31	74.5	1.9	170.0	90.8	0.54	0.63	8.2	12.3	0.26	17.9
	G104	2.31	75.8	2.0	169.2	91.7	0.55	0.6	18.1	9.1	0.26	20.3
	G65	2.28	75.5	1.9	176.7	105.0	0.61	0.61	9.1	5.0	0.28	23.3
	G213	2.28	75.8	1.3	169.6	95.4	0.58	0.61	6.5	17.3	0.29	27.8
	G228	2.08	82.4	-6.5	176.7	98.3	0.57	0.65	8.6	13.7	0.28	22.1
	G21	2.04	76.3	0.7	172.1	92.1	0.53	0.56	7.4	8.1	0.28	22.8
	G154	1.88	76.3	2.8	167.1	86.7	0.53	0.57	6.8	11.0	0.27	29.5
	G157	1.88	74.5	4.2	161.3	87.1	0.53	0.53	10.6	9.1	0.28	19.4
	G69	1.84	75.0	1.3	172.9	99.2	0.58	0.50	8.4	16.6	0.32	22.3
	Mean	2.98	75.1	0.58	170.0	94.3	0.56	0.62	8.28	11.4	0.28	22.5
	LSD	0.19	1.54	0.60	2.80	4.74	0.04	0.04	3.22	3.51	0.05	8.47
	CV%	8.01	3.05	9.10	2.46	7.48	9.38	10.75	57.98	45.84	29.00	55.91
	R^2	0.99	0.98	0.69	0.99	0.92	0.80	0.97	0.93	0.94	0.97	0.83

GYD=grain yield (t ha^{-1}); AD=Anthesis days; anthesis silking interval (days); PH=plant height (cm); Ear height (cm); EPO=ear position (0-1); EPP=ears per plant (#); RL=root lodging (%); SL=stem lodging (%); SEN=senescence (1-10); ER=ear rot (%); LSD=least significant difference; CV=coefficient of variation; R^2=coefficient of determination.

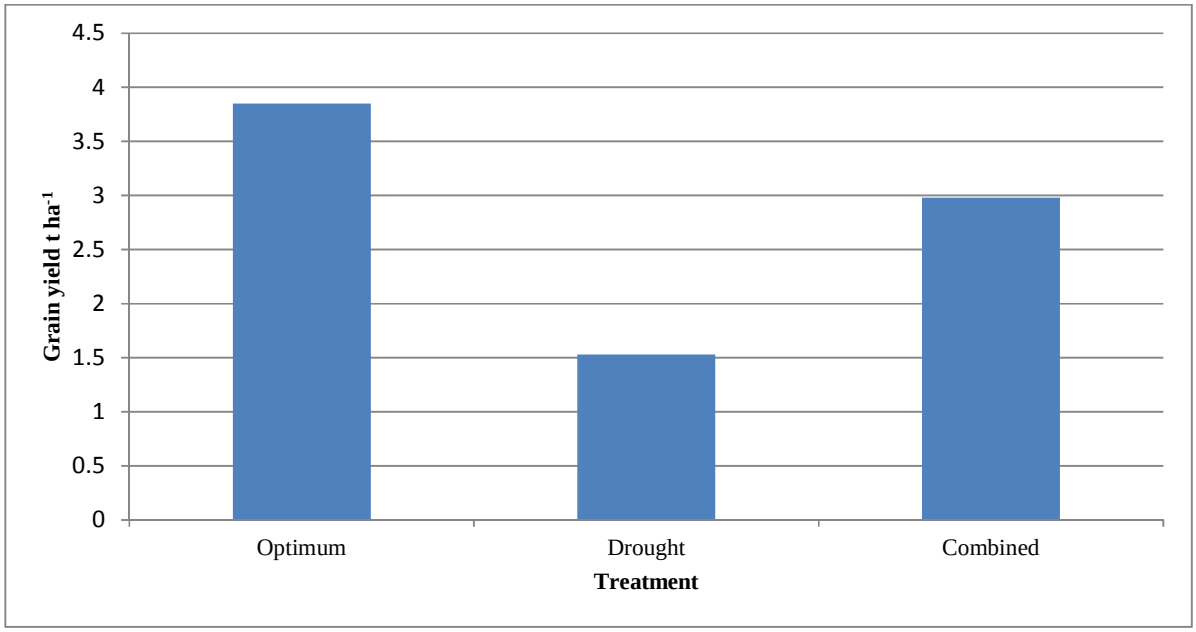

Figure 7.1 Mean grain yield for early maturing testcrosses under well-watered, drought and combined sites.

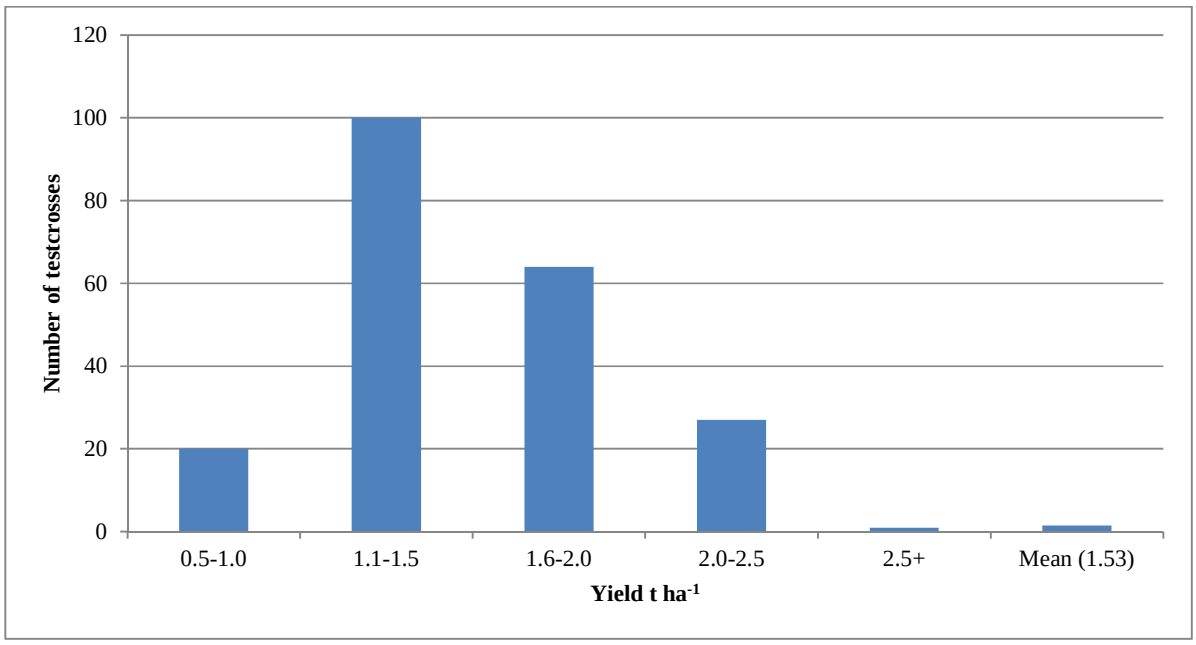

Figure 7.2 Mean grain yield for early maturing testcrosses under drought conditions across two sites in the 2011 winter season.

Table 7.12 Genetic and phenotypic variance, repeatability and genetic gain for early maturing testcrosses for the measured traits

Trait	σ^2_g	σ^2_p	b	Δg
GYD	0.03	0.21	0.14	0.01
AD	0.11	6.28	0.02	0.03
ASI	1.45	599.21	0.002	0.24
PH	9.07	44.27	0.20	1.77
EH	23.39	66.18	0.35	4.6
EPP	0.00125	0.004	0.31	0.0002

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH= ear height; EPP=ears per plant; σ^2_g= genetic variance; σ^2_p=phenotypic variance; b=repeatability; Δg=genetic gain.

Table 7.13 Correlation coefficients between grain yield and secondary traits under managed drought conditions

	GYD	AD	ASI	EPP
AD	-0.1			
ASI	-0.178**	-0.159*		
EPP	0.632**	-0.36	-0.086	
SEN	-0.38	-0.022	-0.143*	-0.177**

****P≤0.01; *P≤0.05; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; EPP=ears per plant; SEN=senescence.**

7.3.4 Performance of late maturing testcrosses under drought conditions

The ANOVA for grain yield and other agronomic traits for late maturing testcrosses are presented in Table 7.14. Environments were significantly different (P≤0.001) for grain yield, anthesis days, ear height, ear position, ears per plant, ear rot and ear aspect. Genotype and the G x E interaction mean squares were significant (P≤0.001) for all traits except anthesis days and anthesis silking interval. The late maturing testcrosses were mainly dominated by SC5522 derived lines. G107 (CZH0837), a CIMMYT experimental three-way hybrid used as a check variety, was the best performer in terms of grain yield under drought conditions with a mean yield of 3.21 t ha^{-1} (Table 7.15). The second best performing testcross was G29 ((DTPWC9-F104-5-4-1-1-BBB/SC5522)-B-2//CML539/CML442) with a mean yield of 3.05 t ha^{-1} and it performed 40.5% above the trial mean of 2.17 t ha^{-1}. The rest of the testcrosses within the ten best performing genotypes all performed above the trial mean.

Table 7.14 Analysis of variance for grain yield and other agronomic traits for late maturing testcrosses under drought conditions in the 2011 winter season

Source	DF	GYD	AD	ASI	EH	EPO	EPP	ER	EA
Site	2	180.48***	29188.95***	18.64	32277.30***	0.19***	1.89***	14050.50***	83.17***
Genotype	107	0.94***	128.43	123.24	506.50***	0.009***	0.04***	159.74***	0.44***
G x E	214	0.68***	119.42	122.59	180.63***	0.005***	0.024***	131.23***	0.47***
Residual	219	0.09	109.94	118.69	118.04	0.004	0.015	68.68	0.24

*****P≤0.001; DF=degrees of freedom; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; EH=ear height; EPO=ear position; EPP=ears per plant; ER=ear rot; EA=ear aspect; Env=environment; G x E=genotype by environment interaction.**

Table 7.15 Performance of late maturing testcrosses for grain yield and other agronomic traits under drought conditions

	Genotype	GYD	EH	EPO	EPP	ER	EA
Ten best testcrosses	G107	3.21	100	0.52	0.73	16.1	4.1
	G29	3.05	95.8	0.50	0.68	15.0	3.7
	G92	2.87	120.8	0.60	0.67	9.4	3.8
	G14	2.86	95.0	0.49	0.69	14.3	3.8
	G32	2.81	95.0	0.49	0.64	8.7	4.0
	G26	2.78	103.3	0.54	0.75	8.1	3.5
	G38	2.75	88.3	0.51	0.67	10.9	3.3
	G39	2.74	91.7	0.50	0.75	9.4	4.0
	G88	2.69	115.0	0.59	0.79	9.5	3.8
	G51	2.69	109.2	0.54	0.64	10.7	3.7
Ten poorest testcrosses	G105	1.62	95.0	0.51	0.64	15.9	3.8
	G48	1.60	101.7	0.54	0.53	19.5	4.3
	G50	1.58	85.8	0.49	0.49	15.7	4.2
	G99	1.56	104.2	0.55	0.58	12.1	3.8
	G95	1.54	106.7	0.56	0.52	10.1	3.2
	G66	1.50	113.3	0.60	0.52	9.3	4.3
	G11	1.34	100.0	0.53	0.47	5.7	4.2
	G103	1.28	105.8	0.56	0.33	29.2	3.3
	G56	1.21	80.8	0.44	0.56	27.9	4.6
	G85	1.16	115.0	0.61	0.51	10.7	4.2
	Mean	2.17	102.0	0.54	0.63	13.6	3.9
	LSD	0.29	10.36	0.05	0.12	7.90	0.47
	CV%	14.05	10.65	10.96	19.53	61.00	12.48
	R^2	0.97	0.88	0.79	0.85	0.86	0.88

GYD=grain yield (t ha^{-1}); EH=ear height (cm); EPO=ear position (0-1); EPP=ears per plant (#); ER=ear rot (%); EA=ear aspect (1-5); LSD=least significant difference; CV=coefficient of variation; R^2=coefficient of determination.

The poorest performing testcross was G85 ((DTPWC9-F115-1-2-1-2-BBB/NAW5885)-B-7//CML444/CML395) with a mean yield of 1.16 t ha^{-1} and the second poorest performing testcross was G56 ((MAS[206/312]-23-2-1-1-B*7/RA214P)-B-1//CML539/CML442) with a mean yield of 1.21 t ha^{-1}. Testcrosses generally showed low ear position values. The ear aspect scores for most testcrosses were above 3.5.

7.3.5 Performance of late maturing testcrosses under optimum conditions

The testcrosses were significantly different ($P \leq 0.001$) for all traits except for ear aspect (Tables 7.16 and 7.17). Environments were significantly different ($P \leq 0.001$) for all measured traits. The G x E interaction was significant for all traits except ear aspect. The best performing testcross in terms of grain yield under optimum conditions was G12 ((DTPWC9-F16-1-1-1-1-BBB/SC5522)-B-6//CML539/CML442) with a mean yield of 5.41 t ha^{-1} followed by G76 ((DTPWC9-F16-1-1-1-1-BBB/NAW5885)-B-9//CML444/CML395) with a mean yield of 5.38 t ha^{-1} (Table 7.18). The two testcrosses generally showed good values for anthesis silking interval, root and stem lodging and ear rot. The poorest performing genotype was G56 ((MAS[206/312]-23-2-1-1-B*7/RA214P)-B-1//CML539/CML442) with a mean yield of 1.88 t ha^{-1} and the same genotype was the second poorest performer under drought conditions. Testcrosses amongst the ten best performers had mean grain yields above the trial mean of 3.94 t ha^{-1}. Genotypes G102 (013WH03) and G103 (013WH01), DR&SS three-way experimental hybrids used as check varieties, were among the poorest performing genotypes and recorded high ear rot scores. The trial mean for grain texture was 2.2, indicating that genotypes were mainly semi-flint in texture. High R^2 values were recorded for all traits. Both genotypes and the environment played a critical role in the expression of plant and ear height as indicated by high genetic and phenotypic variances.

Table 7.16 Analysis of variance for grain yield for late maturing testcrosses under well-watered conditions in the 2011 winter season

Source	DF	MS
Site	1	14.58***
Genotype	107	2.04***
G x E	107	1.53***
Residual	146	0.38

*****P≤0.001; DF=degrees of freedom; MS=mean square; Env=environment; G x E=genotype by environment.**

7.3.6 Performance of late maturing testcrosses under combined environments

The combined ANOVA showed that testcrosses were significantly different (P≤0.001) for all traits (Tables 7.19 and 7.20). Sites were significantly different for all traits except anthesis silking interval. The G x E interaction was highly significant (P≤0.001) for all traits. The other interactions were also significant. The best performing genotype in terms of grain yield in the combined analysis was G26 ((DTPWC9-F16-1-1-1-1-BBB/RS61P)-B-32//CML539/CML442) with a mean yield of 4.24 t ha^{-1} (Table 7.21). The genotype performed above the trial mean which was 3.06 t ha^{-1}. The testcrosses G26 ((DTPWC9-F16-1-1-1-1-BBB/RS61P)-B-32//CML539/CML442) and G29 ((DTPWC9-F104-5-4-1-1-BBB/SC5522)-B-2//CML539/CML442) were among the ten best performers under drought and optimum conditions as well as under combined environment analysis. These testcrosses also exhibited good ear position and root and stem lodging scores. The poorest performing testcross was G56 ((MAS[206/312]-23-2-1-1-B*7/RA214P)-B-1//CML539/CML442) with a mean yield of 1.32 t ha^{-1} with a ears per plant value of less than 0.50 and anthesis silking interval of 4.5. The R^2 values were again very high for all measured traits.

Table 7.17 Analysis of variance for anthesis silking interval and other agronomic traits for late maturing testcrosses under well-watered conditions in the 2011 winter season

Source	DF	ASI	PH	EH	EPO	EPP	RL	SL	ER	EA	TEX
Site	2	11.96***	306516.29***	63852.28***	0.09***	41.40***	12977.14***	11332.01***	8172.72***	951.03***	778.19***
Genotype	107	3.94***	422.57***	439.58***	0.009***	0.016***	74.16***	71.62***	85.52***	4.23	0.18***
G x E	214	2.16***	229.11***	198.42***	0.005***	0.01**	59.53***	54.99***	53.00***	4.29	0.21***
Residual	219	1.18	87.79	53.76	0.003	0.008	33.67	11.88	32.09	4.02	0.05

*****P≤0.001; **P≤0.01; DF=degrees of freedom; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; EPP=ears per plant; RL=root lodging; SL=stem lodging; ER=ear rot; EA=ear aspect; TEX=texture; Env=environment; G x E=genotype by environment interaction.**

Table 7.18 Performance of late maturing testcrosses for grain yield and other agronomic traits under well-watered conditions

	Genotype	GYD	AD	ASI	PH	EH	EPO	EPP	RL	SL	ER	TEX
Ten best testcrosses	G12	5.41	70.0	1.2	176.7	96.7	0.56	0.5	4.3	10.5	8.7	2.3
	G76	5.38	70.5	0.5	178.3	103.3	0.58	0.57	1.1	3.6	3.9	2.0
	G26	5.38	70.0	1.2	175.0	92.5	0.54	0.62	3.1	4.1	5.1	2.0
	G29	5.22	70.0	1.2	175.0	82.5	0.47	0.56	9.4	6.8	8.8	2.2
	G92	5.14	71.0	1.5	187.5	123.3	0.65	0.54	10.0	4.2	7.2	2.3
	G21	5.14	71.0	1.2	187.5	96.7	0.51	0.56	5.5	6.4	4.7	2.0
	G22	5.06	71.0	1.3	186.7	102.5	0.56	0.51	1.9	2.9	4.9	2.1
	G19	5.03	71.5	0.3	160.0	95.8	0.66	0.53	0.0	4.9	5.4	2.3
	G75	4.90	69.0	1.8	168.3	106.7	0.63	0.55	3.3	10.6	6.2	2.3
	G38	4.89	69.0	1.3	171.7	92.5	0.54	0.51	4.1	8.4	4.9	2.0
Ten poorest testcrosses	G71	2.99	70.5	3.2	179.2	92.5	0.52	0.47	8.2	6.2	9.5	2.7
	G11	2.91	74.5	2.5	170.8	96.7	0.57	0.44	8.8	6.6	9.1	2.3
	G45	2.91	71.5	0.8	173.3	90.8	0.53	0.52	0.9	1.1	6.2	2.3
	G58	2.84	70.0	0.8	179.2	98.3	0.55	0.43	3.0	5.2	13	2.3
	G50	2.83	70.5	0.7	170.0	92.5	0.55	0.34	9.5	5.9	2.7	2.5
	G47	2.83	71.5	0.8	176.7	109.2	0.62	0.35	4.2	4.2	1.9	2.4
	G55	2.75	71.0	4.8	165.8	77.5	0.47	0.47	5.4	6.9	8.9	2.3
	G102	2.67	66.5	1.7	180.8	94.2	0.52	0.35	16.6	6.2	33.2	2.6
	G103	2.59	72.0	2.0	187.5	100.8	0.54	0.46	7.9	10.1	18.1	2.6
	G56	1.88	71.5	3.0	176.7	84.2	0.48	0.38	2.3	3.3	7.3	2.2
	Mean	**3.94**	**70.87**	**1.4**	**178.4**	**97.6**	**0.55**	**0.51**	**5.9**	**6.5**	**6.9**	**2.2**
	LSD	**0.72**	**1.46**	**1.03**	**8.93**	**6.99**	**0.05**	**0.08**	**5.53**	**3.29**	**5.4**	**0.21**
	Heritability	**0.25**	**0.13**	**0.45**	**0.46**	**0.55**	**0.38**	**0.32**	**0.19**	**0.23**	**0.38**	**0.17**
	CV%	**15.66**	**1.24**	**29.12**	**5.25**	**7.51**	**10.08**	**17.14**	**38.24**	**33.26**	**31.44**	**9.94**
	R^2	**0.92**	**0.94**	**0.82**	**0.98**	**0.96**	**0.81**	**0.98**	**0.89**	**0.95**	**0.87**	**0.99**

GYD=grain yield (t ha^{-1}); AD=anthesis days; ASI=anthesis silking interval (days); PH=plant height (cm); EH=ear height (cm); EPO=ear position (0-1); EPP= ears per plant (#); RL=root lodging (%); SL=stem lodging (%); ER=ear rot (%); TEX=texture (1-5); LSD=least significant difference; CV=coefficient of variation; R^2=coefficient of determination.

Table 7.19 Analysis of variance for grain yield for late testcrosses under both drought and well-watered conditions in the 2011 winter season

Source	DF	MS
Site	1	115.1***
Genotype	107	2.21***
Treatment	1	661.61***
G x E	107	1.25***
E x T	1	260.13***
G xT	107	0.89***
G x E x T	107	0.97***
Residual	431	0.13

*****P≤0.001; DF=degrees of freedom; MS=mean square; Env=environment; G x E=genotype by environment interaction; G x T=genotype by treatment interaction; G x E x T=genotype by environment by treatment.**

The mean grain yield for late maturing testcrosses under drought conditions was 55% of the mean yield under well-watered conditions (Figure 7.3). The testcrosses showed good performance under drought conditions with more than 50% of them performing above the trial mean (2.71 t ha^{-1}) (Figure 7.4).

7.3.7 Variance components for late maturing testcrosses

The phenotypic variances were higher than the genetic variances for all traits (Table 7.22). Grain yield, plant height, ear height and ears per plant showed good repeatability values. The expected genetic gains were higher for plant and ear height and lower for the other traits. Plant and ear height had repeatability estimates of above 0.50; however grain yield and ears per plant also had good repeatability values of 0.41 and 0.49 respectively. Expected genetic gain was higher for plant (11.3) and ear (15.25) height and for grain yield it was 30 g grain yield per plant, which converted to 3% genetic gain.

7.3.8 Correlation between grain yield and secondary traits under managed drought for late maturing testcrosses

Grain yield was significantly correlated with all secondary traits under managed drought conditions (Table 7.23). The correlation with anthesis days and anthesis silking interval was negative whilst the correlation with ears per plant was positive. Both anthesis days and anthesis silking interval were significantly but negatively correlated with ears per plant.

Table 7.20 Analysis of variance for anthesis days and other agronomic traits for late maturing testcrosses under drought and well-watered conditions in the 2011 winter season

Source	DF	AD	ASI	PH	EH	EPO	EPP	RL	SL
Site	2	44202.88***	0.45	264997.19***	88260.69***	0.01***	16.35***	25733.39***	86739.47***
Genotype	107	72.11***	63.00***	652.57***	795.96***	0.01***	0.04***	113.08***	250.90***
Treatment	1	990.50***	553.56***	32320.05***	6219.08***	0.02***	4.76***	677.88***	181594.35***
G x E	214	61.57***	61.96***	214.65***	208.87***	0.01***	0.02***	74.69***	174.91***
E x T	2	1532.06***	30.14	84746.58***	7868.89***	0.27**	26.95***	231.84***	40979.91***
G x T	107	65.06***	64.17***	197.54***	150.12***	0.01***	0.02***	59.55***	179.43***
G x E x T	214	59.98***	62.79***	213.75***	170.18***	0.01***	0.02***	57.91***	166.17***
Residual	646	37.26	40.64	50.67	58.24	0.002	0.01	19.96	19.38

*****P≤0.001; **P≤0.01; DF=degrees of freedom; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; EPP=ears per plant; RL=root lodging; SL=stem lodging; Env=environment; G x E=genotype by environment; G x T= genotype by treatment; G x E x T= genotype by environment by treatment.**

Table 7.21 Performance of late maturing testcrosses for grain yield and other agronomic traits under drought and well-watered conditions in the 2011 winter season

	Genotype	GYD	AD	ASI	PH	EH	EPO	EPP	RL	SL
Ten best testcrosses	G26	4.24	73.8	1.3	184.2	97.9	0.54	0.69	5.1	18.1
	G29	4.21	73.1	1.8	181.7	89.2	0.49	0.62	7.3	20.9
	G92	4.13	76.4	2.1	195.0	122.1	0.63	0.61	10.8	12.3
	G76	4.00	75.3	0.5	186.7	106.3	0.57	0.61	5.5	14.5
	G38	3.94	73.8	1.2	171.7	90.4	0.53	0.59	3.1	22.6
	G75	3.87	73.5	2.3	180.4	110.8	0.61	0.63	8.5	20.3
	G51	3.82	74.8	1.3	191.3	106.3	0.56	0.58	4.7	12.9
	G28	3.78	74.6	1.4	182.9	105.8	0.58	0.58	6.9	14.1
	G12	3.77	73.5	1.5	180.4	94.6	0.53	0.56	4.4	21.2
	G21	3.69	73.3	1.8	187.1	95.0	0.51	0.54	6.2	19.6
Ten poorest testcrosses	G48	2.36	74.9	4.5	185.8	102.1	0.55	0.52	3.8	17.0
	G47	2.34	74.2	1.7	180.8	108.3	0.60	0.46	5.6	23.6
	G55	2.31	73.3	2.6	168.8	84.6	0.50	0.56	4.4	13.1
	G57	2.25	74.6	1.6	172.9	84.2	0.49	0.51	4.2	20.9
	G85	2.21	76.0	2.4	183.8	112.5	0.61	0.51	7.8	22.4
	G58	2.10	74.4	2.0	183.8	93.8	0.51	0.42	4.1	22.8
	G11	2.05	75.9	3.3	180.4	98.3	0.55	0.46	9.5	15.1
	G50	2.00	73.8	1.8	170.4	89.2	0.52	0.41	10.8	18.9
	G103	1.93	74.9	3.8	187.1	103.3	0.55	0.39	11.3	19.7
	G56	1.32	74.9	4.5	179.2	82.5	0.46	0.47	1.7	21.5
	Mean	**3.06**	**74.1**	**2.0**	**183.4**	**99.8**	**0.55**	**0.56**	**6.6**	**18.3**
	LSD	**0.29**	**4.10**	**4.20**	**4.70**	**5.10**	**0.03**	**0.05**	**3.00**	**2.96**
	CV%	**11.53**	**8.24**	**31.4**	**3.88**	**7.64**	**8.66**	**15.42**	**37.39**	**24.04**
	R^2	**0.97**	**0.86**	**0.67**	**0.96**	**0.92**	**0.80**	**0.96**	**0.91**	**0.98**

GYD=grain yield (t ha^{-1}); AD=anthesis days; ASI=anthesis silking interval (days); PH=plant height (cm); EH=ear height (cm); EPO=ear position (0-1); EPP=ears per plant (#); RL=root lodging (%); SL=stem lodging (%); LSD=least significant difference; CV=coefficient of variation; R^2=coefficient of determination.

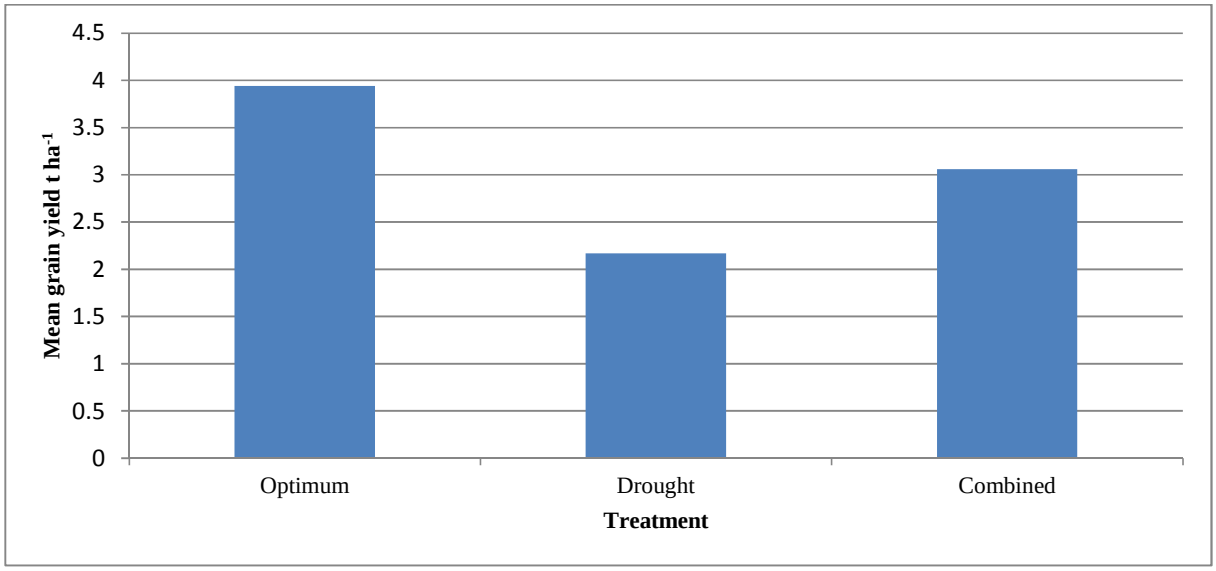

Figure 7.3 Mean grain yield for late maturing testcrosses under optimum, drought and combined environments in the 2011 winter season.

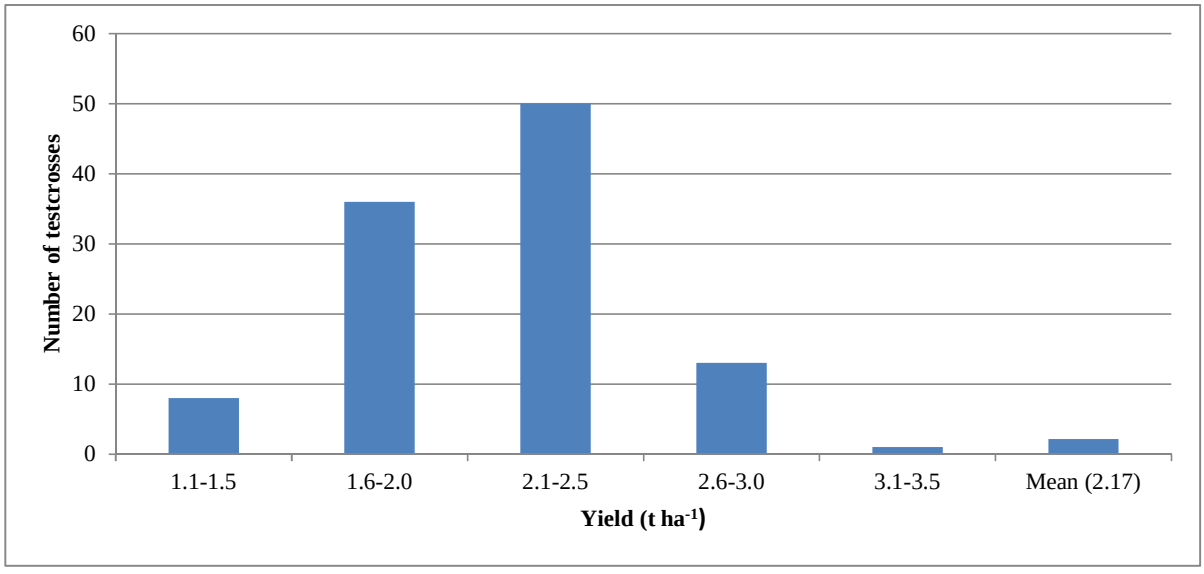

Figure 7.4 Mean grain yield of late maturing testcrosses under drought conditions across three environments in the 2011 winter season.

Table 7.22 Genetic and phenotypic variances, repeatability and genetic gain for grain yield and other agronomic traits measured in late maturing testcrosses

Trait	σ^2_g	σ^2_p	b	Δg
GYD	0.13	0.32	0.41	0.03
AD	0.68	9.01	0.08	0.14
ASI	0.04	8.48	0.0047	0.80
PH	56.77	85.63	0.66	11.30
EH	75.89	104.43	0.73	15.25
EPP	0.0025	0.0051	0.49	0. 10

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPP=ears per plant; σ^2g=genetic variance; σ^2p=phenotypic variance; b=repeatability; Δg=genetic gain.

Table 7.23 Correlation of grain yield and secondary traits for late maturing testcrosses under managed drought

	GYD	AD	ASI
AD	-0.274**		
ASI	-0.360**	0.254**	
EPP	0.616**	-0.234*	-0.341**

****P≤0.01; *P≤0.05; GYD =grain yield; AD=anthesis days; ASI=anthesis silking interval; EPP=ears per plant.**

7.4 Discussion

It is critical to testcross segregating lines in a breeding programme so that inferior lines can be discarded in early stages of breeding and this saves time and resources. Results indicated that environments, genotypes and G x E interaction effects were highly significant for the given growing conditions. Highly significant differences observed among testcrosses in this study were an indication of genetic variability available that can be exploited for further improvement. However, breeders need the information on the magnitude of genetic variation that exists for a given trait in a set of germplasm to justify selection for that trait (Nachit *et al.*, 1992). This can be estimated by partitioning of the total variation (total sum of squares) into its various components from the ANOVA and a higher magnitude implies a greater genetic potential for improvement for the trait. The mean grain yield reported in combined analysis for early maturing testcrosses was 2.98 t ha^{-1} and 3.06 t ha^{-1} for late maturing testcrosses. The yield reductions under drought conditions were 40% for early maturing

testcrosses and 55% for late maturing testcrosses and the stress could be classified as severe. The yield reduction in early maturing testcrosses is in agreement with results reported by Azeez *et al*. (2005). The reported yield reduction of 55% for late maturing testcrosses was within the value range reported by Campos *et al*. (2006) of 45-60% yield losses when drought occurred at silk emergence. However, Banziger *et al*. (2000) reported yield reductions of 15-20% under moderate drought stress. According to Azeez *et al*. (2005) yield reductions reported in the current study could be due to reduced ears per plant, reduction in individual kernel weight and reduced translocation of the photosynthates to the grain. Reduction in other yield determining traits significantly affected grain yield of the testcrosses such as high ear rot percentages and poor ear aspect scores though high maize yield under drought stress has been reported to be associated with a high number of fertile ears per plant (Bolanos and Edmeades, 1996; Bolanos *et al*., 1993).

The presence of significant G x E interactions for most traits recorded in this study is an indication that the testcrosses did not have consistent performance in different environments. Results are contrary to findings by Grauffret *et al*. (2000) and Menkir *et al*. (2007). Morphological, phenological and physiological traits of varieties contribute to G x E interactions (Nachit *et al*., 1992). The presence of significant G x E interaction under drought and non-drought environments underlines the importance of conducting multi-location trials in representative environments to identify yield stable hybrids under both conditions. The variation in performance of genotypes from environment to environment, especially changes in rank, hinder identification of superior stable hybrids (Hyrkas and Carena, 2005). In any plant breeding programme selection of genotypes without crossover G x E interaction and identification of sites with similar or different characteristics is of principal importance (Setimela *et al*., 2007). Significant G x E interaction under drought stress impedes breeding progress (Ribaut *et al*., 2009). It therefore appears that G x E effects would present challenges in breeding for drought tolerance in the given germplasm.

Testcrosses constituted from inbred lines K64r, RS61P and N3.2.3.3 with DTPWC9 based donors generally showed superior performance over other testcrosses. The testcross (DTPWC9-F104-5-4-1-1-BBB/K64r)-B-33//CML444/CML395 was the overall best

performing testcross in the early maturing category whilst (DTPWC9-F16-1-1-1-1-BBB/RS61P)-B-32//CML539/CML442 was the overall best performer in the late maturing category. The testcrosses ranked the best mainly because of the good performance of the inbred lines K64r and RS61P reported earlier in the study. The best testcrosses were generally consistent in their performance as they were also among the best performing genotypes under drought and optimum conditions. Of the nine drought tolerant donors used, the DTPWC9 based donors showed superior performance, especially against G16BNSeqC4 and LaPostaSeqC7 based donors. The identified superior lines can therefore be selected for further generation advancement for the development of homozygous lines. Two main goals of any maize population improvement programme include improving the mean of a quantitative trait by concentrating favourable alleles and maintaining genetic variability (Hyrkas and Carena, 2005). The superior individual lines identified after crossing with a tester can be inbred lines for potential use as parents of synthetic or hybrid cultivars (Fehr, 1987).

Correlation coefficient analysis has been used in selection of secondary traits affecting yield (Menkir, 2008). Grain yield was significantly correlated with the secondary traits anthesis days, anthesis silking interval and ears per plant in this study. Ears per plant showed good heritability estimates compared to anthesis days and anthesis silking interval that showed very low heritability estimates. Indirect selection for secondary traits correlated with grain yield rather than grain yield alone has been shown to increase selection efficiency by about 20% in maize grown under low soil nitrogen stress (Banziger and Lafitte, 1997). Results are consistent with findings by other researchers (Bolanos and Edmeades, 1996; Magorokosho *et al*., 2003; Zaidi *et al*., 2004; Monneveux and Ribaut, 2006; Ribaut *et al*., 2009). Therefore the exploitation of highly heritable components, which are highly correlated to grain yield, is therefore a more effective option than direct selection of yield *per se* (Kashiani and Saleh, 2010). In this study anthesis days was negatively correlated to grain yield for both early and late maturing testcrosses and similar results were reported by Magorokosho *et al*. (2003). This may have been due to high temperatures experienced during the growing season at the Lowveld research stations, which subjected testcrosses to drought stress during grain filling.

The genetic variances and repeatability estimates in this study were generally low. The genetic variance for grain yield recorded for early maturing testcrosses was 0.03 and for late maturing testcrosses was 0.13 and the repeatability estimates were 0.14 and 0.41 respectively. Results are in agreement with findings by other researchers that genetic variance and repeatability of grain yield often decline with increasing moisture stress (Bolanos and Edmeades, 1996; Magorokosho *et al*., 2003). Correlation of ears per plant with grain yield under drought stress was high, but because of the small variances, selection for this trait may not be effective. Similar results were reported by Magorokosho *et al*. (2003); however, these findings are contrary to findings by Edmeades *et al*. (1995) and Bolanos and Edmeades (1996). Plant and ear height had higher genetic variances and consequently higher repeatability estimates in this study. The genetic gains for these traits were also higher compared to other traits. Falconer and Mackay (1996) stated that genetic gain of selection for given traits depend on the heritability estimates. Traits with high repeatability can be selected on individual plant basis (El-Badawy, 2011), whilst on the other hand single plant selection would be inefficient for low repeatability traits and a type of family selection would be required. Good repeatability values were recorded for grain yield, plant height, ear height and ears per plant for the late maturing testcrosses in the study. Large heritability for late hybrids support direct selection for yield whilst the reverse is true for early testcrosses. El-Badawy (2011) postulated that high repeatability values indicate the possibility of predicting the real individual value with a relatively small number of measurements. Therefore the knowledge of the repeatability coefficient allows an efficient use of resources and time in the evaluation phase.

7.5 Conclusions

Testcross performance evaluation is critical in that it enables the breeder to screen and select superior lines even at the early stages of recurrent selection. Significant differences amongst the genotypes in both sets of trials for the majority of traits are an indication that there is existence of variability that can be exploited during selection. However, existence of highly significant G x E interaction is evidence that the performance of testcrosses was not consistent across the production environments therefore complicating the selection process. Generally the best performing testcrosses were identified to be mostly constituted of DR&SS

inbred lines K64r, RS61P and N3.2.3.3 with the CIMMYT drought tolerant donors based on DTPWC9. The best performing testcrosses included testcrosses from both the CIMMYT group A (CML539/CML442) and group B (CML444/CML395) testers. The information can now be used in selecting segregating lines for further generation advancement. Highly significant correlation of grain yield with important secondary traits such as anthesis days and anthesis silking interval under drought conditions was recorded in the study. This therefore means that these traits can be exploited in the selection process instead of just directly selecting for yield *per se*. High and positive correlations of grain yield with ears per plant under drought conditions were recorded for both sets of trials, however because of small genetic variances, selecting for the trait may not be effective. On the contrary, ears per plant showed good repeatability, which means that if the trait is selected for there are higher chances that similar results may be realised again. The genetic variances and repeatability estimates for grain yield were low compared to traits such as plant and ear height, which implies that a slow genetic progress will be realised in terms of selecting for the trait. Repeatability for grain yield for the late maturing testcrosses under drought conditions was good, which therefore means that there are higher chances that the performance will be consistent over years compared to the early maturing testcrosses and the finding supports direct selection.

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CHAPTER 8

Performance and yield prediction of three-way hybrids from drought tolerant single cross hybrids

Abstract

Drought is one of the most limiting factors in maize production in Zimbabwe and the use of poorly adapted varieties has been of great concern. The objectives of this study were to evaluate the agronomic performance of three-way hybrids predicted from drought tolerant single cross hybrids and to investigate the correlation between the predicted and observed means. Eleven single cross hybrids were crossed to different males using a North Carolina Design I. A total of 77 three-way hybrids were successfully produced. Hybrids were then evaluated across three sites under optimum and managed drought conditions in the 2011 winter season. Results revealed varied performance of hybrids for grain yield and other yield related traits. Grain yield was significantly ($P \leq 0.01$) and negatively correlated with anthesis days ($r=-40$) under drought conditions and there was a tendency that the earlier maturing hybrids produced higher yields compared to the later maturing hybrids. The correlation between grain yield and ears per plant was significant ($P \leq 0.01$) and higher under drought conditions ($r=0.76$) compared to optimum conditions. The study further confirmed the utility of anthesis silking interval as indirect selection criteria for grain yield under drought conditions. However, the utility of ears per plant was not conclusive because of low genetic variance and negligible broad sense heritability estimates. A significant ($P \leq 0.05$) but weak correlation ($r=0.27$) was obtained between the predicted and the observed grain yield means and this could be explained by the epistatic and significant G x E interaction effects, which were not taken into account in the prediction equation used. Results show that the three-way hybrids with superior predicted yields can be evaluated in multi-location trials and superior hybrids identified and released for commercial use. Three hybrids, RA214P/CML538//RS61P, RS61P/CML444//CML538 and RS61P/CML444//CML539 were identified as having superior performance. However, there is still need for further evaluation of hybrids in multi-location advanced variety trials before considering them for release. There is also a need to evaluate the hybrids under low nitrogen conditions as this was not done in the current study.

8.1 Introduction

Maize ranks first in terms of the number of hectares grown and total cereal production in Zimbabwe. It is the staple food and an important cash crop. Zimbabwe maize breeding has been a success story over the years with hybrids being developed for both small scale and commercial production. The maize breeding programme evolved through four phases, namely, OPV, double cross hybrid, single cross hybrid, and the three-way hybrid development phases. The three-way hybrid phase started in the late 1970s and the first three-way hybrids, R201 and R215, were registered in 1988. These two hybrids became very popular with farmers but their weakness was that they were not bred for drought tolerance as a result they would succumb to drought.

The main maize production constraint in the country has been the use of poorly adapted varieties since most of the previous maize breeding work was focusing on high input environments. According to CIMMYT-Zimbabwe (2000) improved yields, variety yield stability, pest and disease resistance, tolerance to drought and low soil fertility, generally produce yield improvements of 30-50%. Breeding for abiotic stress tolerance (drought and low N) is now being incorporated into the inbred line and variety development process. The expected genetic variance and predicted yield potential decline from single, three-way, double to top crosses (Cockerham, 1961). Performance evaluation trials performed by Weatherspoon (1970) showed that single crosses showed higher grain yields followed by three-way crosses, while the double crosses showed lower grain yields. Single crosses result in maximum hybrid vigour, but low seed yields of inbred lines make the cost of single cross hybrid seed prohibitive. Single crosses are more sensitive or responsive to environmental conditions, whilst stable high average yield is important for producer consistency in performance across years and locations. According to Allard and Bradshaw (1964) the uniformity of single crosses causes a lack of population buffering as they possess only individual buffering, whilst three-way crosses have both population and individual buffering.

Maize breeders have continued to develop a large number of inbred lines to facilitate efficient hybrid variety development and in recent years this has been expedited by doubled haploid technology (Seitz, 2005). Predicting the performance of hybrids from *per se*

performance of their parental inbred lines has not been effective due to masking dominance effects (Smith, 1986; Hallauer, 1990). With many inbred lines it is often impractical to test and compare all possible three-way cross hybrids. In commercial maize breeding programmes, identification of single cross hybrids with superior yield performance is of fundamental importance. Many methods of prediction have been proposed and some are currently in use (Eberhart, 1964; Eberhart and Gardner, 1966; Hinkelmann, 1968).

Predicting the performance of all hybrid combinations between a number of inbred lines is a practical problem because the number of these combinations usually exceeds the practical limits of field evaluation. Questions arise, however, as to the best methods of prediction and the accuracy of predictions. In various studies Jenkins' method B (Jenkins, 1934), which employs the mean of the non-parental single crosses, proved more suitable and is consequently used in hybrid maize breeding for prediction of three-way and double crosses (Melchinger *et al*., 1987). The single crosses used for prediction are anticipated to have originated from a comprehensive factorial mating design. The number of single crosses is considerably fewer and it is logical that they can be used to estimate or predict the performance of three-way cross hybrids. It is common to predict performance of three-way hybrids from single cross test results (Melchinger *et al.*, 1987). However, results obtained using the different methods did not always agree sufficiently with the observed values. In an earlier study (Chapter 3) superior single cross hybrids developed using a NCDII were identified and they are the ones used for predicting three-way hybrid performance in the current study. Therefore the objectives of this study were to evaluate the agronomic performance of three-way hybrids predicted from superior drought tolerant single cross hybrids and to determine the relationship between the observed and estimated means.

8.2 Materials and methods

8.2.1 Germplasm

In the previous study (Chapter 3) 11 single cross hybrids were identified in terms of good SCA and 12 inbred lines were identified in terms of good GCA effects (Table 8.1). The

three-way hybrids were then constituted in the field using North Carolina Design I. A total of 77 three-way hybrids were successfully produced.

Table 8.1 Germplasm used in constituting the three-way hybrids

Single cross hybrids	Code	Inbred lines	Code
K64r/CML444	SC1	NAW5885	L1
K64r/CML537	SC2	CML442	L2
K64r/CML539	SC3	RA214P	L3
N3.2.3.3/CML545	SC4	N3233	L4
N3.2.3.3/CML444	SC5	CML539	L5
NAW5885/CML442	SC6	CML538	L6
NAW5885/CZL03007	SC7	CML548	L7
RA214P/CML538	SC8	CML545	L8
RA214P/CML548	SC9	CML444	L9
RS61P/CML444	SC10	RS61P	L10
RS61P/CZL03007	SC11	CZL03007	L11
		K64r	L12

8.2.2 Evaluation sites

The three-way hybrids were evaluated under well-watered and managed drought conditions across three sites namely Chiredzi, Chisumbanje and Save Valley Research Stations. The sites are as described in section 7.2.2.

8.2.3 Trial management

A 0.1 alpha lattice design was used in planting the trials. Trials were planted in 4.0 m one row plots with an inter-row spacing of 0.75 m and in-row spacing of 0.25 m. Two seeds were planted per station and later thinned to one plant per station to achieve a plant population of 53 000 plants ha^{-1}. Compound D basal dressing of 400 kg ha^{-1} and ammonium nitrate topdressing of 350 kg ha^{-1} were applied to the crop. The topdressing was split applied at four and eight weeks after crop emergence. Dipterex 2.5% granules were applied for stalkborer control. Weed control was mainly done through hand weeding.

8.2.4 Management of drought site

The crop was raised offseason (winter) through the use of irrigation. Irrigation was applied in such a way that drought at flowering was severe enough to delay silking and cause ear abortion. Ideally anthesis silking interval should average about four to eight days and ears per plant 0.3 to 0.7, whilst grain yield should be around 1-2 t ha^{-1} (15-20% of well watered yields) for a drought managed site.

8.2.5 Data collection

Data was collected for grain yield (t ha^{-1}), days to 50% anthesis, days to 50% silking, plant and ear height (cm), root and stem lodging, ear rot, senescence, ear aspect and texture. Derived traits such as ear position, ears per plant and anthesis silking interval were calculated. Traits were measured and derived as described in section 3.2.4 (Table 3.2).

8.2.6 Statistical analysis

The data from individual sites was subjected to ANOVA and later the analysis was combined over locations using AGROBASE (2005). Broad-sense heritability for traits was estimated using the ANOVA components of variance procedure as suggested by Becker (1984) and as described in sections 3.2.5 and 5.2.6.

The three-way hybrid prediction from the single crosses was done according to Fehr (1993).

$TWC_{12.3} = ½ [SC_{13} + SC_{23})$

Where: $TWC_{12.3}$ = predicted three-way cross mean

SC_{13} = single cross mean between inbred line 1 and inbred line 3

SC_{23} = single cross mean between inbred line 2 and inbred line 3

Pearson correlation coefficients (r) amongst the different traits were calculated from means across environments. The statistical computations were performed with SPSS 15.0 version for Windows (2006).

8.3 Results

8.3.1 Performance of hybrids under managed drought conditions

Results presented are for the 10 best and 10 poorest hybrids in terms of grain yield and the means presented in the tables are for all entries. Results for all entries under optimum, drought and combined environments are presented in Appendices 13-15. Sites were significantly different (P≤0.001) for anthesis days, anthesis silking interval, plant and ear height, root and stem lodging and senescence (Table 8.2).

Table 8.2 Analysis of variance for grain yield and other agronomic traits under managed drought conditions in the 2011 winter season

Source	DF	GYD	AD	ASI	PH	EH	EPP	RL	SL	SEN
Site	2	6.32**	48134.59***	170.096***	27497.19***	14735.64***	0.166*	6438.63***	6879.46***	5.211***
Genotypes	84	1.21***	18.89***	17.82***	480.80**	482.96***	0.051ns	156.70***	390.25***	0.010***
G x E	168	4.68*	2.84***	9.078ns	244.58**	142.38***	0.044ns	96.93*	189.65***	0.006***
Residual	156	0.182	0.085	7.133	115.34	39.62	0.041	75.25	58.92	0.001

*****P≤0.001; **P≤0.01; *P≤0.05; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPP=ears per plant; RL=root lodging; SL=stem lodging; SEN=senescence; Env=environment; G x E=genotype x environment interaction; DF=degrees of freedom.**

Genotypes were significantly different for all traits except ears per plant. The G x E interaction was significant for grain yield and root lodging (P≤0.05), plant height (P≤0.01) and for anthesis days, ear height, stem lodging and senescence (P≤0.001). The minimum grain yield obtained under managed drought conditions was 0.68 t ha^{-1} from genotype 013WH63 and the maximum yield was 2.89 t ha^{-1} from two genotypes SC7//L5 and SC8//L10 (Table 8.3). The ten best performing hybrids had mean yields above the trial mean. Hybrids generally showed good anthesis silking interval with a trial mean of 2.6 days and this is desirable under drought conditions. The root and stem lodging trial means were also indicative of good stand ability of hybrids. The number of ears per plant was influenced by a high level of barrenness amongst hybrids with a trial mean of 0.59. The poorest performing hybrids generally had ear rot means that were above the trial mean and hence they had poor ear aspect scores. The Pearson's coefficient of correlation showed that there was significant (P≤0.01) correlation between grain yield and other traits such as anthesis days, anthesis silking interval, plant and ear height, ear position and ears per plant under managed drought conditions (Table 8.4).

Table 8.3 Mean performance for grain yield and other agronomic traits under managed drought in the 2011 winter season

	Entry	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER	SEN	TEX	EA
Ten Best hybrids	SC7//L5	2.89	63.3	2.8	178.3	78.0	0.43	10.6	19.8	0.76	5.4	0.5	1.5	2.4
	SC8//L10	2.89	63.9	1.2	174.7	101.0	0.53	11.1	5.1	0.65	16.0	0.5	2.7	3.3
	SC3//L2	2.88	66.4	1.6	167.7	82.7	0.45	15.8	22.0	0.65	30.8	0.6	3.5	3.6
	SC2//L5	2.87	64.9	1.5	170.5	77.4	0.46	11.7	13.1	0.75	6.3	0.6	1.0	2.7
	SC10//L6	2.86	66.4	1.0	177.1	88.9	0.52	-1.5	12.1	0.69	16.3	0.5	2.7	3.0
	SC3//L1	2.63	65.2	5.4	176.3	81.3	0.41	8.0	12.2	0.56	21.9	0.5	3.0	3.0
	SC2//L9	2.61	67.9	1.6	180.3	96.7	0.51	11.7	10.8	0.69	26.0	0.6	3.0	3.8
	SC10//L5	2.59	63.7	0.8	178.4	90.4	0.47	10.4	29.4	0.76	4.9	0.6	3.0	3.2
	CZH0616	2.57	64.7	4.0	174.8	85.8	0.50	6.3	9.9	0.69	11.4	0.5	3.0	2.9
	023WH31	2.54	69.4	1.8	185.9	100.9	0.55	11.8	11.4	0.67	23.3	0.5	2.8	3.2
Ten Poorest hybrids	SC4//L9	1.27	67.6	3.0	165.9	108.2	0.60	1.7	8.6	0.42	40.6	0.5	2.9	4.2
	SC7//L3	1.26	65.9	4.7	184.5	95.1	0.51	8.5	1.6	0.42	18.4	0.5	2.9	3.7
	SC8//L11	1.22	67.6	3.6	179.6	102.3	0.54	6.4	14.6	0.36	31.1	0.6	2.7	3.5
	SC6//L5	1.19	68.2	2.9	181.4	102.5	0.55	7.9	12.3	0.49	21.5	0.5	3.3	4.2
	SC7//L4	1.18	65.7	5.9	195.1	103.0	0.52	9.8	5.6	0.39	34.1	0.5	2.5	3.5
	SC5//L2	1.15	69.6	6.3	187.5	98.1	0.51	8.7	9.0	0.41	27.0	0.5	4.1	4.1
	SC5//L11	0.83	68.9	2.6	168.5	117.7	0.65	0.2	7.3	0.37	26.8	0.6	3.3	4.1
	SC10//L4	0.83	68.1	7.4	190.5	107.3	0.56	6.8	11.9	0.26	48.0	0.5	3.4	4.2
	SC11//L4	0.80	66.1	3.4	178.6	102.2	0.51	22.3	16.8	0.51	22.9	0.5	3.0	3.3
	013WH63	0.68	66.9	5.1	190.2	97.0	0.53	17.2	6.7	0.40	40.6	0.6	3.9	4.3
	Mean	**1.95**	**66.0**	**2.6**	**175.5**	**94.5**	**0.52**	**13.1**	**13.7**	**0.59**	**26.6**	**0.6**	**3.0**	**3.5**
	LSD (0.05)	**1.03**	**2.0**	**2.4**	**17.8**	**13.1**	**0.10**	**20.3**	**13.0**	**0.16**	**46.7**	**0.1**	**0.9**	**0.9**
	MSE	**0.53**	**2.1**	**4.4**	**80.5**	**130.6**	**0.00**	**104.5**	**128.9**	**0.02**	**1651.1**	**0.0**	**0.2**	**0.4**
	Min	**0.68**	**61.8**	**-1.1**	**133.1**	**66.5**	**0.37**	**-2.3**	**-1.1**	**0.26**	**2.3**	**0.5**	**1.0**	**2.4**
	Max	**2.89**	**70.6**	**7.4**	**198.5**	**117.7**	**0.70**	**50.7**	**33.6**	**0.83**	**180.9**	**0.7**	**4.1**	**4.6**

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; RL=root lodging; SL=stem lodging; EPP=ears per plant; ER=ear rot; SEN=senescence; TEX=texture; EA=ear aspect; LSD=least significant difference; MSE=Mean square error; Min=minimum; Max=maximum.

Table 8.4 Pearson's coefficient of correlation between grain yield and other agronomic traits under managed drought conditions

	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER
AD	-0.40**									
ASI	-0.44**	0.30**								
PH	-0.288**	0.25*	0.32**							
EH	-0.45**	0.43**	0.28*	0.27*						
EPO	-0.33**	0.36**	0.19	-0.10	0.78**					
RL	0.10	-0.40**	-0.18	0.05	-0.05	-0.11				
SL	0.17	-0.27*	-0.32**	-0.42**	-0.40**	-0.35**	0.05			
EPP	0.76**	-0.60**	-0.65**	-0.36**	-0.52**	-0.38**	0.24*	0.31**		
ER	-0.18	-0.13	0.03	0.02	0.10	0.13	-0.05	-0.02	-0.30**	
SEN	-0.03	-0.24*	-0.37**	-0.17	-0.08	-0.01	0.27*	0.35**	0.11	0.16

****P≤0.01; *P≤0.05; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; RL=root lodging; SL=stem lodging; EPP=ears per plant; ER=ear rot; SEN=senescence.**

The correlation was, however, negative with all these traits except ears per plant. A highly significant positive (0.76) correlation of grain yield with ears per plant was realised. Ear position was significantly and positively correlated (0.78) with ear height. There was negative and significant correlation of ears per plant with ear rot. The genotypic variances were lower than the phenotypic variances under managed drought conditions and hence the broad sense heritability estimates were also low. Grain yield, stem lodging, ear height and anthesis days had broad sense heritability estimates were above 0.50 (Table 8.5). Ears per plant had low variances compared to all the other traits and the broad sense heritability estimate was negligible.

Table 8.5 Genotypic and phenotypic variances and broad sense heritability estimates for measured traits under managed drought conditions

Trait	σ^2_g	σ^2_p	h^2_B
AD	0.1573	0.1852	0.85
ASI	0.0857	0.175	0.49
PH	2.3159	4.714	0.49
EH	3.339	4.7349	0.71
EPP	0.0001	0.0005	0.14
RL	0.5859	1.5362	0.38
SL	1.9667	3.826	0.51
SEN	0.000041	0.0001	0.41
GYD	0.234	0.456	0.51

σ^2_g=genetic variance; σ^2_p=phenotypic variance; h^2_B=broad sense heritability; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPP=ears per plant; RL=root lodging; SL=stem lodging; SEN=senescence; GYD=grain yield.

8.3.2 Performance of three-way hybrids under optimum conditions

Environments were again significantly different for all traits ($P \leq 0.001$ and $P \leq 0.01$) under optimum conditions (Table 8.6). Genotypes were significantly different ($P \leq 0.001$) for grain yield, anthesis silking interval, plant and ear height, ear position, ear aspect, ear rot, root and stem lodging and texture and significantly at $P \leq 0.01$ for anthesis days. The G x E interaction was also significant for all traits. The best performing genotype was SC9//L10 with a mean yield of 4.79 t ha^{-1} whilst the poorest performing hybrid was 013WH01 with a mean yield of 1.95 t ha^{-1} (Table 8.7).

The overall trial mean was 3.51 t ha^{-1} and all ten best performing genotypes performed above the mean. Genotypes generally outperformed the check varieties. The ear position values showed that genotypes had good ear placement and the mean was 0.55. The stand ability properties of genotypes were good with root and stem lodging means of 5.4 and 16.6 respectively. Prolificacy (ears per plant) also appeared to be good with a trial mean of 0.78 with SC9//L9 having a mean of 1.25 ears per plant. The maturity of hybrids was more inclined toward medium maturity with a trial mean of 71.8 anthesis days. The Pearson correlation coefficient among traits showed a positive and significant correlation between grain yield and ear height, ear position and ears per plant (Table 8.8).

Table 8.6 ANOVA for grain yield and other agronomic traits under optimum conditions in the 2011 winter season

Source	DF	GYD	AD	ASI	PH	EH	EPO	EPP	EA	ER	RL	SL	TEX
Site	2	108.59***	303.22**	117.531***	315379.460***	350.66**	0.141***	0.291***	24.095***	286.024***	18209.290***	19156.42***	40.687***
Genotypes	84	164.52***	50.56**	30.902***	500.838***	363.80***	0.009***	0.039**	0.527***	147.860***	185.640***	121.95***	0.895***
G x E	168	16.56**	21.78***	26.264***	171.971***	140.59**	0.004***	0.036**	0.356***	81.910***	162.603***	120.73***	0.576***
Residual	156	0.035	10.65	15.702	40.375	2.70	0.001	0.025	0.131	24.980	24.462	56.86	0.150

*****P≤0.001; **P≤0.01; DF=degrees of freedom; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; EPP=ears per plant; EA=ear aspect; ER=ear rot; RL=root lodging; SL=stem lodging; TEX=texture; Env=environment; G x E=genotype x environment interaction.**

Table 8.7 Mean performance of three-way hybrids for grain yield and other agronomic traits under optimum conditions in the 2011 winter season

	Entry	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER	TEX	EA
Ten best hybrids	SC9//L10	4.79	69.9	2.1	162.9	90.9	0.57	34.6	17.8	0.66	15.4	3.6	4.1
	SC10//L5	4.65	71.2	2.9	174.0	90.7	0.54	5.2	16.5	0.94	7.8	3.1	3.7
	SC4//L10	4.64	70.8	1.4	171.2	103.1	0.60	12.0	9.6	0.75	8.6	3.7	3.9
	SC6//L10	4.64	71.2	2.0	176.5	98.3	0.56	30.4	7.6	0.83	8.6	3.8	3.8
	SC8///L10	4.63	71.6	1.8	179.6	98.5	0.55	2.2	6.9	0.79	11.0	3.6	4.1
	SC3//L8	4.63	69.7	4.0	157.6	82.2	0.52	0.9	20.2	0.84	2.8	3.3	3.8
	SC11//L5	4.60	69.8	4.0	156.8	89.7	0.58	2.5	22.3	0.91	3.3	2.7	3.5
	SC6//L5	4.58	73.6	0.9	187.0	104.2	0.56	9.7	9.8	0.69	12.1	3.7	3.3
	SC9//L9	4.52	75.1	0.7	177.7	99.6	0.57	2.3	43.4	1.25	6.5	4.8	2.6
	SC1//L6	4.50	71.6	2.1	172.6	89.1	0.53	9.4	11.2	0.79	3.2	3.3	3.6
Ten poorest hybrids	SC9//L12	2.61	72.0	1.0	165.2	80.5	0.49	14.0	16.3	0.79	25.2	3.8	4.4
	013WH63	2.56	72.4	-0.2	171.0	93.0	0.54	24.2	18.9	0.60	5.8	3.8	4.6
	SC3//L6	2.54	70.8	3.3	164.3	83.3	0.52	2.2	12.3	0.77	8.4	3.8	4.0
	SC3//L7	2.43	70.2	3.3	149.5	69.3	0.48	1.1	25.9	0.81	2.9	3.2	3.9
	SC2//L3	2.37	71.6	1.8	174.2	83.6	0.49	0.2	21.9	0.85	18.6	3.9	4.0
	SC7//L4	2.36	73.2	0.3	185.2	104.5	0.58	38.7	20.3	0.65	9.7	4.0	4.2
	SC3//L1	2.24	69.7	7.0	171.0	88.0	0.52	3.7	20.4	0.84	21.6	3.9	4.6
	SC1//L5	2.20	71.3	3.8	158.3	81.3	0.52	5.6	19.4	0.90	10.2	3.0	3.3
	SC1//L4	1.99	72.7	0.0	178.1	100.3	0.57	26.4	16.6	0.66	18.3	4.2	4.6
	013WH01	1.95	74.7	0.5	183.8	106.6	0.59	31.1	10.6	0.65	7.6	3.6	4.1
	Mean	**3.51**	**71.8**	**2.4**	**172.6**	**93.0**	**0.55**	**5.4**	**16.6**	**0.78**	**12.2**	**3.5**	**3.9**
	LSD	**1.33**	**1.6**	**2.6**	**13.0**	**11.6**	**0.06**	**29.4**	**17.5**	**0.21**	**12.8**	**0.7**	**0.8**
	MSE	**0.90**	**2.0**	**1.7**	**128.8**	**101.5**	**0.00**	**218.8**	**154.1**	**0.02**	**41.3**	**0.3**	**0.4**
	Min	**1.95**	**68.5**	**-0.6**	**149.5**	**69.3**	**0.48**	**11.5**	**2.3**	**0.51**	**1.1**	**2.6**	**2.6**
	Max	**4.79**	**75.2**	**7.0**	**190.7**	**116.4**	**0.66**	**57.8**	**43.4**	**1.25**	**27.1**	**4.8**	**4.7**

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; RL=root lodging; SL=stem lodging; EPP=ears per plant; ER=ear rot; TEX=texture; EA=ear aspect; LSD=least significant difference (0.05); MSE=mean square error; Min=minimum; Max=maximum.

Table 8.8 Pearson's coefficient of correlation of grain yield with other agronomic traits under optimum conditions

	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER	TEX
AD	0.00										
ASI	-0.13	-0.64**									
PH	0.02	0.59**	-0.30**								
EH	0.24*	0.47**	-0.45**	0.65**							
EPO	0.27*	0.17	-0.36**	0.11	0.80**						
RL	-0.17	0.10	-0.24*	0.02	0.17	0.23*					
SL	-0.19	0.00	0.10	-0.16	-0.19	-0.11	-0.71				
EPP	0.22*	-0.21	0.29**	-0.23*	-0.23*	-0.12	-0.40**	0.35**			
ER	-0.34**	-0.10	0.17	0.08	-0.09	-0.17	-0.10	0.05	-0.14		
TEX	-0.27*	0.29**	-0.10	0.31**	0.12	-0.09	0.10	0.08	-0.08	0.23	
EA	-0.48**	-0.12	0.01	0.61	0.01	-0.01	0.10	-0.06	-0.31**	0.38**	0.26*

****P≤0.01; *P≤0.05; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; RL=root lodging; SL=stem lodging; EPP=ears per plant; ER=ear rot; TEX=texture; EA=ear aspect.**

The correlation of grain yield with ear rot, texture and ear aspect was negative and significant. The highest correlation of 0.80 (P≤0.01) was observed between ear height and ear position. The heritability estimate for grain yield under optimum conditions was 0.62 (Table 8.9). Other traits with heritability estimates above 0.50 were plant and ear height and ear position. Traits such as ears per plant and root and stem lodging had negligible heritability estimates.

8.3.3 Combined analysis

A combined ANOVA for optimum and managed drought environments was done for grain yield and other yield related agronomic traits (Table 8.10). Environments, genotypes as well as G x E interaction mean squares were significant (P≤0.001) for all traits. The variance components were estimated from ANOVA mean squares in order to estimate heritability. Generally the heritability estimates combined for all trials were lower than estimates realised under drought and optimum conditions separately. The highest heritability estimate of 0.86 was realised for ear height and the lowest heritability estimate of 0.19 for anthesis silking interval (Table 8.11). Grain yield had negative and significant correlation with anthesis days, anthesis silking interval and plant height. The correlation of grain yield with ears per plant was positive and significant (0.59). The highest positive and significant correlation was realised for ear height and ear position (0.83) (Table 8.12).

Table 8.9 Genotypic and phenotypic variance estimates and broad sense heritability of the agronomic traits under optimum conditions

Trait	σ^2_g	σ^2_p	h^2_B
ASI	0.0455	0.303	0.15
PH	3.224	4.91	0.66
EPO	0.0001	0.0001	0.60
EPP	0.000006	0.0001	0.06
EA	0.0017	0.0052	0.33
ER	0.6466	1.4496	0.45
RL	0.2258	1.82	0.12
SL	0.012	1.1956	0.01
TEX	0.0031	0.0088	0.34
EH	10.62	13.32	0.79
GYD	0.0566	0.0917	0.62

σ^2_g=genotypic variance; σ^2_p=phenotypic variance; h^2_B=broad sense heritability; ASI=anthesis silking interval; PH=plant height; EPO=ear position; EPP=ears per plant; EA=ear aspect; ER=ear rot; RL=root lodging; SL=stem lodging; TEX=texture; EH=ear height; GYD=grain yield.

Table 8.10 Combined analysis of variance for agronomic traits in the 2011 winter season

Source	DF	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP
Site	2	3.37***	25.83***	189.52***	221531.61***	46892.45***	0.27***	2879.91***	14157.11***	1.09***
Genotype	84	0.93***	1.17***	26.81***	790.67***	829.79***	0.04***	220.75**	323.64***	0.09***
Treatment	1	5.44***	8.66***	301.99***	26581.23***	1669.63***	0.042	824.76***	216.12***	6.72***
G x E	168	0.47***	0.62***	16.77***	228.35***	147.56***	0.02***	149.99***	150.29***	0.03***
E x T	2	26.14***	27.56***	98.11***	121345.05***	31478.83***	0.035	21768.01***	878.77***	0.24***
G x T	84	0.56***	0.40***	21.91***	190.97***	162.44***	0.02***	121.59***	188.57***	0.04***
G x E x T	168	0.38***	0.39***	18.58***	188.20***	138.00***	0.03***	109.55***	160.09***	0.03***
Residual	508	0.08	0.11	7.01	47.82	11.06	0.01	30.62	35.57	0.008

*****P≤0.01; DF=degrees of freedom; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; RL=root lodging; SL=stem lodging; EPP=ears per plant; Env=environment; G x E=genotype x environment interaction; E x T=environment x treatment; G x T=genotype x treatment G x E x Y=genotype x environment x treatment.**

Table 8.11 Genotypic and phenotypic variances and broad sense heritability for critical agronomic traits in combined analysis

Trait	σ^2_g	σ^2_p	h^2_B
GYD	0.0708	0.1508	0.47
AD	0.088	0.1983	0.45
ASI	1.65	8.66	0.19
PH	61.9042	109.7241	0.56
EH	68.2275	79.2875	0.86
EPO	0.0025	0.0125	0.20
EPP	0.0068	0.0148	0.46

σ^2_g=genotypic variance; σ^2_p=phenotypic variance; h^2_B=broad sense heritability; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; EPP=ears per plant.

In combined analysis genotype SC8//L10 was the best performer with a mean yield of 3.76 t ha^{-1} and the poorest performing genotype was 013WH63 (Table 8.13). The new three-way hybrids generally outperformed the locally grown varieties that were included in evaluations as check varieties such as SC635 and SC513. Hybrids displayed good stand ability properties and good ear placement. The ear per plant mean of 0.66 was obtained in combined analysis indicating that hybrids had good prolificacy.

Table 8.12 Pearson's correlation coefficients among agronomic variables in the combined analysis

	GYD	AD	ASI	PH	EH	EPO
AD	-0.25*					
ASI	-0.39**	0.16				
PH	-0.27*	0.56**	0.35**			
EH	-0.07	0.51**	0.10	0.65**		
EPO	0.10	0.32**	-0.76	0.21	0.83**	
EPP	0.59**	-0.59**	-0.52**	-0.57**	-0.47**	-0.25*

****P≤0.01; *P≤0.05; GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; EPP=ears per plant.**

Table 8.13 Mean performance of hybrids for grain yield and other agronomic traits in combined analysis in the 2011 winter season

	Entry	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER	SEN	TEX	EA
Ten best hybrids	SC8//L10	3.76	68.5	1.3	178.3	99.8	0.54	6.6	5.8	0.70	14.8	0.5	3.4	3.7
	SC10//L6	3.68	69.5	1.1	169.5	90.7	0.54	4.2	10.9	0.71	13.8	0.5	3.5	3.5
	SC10//L5	3.62	68.2	1.3	175.1	90.6	0.51	2.6	24.2	0.83	5.6	0.6	3.1	3.4
	SC4//L10	3.58	67.7	1.4	170.9	101.3	0.58	22.5	3.2	0.74	19.1	0.6	3.7	3.7
	SC2//L9	3.50	70.7	1.5	181.5	96.3	0.54	0.1	12.8	0.72	22.5	0.6	3.2	3.8
	SC3//L8	3.44	66.9	1.2	159.6	82.0	0.51	3.8	23.2	0.66	136.4	0.6	3.1	3.8
	SC11//L5	3.39	67.4	1.6	160.3	92.1	0.53	7.5	26.1	0.75	15.5	0.6	2.4	3.1
	SC1//L6	3.36	70.1	1.5	170.8	90.4	0.52	7.1	6.0	0.68	19.0	0.6	3.2	3.5
	013WH29	3.32	69.2	1.8	164.7	95.0	0.57	6.6	5.5	0.72	14.8	0.5	3.1	3.5
	SC7//L5	3.31	67.1	2.9	167.3	78.9	0.48	0.3	20.6	0.80	8.4	0.5	2.3	3.2
Ten poorest hybrids	SC7//L3	2.19	69.1	4.8	178.4	90.2	0.50	11.7	5.2	0.51	19.4	0.5	3.2	4.0
	SC2//L1	2.09	69.0	6.6	181.0	91.6	0.51	12.3	9.0	0.56	35.4	0.6	3.6	4.1
	SC3//L6	2.08	68.9	1.5	167.1	80.9	0.50	6.4	15.0	0.65	11.8	0.6	3.5	3.7
	SC10//L4	2.08	72.4	5.5	190.1	109.7	0.59	9.9	15.5	0.43	37.3	0.5	3.6	4.0
	SC1//L7	2.05	70.0	2.5	166.0	86.1	0.50	2.3	26.0	0.61	12.6	0.6	3.3	3.9
	SC11//L4	1.91	70.2	2.9	184.4	104.1	0.56	38.1	19.0	0.56	19.6	0.5	3.5	3.6
	SC1//L4	1.79	70.2	3.1	181.3	98.2	0.54	14.0	12.7	0.58	28.5	0.6	3.9	3.9
	SC7//L4	1.77	70.2	4.5	187.7	103.7	0.56	24.2	11.4	0.50	28.0	0.5	3.6	3.9
	013WH01	1.70	72.2	4.0	187.5	107.9	0.57	19.5	7.0	0.46	32.1	0.6	3.3	3.6
	013WH63	1.62	70.2	3.8	175.8	95.0	0.54	20.7	11.6	0.48	31.9	0.6	3.9	4.4
	Mean	**2.73**	**69.5**	**2.5**	**173.3**	**93.7**	**0.53**	**9.3**	**14.9**	**0.66**	**23.0**	**0.6**	**3.4**	**3.7**
	LSD (0.05)	**0.84**	**1.3**	**1.9**	**10.7**	**8.7**	**0.05**	**17.9**	**10.5**	**0.13**	**35.1**	**0.1**	**0.5**	**0.6**
	MSE	**0.72**	**2.0**	**3.8**	**116.7**	**116.1**	**0.00**	**161.6**	**139.0**	**0.02**	**1248.7**	**0.0**	**0.3**	**0.4**
	Min	**1.62**	**65.9**	**-0.2**	**148.1**	**75.8**	**0.46**	**0.1**	**2.0**	**0.43**	**2.4**	**0.5**	**2.3**	**2.9**
	Max	**3.76**	**72.8**	**6.6**	**190.1**	**117.0**	**0.64**	**40.5**	**29.4**	**0.84**	**136.4**	**0.7**	**4.3**	**4.5**

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; EPO=ear position; RL=root lodging; SL=stem lodging; EPP= ears per plant; ER=ear rot; SEN=senescence; TEX=texture; EA=ear aspect; LSD=least significant difference; MSE= mean square error; Min=minimum; Max=maximum.

8.3.4 Correlation between the predicted and observed mean yield

The predicted mean yields were positively and significantly (0.27) correlated with observed mean yield although the correlation was relatively small (data not shown). The predicted means followed a similar trend with the observed means (Figure 8.1). The arrow shows the drop in yield within the predicted and observed means where yield drops as the graph approaches the poorest performing hybrids. The ten best performing hybrids in the combined analysis also had high predicted means compared to the poorest performing hybrids.

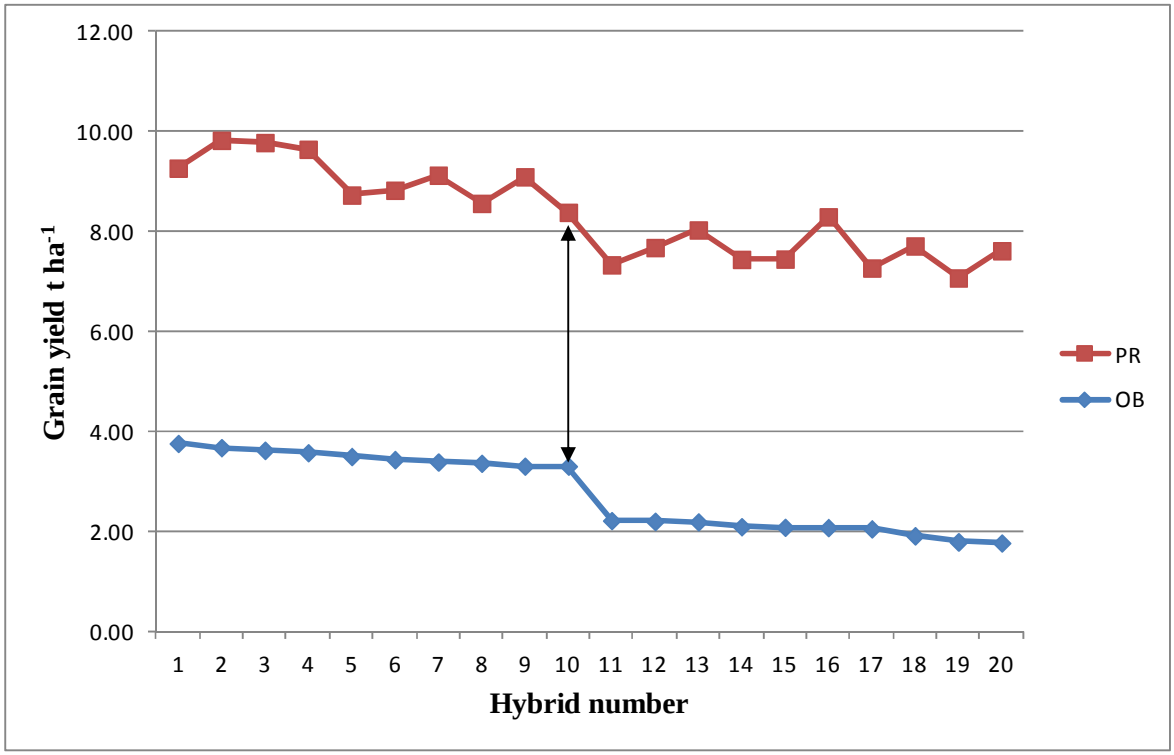

Figure 8.1 Predicted and observed mean yield for the best 10 and poorest 10 hybrids for combined environments.

PR=predicted; OB=observed.

8.4 Discussion

Hybrid varieties evaluated in this study revealed varied performance for yield and yield components. The final grain yield is a function of the combined individual yield components, which are likely to be influenced by genetic as well as environmental factors. The good performance demonstrated by genotypes was an indication that they were able to adapt to the environments in which they were evaluated. Results therefore suggest different genetic backgrounds among hybrids studied, as was also indicated by Khan *et al*. (2002). This is ideal as it provides an opportunity for selecting genotypes based on their performance for the different measured traits. In this study grain yield under drought conditions was 55% of the grain yield realised under optimum conditions and this was equivalent to severe stress. The new three-way hybrids were evaluated against check varieties, which included the current experimental hybrids and locally grown maize varieties. The new hybrids generally outperformed the check varieties both under drought and optimum conditions. Three hybrids namely RA214P/CML538//RS61P, RS61P/CML444//CML538 and RS61P/CML444//
CML539 showed stable performance across different conditions and were amongst the ten best performing hybrids under drought, optimum and combined analysis. This therefore means that there is no yield penalty if good rainfall occurs in a drought prone area. Hybrids outperformed the locally grown varieties such as SC635 and SC513 and also outperformed the recently released hybrid from the national programme, 013WH63. RA214/CML538//
RS61P outperformed the check varieties SC635, SC513 and 013WH63 by 1.7%, 19.5% and 88.9% respectively whilst RS61P/CML444//CML538 by 22.2%, 43.8% and 127.2% respectively and RS61P/CML444//CML539 by 20.2%, 41.4% and 123.5% respectively. The results show that there was a significant progress made in terms of producing superior hybrids.

The maturity range of hybrids in the study was early to medium and displayed short anthesis- silking intervals. The environmental mean for anthesis-silking interval ranged from -0.2 to 6.6 and results are similar to results reported by Betran *et al*. (2003) and Salami *et al*. (2007). The shortened anthesis-silking interval observed in this study is desirable and according to Edmeades *et al*. (1993) low anthesis-silking intervals enhance maize tolerance to stresses during flowering and ensures good grain filling. Results in this study showed that

hybrids had low root and stem lodging means, which is an indication of good stand ability properties. The ability of maize hybrids to withstand root and stem lodging at optimum plant populations is vital to obtaining high harvestable yields. Prolificacy (ears per plant) ranged from 0.51-1.25 under optimum conditions to 0.26-0.83 under drought conditions. Betran *et al.* (2003) and Derera *et al*. (2008) also reported reduced ears per plant under drought conditions. Salami *et al.* (2007) reported no prolificacy since none of the cultivars evaluated had ears per plant exceeding one. Decreased number of ears per plant may occur as a result of failure of fertilisation caused by large anthesis-silking intervals or increased rate of kernel abortion due to water stress (Westgate and Bassetti, 1990).

Significant negative correlation between grain yield and maturity under drought conditions was observed and there was a tendency for hybrids with shorter maturity periods to produce higher grain yield. This might have been due to the shorter growing period where the later maturing hybrids needed a longer growing season for different developmental stages. In some cases earliness is associated with drought escape as hybrids tend to reach the grain filling stage before being severely stressed. On the contrary Salami *et al.* (2007) reported negative and non-significant correlation between grain yield and days to 50% anthesis. The effectiveness of stress around flowering and the controlling of drought intensity are important in order to reveal genetic variability for anthesis-silking interval and ears per plant. Grain yield under drought conditions showed strong correlation with anthesis-silking interval (-0.44) and ears per plant (0.76). Similar results were reported by Bolanos and Edmeades (1996), Betran *et al*. (2003) and Magorokosho *et al*. (2003). In this study, the correlation of grain yield with anthesis-silking interval and ears per plant became stronger under drought conditions and increased from -0.13 and 0.22 respectively under optimum conditions to -0.44 and 0.76 respectively under drought conditions. These results further confirm that anthesis- silking interval and ears per plant are useful secondary traits to select for grain yield under drought conditions but that they are less useful under optimum conditions. Positive correlations between yield and ears per plant are expected because yield is a dependent variable of ears per plant. The importance of secondary traits in screening germplasm for drought tolerance has been determined by observing genetic correlations with grain yield (Betran *et al*., 2003).

Heritability estimates provide guidelines for the development of effective breeding strategies (Smalley *et al*., 2004). In this study lower broad sense heritability estimates for grain yield (0.47) than for ear (0.86) and plant (0.56) height were recorded. Results are consistent with previous findings by Hallauer and Miranda (1988) and Smalley *et al*. (2004). Traits that are more closely related to reproductive fitness have lower heritability and grain yield is critical to reproductive fitness in maize whilst ear and plant heights are considered of less importance to reproductive fitness. Broad sense heritability estimates for grain yield decreased from 0.62 under optimum conditions to 0.51 under drought conditions, whilst broad sense heritability estimates for anthesis silking interval increased from 0.15 under optimum conditions to 0.49 under drought conditions. Heritability of 0.51 for grain yield under drought was large and this implies that direct selection would be effective. Similar results were reported by Rosielle and Hamblin (1981) and Magorokosho *et al*. (2003). These results further confirm the importance of anthesis silking interval as a secondary trait used to increase grain yield when selecting under drought conditions.

The performance of three-way cross hybrids can be predicted from single cross hybrids that have been evaluated in as many environments as feasible. In this study the correlation between the predicted and observed means was significant but the correlation was weak. This might have been due to significant G x E interaction. Otsuka *et al*. (1972) found that G x E interaction affected the correlation of observed and predicted performance much more than did epistatic effects. The epistatic and G x E interaction effects were not considered during yield prediction of the three-way hybrids in this study. Otsuka *et al*. (1972) also showed that heritability of three-way crosses was higher than the correlation between the genotypic and predicted three-way cross values probably because of epistasis. Contrary to results obtained in this study Melchinger *et al*. (1987) reported high (0.86) correlation between the predicted and observed three-way cross means. Results show that the three-way hybrids with superior predicted yields can be evaluated in several environments and superior ones released for commercial use. Genetic gain can be increased through employing higher selection intensity among the predicted hybrids (Melchinger *et al.*, 1987).

8.5 Conclusions

Generally the performance of hybrids indicated that they have good genetic potential, as some showed high yield performance and good performance in other traits. Three hybrids, RA214P/CML538//RS61P, RS61P/CML444//CML538 and RS61P/CML444//CML539 were identified to have better performance compared to other hybrids. However, there still exists a need for further evaluation of these hybrids on a larger scale in advanced variety trials before considering them for release. There also exists a need to evaluate hybrids under low nitrogen conditions as well. The amount of genetic variance, heritability and genetic correlation with grain yield determine the relative usefulness of secondary traits such as ears per plant and anthesis silking interval as indirect selection criteria for grain yield. This study therefore identified the potential of anthesis silking interval as an indirect selection criterion for grain yield under drought stress conditions. The usefulness of ears per plant as an indirect selection criterion for grain yield under drought conditions was not convincingly determined in this study due to low genetic variance and a negligible heritability estimate. The low and negligible heritability estimates for ears per plant might have been an indication that the trait was influenced by environmental factors. However heritability for grain yield under drought conditions was larger relative to secondary traits such as ears per plant hence direct selection would be the most appropriate. Results have shown that the number of three-way hybrids handled by a breeding programme can be reduced by predicting their performance from superior single cross hybrids followed by evaluating them in multi-location trials to identify superior hybrids for release.

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CHAPTER 9

General conclusions and recommendations

Since maize is the staple food crop in Zimbabwe, the country requires 1.8 million ton for consumption and 300 000 ton as national strategic reserve per annum. However, biotic (insect pests and diseases) and abiotic (drought and low nitrogen) stresses pose many challenges for maize production in the country. The crop is produced by large and small scale commercial as well as communal farmers. The communal sector is, however, the largest producer of maize in the country. The yield of maize in the communal sector has remained below 0.5 t ha^{-1} and the sector is faced with major challenges, amongst them occurrence of dry spells, unavailability of inputs and poorly adapted varieties. Therefore germplasm improvement for drought tolerance remains a high priority in the country. The Zimbabwe National Breeding Programme under the DR&SS has partnered with CIMMYT in producing drought tolerant maize varieties. Drought tolerance is required for farmers to achieve high and stable maize yields, especially for the communal farmers who are mostly located in the drier parts of the country. This study was thus conducted to (i) estimate combining ability and heterosis for grain yield and other agronomic traits between DR&SS and CIMMYT white maize inbred lines under stress and non-stress environments, (ii) analyse G x E interaction and stability of single cross hybrids for grain yield, (iii) examine genetic diversity among DR&SS and CIMMYT white maize inbred lines using morphological traits and SNP markers, (iv) assess the relationship between genetic diversity of DR&SS and CIMMYT parental inbred lines and F_1 performance, heterosis and SCA effects of hybrids under abiotic and optimal environments, (v) estimate test-cross performance of F_3 segregating populations developed from CIMMYT drought tolerant donors and DR&SS elite inbred lines under drought and optimal conditions and (vi) estimate performance and yield prediction of three-way hybrids from drought tolerant single cross hybrids.

Outputs from the study indicated that an average of 112.29% MPH and 76.40% HPH were realised across environments and was an indication of the potential of these inbred lines for hybrid development. The negative heterosis (-5.1% MPH and -7.4% HPH) for days to anthesis was an indication that hybrids were earlier compared to their parental inbred lines.

Significant positive correlations and regressions were recorded for SCA with MPH, HPH and *per se* performance of hybrids under optimum and drought conditions. Results imply that an improvement in selection for SCA, which is a good predictor of grain yield, will result in indirect improvement of MPH and HPH under both optimum and drought conditions. The HPH and MPH also showed significant positive association and linear regression along with high coefficient of determination with *per se* performance of hybrids especially under drought conditions.

Pearson's correlation coefficients between grain yield and anthesis silking interval were smaller under optimum conditions and they became larger under drought and low N conditions. A strong and negative correlation of grain yield and anthesis silking interval under stress conditions showed that genotypes with negative anthesis silking interval would also have good grain yield performance. Reduced or negative anthesis silking interval under stress environments is desirable as silking delay has been seen as the cause for poor pollination and grain filling in maize. Similarly the relationship between grain yield and ears per plant became stronger under stress environments. Positive and strong correlation of grain yield and ears per plant under stress environments showed that genotypes with a high number of ears per plant would also have high grain yield. Results confirmed ears per plant and anthesis silking interval as important secondary traits to select for under stress environments. Therefore these two traits can be used together with grain yield in selecting superior genotypes under stress environments. Anthesis days were negatively correlated with grain yield under both drought and low N. Early maturing genotypes tended to produce higher yields as stress did not coincide with their critical stage of development. Results have shown that selection for earliness in the breeding programme have produced genotypes that perform well under short rainy seasons or where mid-season dry spells coincide with flowering of late maturing genotypes. In some cases genotypes that performed well under optimum conditions also performed well under drought conditions but this was not always true. It is thus critical for the programme to focus on breeding for varieties that are drought tolerant and also perform well under optimum conditions.

The genetic variability of lines was studied using morphological data and SNP markers. Both methods indicated that there was variability amongst lines. UPGMA cluster analysis based on morphological data grouped lines into five clusters mainly based on grain yield, anthesis days, anthesis silking interval and plant height. SNP markers grouped lines into two major groups and a number of subgroups. Within subgroups some lines clustered in accordance with known heterotic groups and pedigree relationships. DR&SS and CIMMYT have been using heterotic groups based on combining ability studies from diallel and NCDII experiments over the years. Morphological traits have been used in defining heterotic groups. These heterotic groups have proved to be useful as high levels of heterosis have been realised from crossing lines from different defined groups. As a result a number of good hybrids have been registered and availed to farmers for production. New testers that have been identified can be used together with the old testers in predicting heterotic groups. However to complement the currently used method, SNP markers can also be used in further defining the heterotic groups. Information generated using SNP markers can confirm the existing groups or can assist in defining new groups that could otherwise not be possible to define using only morphological traits. The National Breeding Programme is recommended to use both morphological and SNP markers in defining efficient and effective heterotic groups in order to maximise on utilisation of existing and newly acquired germplasm.

Therefore superior germplasm identified in this study can be put into use in germplasm improvement for stress environments. Lines RS61P, NAW5885 (from DR&SS) and CML444, CML539, CML442, CML537 and CML548 (from CIMMYT) were identified as having desirable GCA effects under both drought and low N conditions. The single cross RS61P/CML444 was identified as a potential tester for the SC heterotic group, whilst 2N3d/CML548 was identified as a potential tester for the N3 heterotic group. The testcrosses containing lines derived from DR&SS lines K64r, RS61P, NAW5885, SC5522 and CIMMYT drought tolerant donors based on DTPWC9 were amongst the best performing testcrosses in early and late maturing trials under drought, optimum and across environments. Three-way cross hybrids namely RA214P/CML538//RS61P, RS61P/CML444 //CML538 and RS61P/CML444//CML539 were identified as having superior performance.

The two single crosses identified as potential testers were also identified as the most stable genotypes across test environments. Therefore the national programme can now engage in validation studies for these testers before they are used in predicting heterotic groups. Identification of good single cross hybrids with good SCA effects and lines with good GCA effects proved to be an important intervention for the breeding programme. The single crosses and inbred lines were used to produce three-way hybrids which showed good performance under both optimum and drought conditions. By doing so the breeding programme has managed to produce final products within a short space of time. Results revealed that additive and non-additive gene effects were important in expression of traits. However, non-additive gene action assumed a more important role in expression of grain yield and secondary traits (anthesis silking interval, ears per plant and senescence) under both drought and low N conditions. Exotic germplasm was furthermore acquired from CIMMYT Mexico and used to enhance the National Breeding Programme germplasm. By doing this, a segregating population was developed to facilitate development of new inbred lines. Early testcrossing has been used to assist in selecting superior segregating lines for further generation advancement. Genetic variances and genetic gains for plant and ear height were generally higher than those for other traits. Grain yield had a repeatability value of 0.41 for late maturing testcrosses and good repeatability values were also recorded for plant and ear height and ears per plant. It is important for breeding programmes to constantly acquire germplasm from other breeding programmes to improve on genetic diversity and enhance quality of breeding material.

Finally it is recommended that the national programme utilises lines identified as having good GCA as parental lines in the hybrid breeding programme. Genetic relationships determined using morphological and SNP data will further assist breeders in identifying divergent parents and designing an effective crossing programme. Identified single cross testers can be used in grouping new inbred lines into heterotic groups, however there exists a need to validate testers through further combining ability studies before they can be put to use in the breeding programme. The information generated from testcross evaluation will assist breeders in selecting segregating lines for further generation advancement until homozygosity is attained. The identified three-way hybrids can be included in multi-location

national advanced variety trials before they are considered for release. There is also need to evaluate the testcrosses and the three-way hybrids under low N conditions. With the current climate change, improving germplasm for drought tolerance is an important intervention therefore the National Breeding Programme will in the near future be able to register drought tolerant lines and hybrids as products of this study.

SUMMARY

Breeding for drought and low N tolerant genotypes in Zimbabwe is an important intervention that will see the country curbing the food in-security problem. Both CIMMYT and DR&SS maize germplasm was used in this study that was conducted in Zimbabwe in the 2009/10 and 2010/11 seasons. Evaluations were done under optimum, drought and low N conditions. One of the objectives was to estimate combining ability and heterosis for grain yield and other agronomic traits of white maize inbred lines under stress and optimal environments. Line x tester analysis of 23 inbred lines identified RS61P, NAW5885 (from DR&SS) and CML444, CML539, CML442, CML537 and CML548 (from CIMMYT) as having desirable GCA effects under both drought and low N conditions. Additive and non-additive gene effects were important in the expression of traits across all environments; however non-additive gene effect assumed a more important role in the expression of traits under stress environments. The single crosses RS61P/CML444 and 2N3d/CML548 were identified as potential testers for the SC and N3 heterotic groups respectively. In the analyses of G x E and stability using AMMI and GGE biplot the same single crosses were identified as the most stable. There were three mega environments identified within the test environments and Agricultural Research Trust farm site was the most powerful in discriminating genotypes. Genetic diversity amongst the 23 inbred lines was examined using 14 morphological traits and 1 129 SNP markers. The morphological data revealed variability amongst inbred lines that could be manipulated through selection and hybridisation. Variability was further substantiated using PCA where the overall diversity could not be explained by a few eigenvectors and the major contributors were grain yield, texture, ear aspect, common rust, GLS and anthesis days. Euclidean and Rogers' dissimilarity matrices based on morphological and SNP data respectively clustered lines related by pedigree together in some cases. The SNP dendrogram had a high goodness of fit value (r=0.87) compared to the morphological dendrogram, which showed that it grouped the lines efficiently, although at times it was not in agreement with the known heterotic grouping that was previously established using testers. The assessment of correlation between genetic distances, F_1 performance, heterosis and SCA revealed significant positive correlations and regressions between SCA, MPH, HPH and *per se* performance of hybrids.

The HPH and MPH also showed significant positive association and linear regression along with high coefficient of determination with *per se* performance of hybrids, especially under drought conditions. Correlations of genetic distances with MPH and HPH were too low to be of predictive value. An average of 112.29% MPH and 76.40% HPH were realised across environments and this was an indication of the potential of these inbred lines for hybrid development. The segregating lines at F_3 stage were testcrossed to group A (CML539/CML442) and B (CML444/CML395) testers and testcrosses containing lines derived from DR&SS lines K64r, RS61P, NAW5885, SC5522 and CIMMYT drought tolerant donors based on DTPWC9 were generally amongst the best performing testcrosses in early and late maturing trials. Three-way hybrid performance was predicted from 11 single cross hybrids and results showed that there was significant but weak correlation between the predicted and the observed grain yield means and this could be explained by epistatic and significant G x E interaction, which were not taken into account in the prediction equation. Three-way cross hybrids identified as having superior performance under drought and well-watered conditions included RA214P/CML538//RS61P, RS61P/ CML444//CML538 and RS61P/CML444//CML539. However, there is still need to evaluate these hybrids under low N conditions before they can be recommended for release.

Key words: combining ability, genetic diversity, heterosis.

OPSOMMING

Die ontwikkeling van genotipes vir Zimbabwe wat droogte en lae N tolerant is kan ‘n belangrike bydrae maak om die probleem van voedselsekuriteit aan te spreek. Beide CIMMYT en DR&SS mieliekiemplasma is in hierdie studie gebruik wat uitgevoer is in Zimbabwe in die 2009/10 en 2010/11 seisoene. Die proewe is uitgevoer onder optimum, droogte en lae N toestande. Een van die belangrikste doelwitte was om kombineervermoë en heterose vir graanopbrengs en ander agronomiese eienskappe van wit mielie ingeteelde lyne onder stremmings en optimale toestande te bepaal. Lyn x toetser analise van 23 ingeteelde lyne het RS61P, NAW5885 (van DR&SS) en CML444, CML539, CML442, CML537 en CML548 (van CIMMYT) geïdentifiseer as lyne met die beste GCA effekte onder beide droogte en lae N toestande. Beide additiewe en nie-additiewe geeneffekte was belangrik by die uitdrukking van eienskappe oor alle omgewings; maar nie-additiewe geeneffekte was meer belangrik by die uitdrukking van eienskappe onder stremmingstoestande. Die enkelkruise RS61P/CML444 en 2N3d/CML548 is geïdentifiseer as potensiële toetsers vir die SC en N3 heterotiese groepe onderskeidelik. In die analise van G x E en stabiliteit met die gebruik van AMMI en GGE biplotte is dieselfde enkelkruise as die mees stabiel geïdentifiseer. Daar was drie mega-omgewings binne die toetsomgewings en die “Agricultural Research Trust” plaas omgewing was die mees effektief om te onderskei tusssen genotipes. Genetiese diversiteit tussen die 23 ingeteelde lyne is ondersoek met die gebruik van 14 morfologiese eienskappe en 1 129 SNP merkers. Die morfologiese data data het variasie tussen ingeteelde lyne gewys wat gemanipuleer kan word deur seleksie en hibridisasie. Variasie is verder bevestig met die gebruik van PCA waar totale variasie nie verklaar kon word deur enkele eigenvektore nie, en waar die meeste variasie verklaar is deur graanopbrengs, tekstuur, kopaspek, gewone roes, GLS en dae tot antese. Euklidiese en Rogers se matrikse van verskille gebasseer op morfologiese en SNP data onderskeidelik het in sommige gevalle die lyne gegroepeer volgens stambome. Die SNP dendrogram het die hoogste akkuraatheid (r=0.87) getoon in vergelyking met die morfologiese dendrogram, wat gewys het dat lyne effektief gegroepeer is, alhoewel dit soms nie in ooreenstemming was met bekende heterotiese groeperings wat vroeër bepaal is met toetsers nie. Die evaluasie van korrelasies tussen genetiese afstande, F_1 prestasie, heterose en SCA het betekenisvolle positiewe korrelasie en regressie tussen SCA, MPH,

HPH en *per se* prestasie van basters getoon. Die HPH en MPH was ook betekenisvol positief geassosieër en liniêre regressie sowel as ‘n hoë koeffisiënt van bepaling is gesien met die *per se* prestasie van basters, veral onder droogtetoestande. Korrelasies van genetiese afstande met MPH en HPH was te laag om voorspellingswaarde te hê. ‘n Gemiddeld van 112.29% MPH en 76.40% HPH is gesien oor omgewings. Dit is ‘n aanduiding van die potensiaal van hierdie ingeteelde lyne vir basterontwikkeling. Die segregerende F_3 lyne is getoetskruis met groep A (CML539/CML442) en B (CML444/CML395) toetsers en toetskruise van lyne afkomstig van DR&SS lines K64r, RS61P, NAW5885, SC5522 en CIMMYT droogtetolerante skenkers gebasseer op DTPWC9 was oor die algemeen die beste presterende toetskruise in die vroeë en laat rypheidstyd proewe. Drierigting basterprestasie is voorspel vanaf 11 enkelkruise en resultate het getoon dat daar betekenisvolle maar lae korrelasies was tussen die voorspelde en die werklike graanopbrengs. Dit kon verklaar word deur die epistatiese en betekenisvolle G x E interaksies wat nie in ag geneem is in die voorspellingsformule nie. Drierigtingkruisbasters wat geïdentifiseer is wat baie goed presteer het onder beide droogte en optimum toestande, was RA214P/CML538//RS61P, RS61P/CML444//CML538 and RS 61P/CML444//CML539. Dit is egter nog steeds nodig om hierdie basters onder lae N toestande te evalueer voor hulle aanbeveel kan word vir vrystelling.

Sleutelwoorde: kombineervermoë, genetiese diversiteit, heterose

Appendices

Appendix 1 Single cross hybrids produced

Entry	Pedigree
1	N3233-B/CML536
2	CML395-B/N3233-B
3	CML442-B/N3233-B
4	CML442-B/CML539
5	CML444-BB/N3233-B
6	CML537/N3233-B
7	CML538/N3233-B
8	CML539/N3233-B
9	CML548/N3233-B
10	CML545/N3233-B
11	CZL03007/N3233-B
12	SC5522-B/CML536
13	CML395-B/SC5522-B
14	CML444-BB/SC5522-B
15	CML545/C5522-B
16	CZL03007/SC5522-B
17	2Kba-B/CML444-BB
18	CML395-B/2Kba-B
19	CML442-B/2Kba-B
20	CML537/2Kba-B
21	CML538/2Kba-B
22	CML539/2Kba-B
23	CML545/2Kba-B
24	CZL052/2Kba-B
25	CZL03007/2Kba-B
26	K64r-B/CML312-B
27	K64r-B/CML536
28	K64r-B/CZL052
29	CML395-B/K64r-B
30	CML442-B/K64r-B
31	CML444-BB/K64r-B
32	CML537/K64r-B
33	CML538/K64r-B
34	CML539/K64r-B
35	CML548/K64r-B
36	CML545/K64r-B
37	CZL052/K64r-B
38	CML442-B/NAW5885-B
39	CML444-BB/NAW5885-B
40	CML537/NAW5885-B

Appendix 1 72 single cross hybrids produced

41	CML538/NAW5885-B
42	CML539/NAW5885-B
43	CML548/NAW5885-B
44	CML545/NAW5885-B
45	CZL03007/NAW5885-B
46	CML395-B/2N3d-B
47	CML442-B/2N3d-B
48	CML444-BB/2N3d-B
49	CML537/2N3d-B
50	CML538/2N3d-B
51	CML539/2N3d-B
52	CML548/2N3d-B
53	CML545/2N3d-B
54	CML395-B/RS61P-B
55	CML442-B/RS61P-B
56	CML442-B/CML537
57	CML444-BB/RS61P-B
58	CML537/RS61P-B
59	CML538/RS61P-B
60	CML539/RS61P-B
61	CML548/RS61P-B
62	CML545/RS61P-B
63	CZL03007/RS61P-B
64	RA214P-B/CML444-BB
65	CML395-B/RA214P-B
66	CML442-B/RA214P-B
67	CML537/RA214P-B
68	CML538/RA214P-B
69	CML539/RA214P-B
70	CML548/RA214P-B
71	CML545/RA214P-B
72	CZL03007/RA214P-B

Appendix 2 Performance of genotypes for grain yield and other agronomic traits across 14 environments in the 2009/10 and 2010/11 seasons

ENTRY	GYD t ha^{-1}	AD d	ASI d	PH cm	EH cm	RL %	SL %	EPP #	ER %	GLS 1-5	RUST 1-5	ET 1-5	SEN 1-10
74	5.62	75.3	1.4	266.7	138.6	5.7	5.0	0.80	8.4	1.5	1.3	2.2	1.9
61	5.31	69.7	0.6	230.7	119.5	11.0	3.7	0.92	5.0	2.7	1.0	1.5	2.1
57	4.94	72.4	1.5	240.5	133.3	6.9	6.7	0.91	5.2	1.6	1.0	2.6	1.9
54	4.89	70.2	1.6	242.2	138.7	8.3	4.6	0.87	6.9	2.1	0.9	1.8	1.9
48	4.88	73.0	1.1	267.9	147.0	7.7	3.7	0.96	8.1	3.9	1.4	1.1	1.8
63	4.78	69.8	1.2	227.3	121.1	19.4	6.7	0.85	4.7	1.3	1.0	1.6	2.1
52	4.76	71.0	1.0	244.4	125.3	9.4	1.8	0.86	12.7	3.1	1.3	2.0	2.2
79	4.74	75.1	0.9	243.3	135.6	5.4	5.5	0.83	4.4	1.9	1.0	1.5	1.8
45	4.74	70.2	1.9	239.6	122.5	7.7	7.9	0.84	6.3	2.7	1.0	1.5	2.1
68	4.69	70.7	1.5	235.5	114.6	10.0	0.6	0.89	7.4	1.8	1.0	1.5	2.3
10	4.69	69.6	1.2	235.2	124.3	5.5	2.5	0.82	7.6	3.3	1.0	2.2	2.0
72	4.63	69.9	-0.3	229.0	112.0	6.5	3.1	0.93	4.6	1.5	1.0	1.5	2.1
34	4.63	66.7	0.2	213.8	101.2	11.9	15.7	0.94	5.8	3.0	1.0	2.1	2.2
38	4.63	69.1	2.4	242.4	121.4	7.2	7.1	0.88	5.4	3.4	1.1	2.0	1.7
60	4.62	66.9	0.5	223.7	117.8	7.7	3.1	0.98	6.6	2.7	0.9	1.5	2.0
7	4.61	70.0	2.0	244.2	120.3	10.7	5.6	0.77	8.3	1.8	0.9	1.8	2.0
58	4.61	69.4	1.5	226.4	113.5	8.6	5.7	0.95	5.0	2.2	1.0	1.9	1.9
59	4.56	69.3	0.0	220.6	119.2	2.6	8.4	0.88	6.9	1.4	0.9	2.3	2.1
70	4.55	72.9	3.0	244.1	128.4	4.5	4.8	0.73	8.3	1.6	1.0	1.1	1.9
8	4.49	69.8	1.8	236.2	122.3	11.5	7.0	0.90	4.8	4.2	0.9	1.7	2.3
51	4.48	70.3	0.8	237.4	115.7	11.2	7.6	0.84	8.1	3.6	1.0	1.6	2.0
62	4.47	68.0	0.0	229.5	125.3	10.0	2.7	1.00	8.8	1.4	0.9	1.1	1.9
5	4.41	73.0	1.5	250.4	138.7	12.7	13.1	0.79	7.3	3.4	1.1	2.3	2.0
47	4.41	71.3	0.8	242.5	120.3	6.8	7.6	0.85	8.2	4.3	1.5	2.1	1.8
77	4.32	72.3	1.6	235.9	116.5	13.0	-0.6	0.82	9.7	1.9	0.9	2.2	2.1
4	4.30	70.0	1.5	217.8	105.4	20.8	3.5	0.99	5.6	2.7	1.3	2.1	2.1
27	4.29	71.1	-0.4	241.1	119.6	12.1	3.3	0.82	7.2	2.5	1.4	2.0	1.9
28	4.28	68.3	1.1	227.9	114.7	10.7	5.7	0.86	4.8	1.7	1.7	3.0	2.2
26	4.27	69.8	1.2	235.2	122.2	3.8	3.2	0.88	7.2	1.9	0.9	1.6	2.0
78	4.27	72.6	1.5	241.2	122.1	7.3	4.9	0.79	7.9	2.0	1.0	2.5	1.9
31	4.21	71.7	1.2	239.9	126.6	13.1	5.1	0.82	10.3	2.5	1.5	2.2	2.0
71	4.18	69.2	0.5	232.9	115.9	7.7	1.9	0.89	9.3	2.3	0.9	1.1	2.2
6	4.17	71.6	1.6	254.1	130.1	7.6	1.5	0.83	10.2	3.7	1.0	2.0	2.2
32	4.15	69.1	0.6	232.1	113.9	13.7	2.2	0.86	6.2	3.2	1.1	2.7	2.3
1	4.12	72.1	1.7	253.6	138.3	4.3	5.8	0.90	6.3	2.1	1.6	2.0	2.0
80	4.10	70.5	1.3	229.9	110.6	5.5	2.6	0.84	3.8	1.7	1.0	2.0	1.8
3	4.09	70.1	2.4	246.4	123.3	17.1	7.7	0.84	5.3	3.9	1.2	1.5	2.1
16	4.08	72.0	1.0	244.7	130.3	6.6	14.5	0.85	7.8	2.6	0.9	2.1	2.0
55	4.07	70.4	0.5	232.6	126.9	7.7	2.9	0.96	5.2	2.6	1.0	2.2	1.7

Appendix 2 Performance of genotypes for grain yield and other agronomic traits across 14 environments in the the 2009/10 and 2010/11 seasons

ENTRY	GYD t ha^{-1}	AD d	ASI d	PH cm	EH cm	RL %	SL %	EPP #	ER %	GLS 1-5	RUST 1-5	ET 1-5	SEN 1-10
11	4.07	70.1	2.0	245.3	128.2	24.7	16.9	0.81	6.1	1.8	1.3	1.6	2.3
49	4.05	72.3	1.8	248.0	126.6	5.3	9.1	0.95	9.3	3.4	1.0	1.9	2.2
15	4.04	70.4	0.7	235.9	126.4	7.2	0.4	0.89	7.3	2.0	1.1	1.7	2.3
36	4.03	66.9	-0.1	228.1	111.8	9.7	4.1	0.90	11.7	2.2	1.2	2.0	2.1
35	4.02	69.1	1.2	230.1	108.8	10.7	6.2	0.86	6.0	2.3	1.2	1.9	2.5
66	4.01	72.2	2.3	239.5	116.5	9.3	0.1	0.82	6.8	1.8	1.0	2.0	2.2
19	4.01	68.1	1.3	231.2	113.5	13.5	4.5	0.85	6.3	3.7	1.6	2.1	1.9
67	4.01	73.5	1.8	256.2	131.1	7.8	1.1	0.88	8.2	2.3	1.1	1.9	2.3
9	3.99	70.4	1.5	237.9	125.4	12.5	9.8	0.83	10.5	3.3	1.0	1.7	2.3
21	3.98	68.2	1.1	233.1	114.2	10.3	4.0	0.82	5.6	1.3	1.0	1.4	2.1
22	3.97	66.6	0.7	230.2	114.1	10.2	4.4	0.89	4.6	3.0	1.0	1.0	2.3
20	3.96	69.3	1.4	233.7	120.6	13.3	2.2	0.83	8.4	2.4	0.9	1.4	2.2
53	3.95	69.6	0.1	240.6	127.2	6.7	13.0	0.92	11.2	4.1	0.9	2.0	2.2
46	3.91	72.9	1.9	261.8	135.9	14.5	11.0	0.88	9.2	2.8	1.1	2.4	2.3
76	3.90	69.0	1.3	240.0	119.3	10.1	0.9	0.99	6.1	2.4	1.2	1.5	2.0
33	3.89	68.2	0.0	219.7	108.2	12.4	4.0	0.78	7.7	2.2	1.0	2.8	2.0
65	3.88	74.9	2.8	258.7	138.5	12.9	5.8	0.75	5.1	2.0	1.1	2.5	2.1
40	3.85	72.4	1.9	241.1	118.7	13.2	3.3	0.80	12.0	2.3	0.9	1.0	2.0
44	3.85	68.6	2.3	241.9	117.6	7.1	3.9	0.77	8.4	2.4	1.1	1.6	1.9
2	3.83	71.3	2.2	249.2	138.1	16.4	9.9	0.69	15.5	3.7	1.6	2.7	2.2
30	3.81	68.3	0.8	224.0	112.1	10.5	2.0	0.86	8.0	3.8	1.2	2.5	2.1
75	3.81	72.0	2.0	253.5	120.1	7.3	8.1	0.93	5.4	2.3	1.5	1.3	2.0
43	3.74	72.1	2.7	240.9	124.1	8.1	4.4	0.81	9.5	1.7	1.4	1.1	2.2
64	3.69	75.4	1.5	259.5	138.1	0.7	5.9	0.86	7.4	1.4	1.2	1.4	2.0
41	3.67	71.0	1.9	245.4	122.7	11.4	8.5	0.76	8.9	2.9	0.9	1.8	1.9
23	3.62	66.7	0.4	227.5	117.4	10.7	9.0	0.88	9.0	2.1	1.3	2.3	2.1
25	3.61	68.4	1.1	229.1	111.0	13.6	13.3	0.77	6.9	2.0	1.0	2.2	2.4
24	3.59	66.7	0.8	228.2	116.7	10.9	4.8	0.87	7.5	2.7	1.0	1.7	2.2
56	3.50	71.4	1.8	224.2	104.3	14.1	9.0	0.83	5.5	3.0	1.0	1.5	2.1
13	3.49	72.7	2.0	253.2	137.8	19.6	6.7	0.68	8.3	2.4	1.9	2.8	2.2
42	3.49	68.2	2.2	229.0	105.4	8.8	1.7	0.81	11.2	2.5	1.3	0.9	2.2
69	3.48	71.7	1.6	191.4	91.6	6.2	20.8	0.72	7.3	2.6	1.0	1.0	2.4
18	3.45	71.0	1.7	249.4	133.7	1.9	6.6	0.86	6.4	2.3	1.4	1.9	2.3
39	3.41	71.4	0.7	251.1	132.2	6.0	0.7	0.87	8.2	3.3	1.5	1.0	1.7
50	3.41	70.7	1.5	232.2	116.2	14.7	3.3	0.69	12.7	3.1	1.3	1.6	2.2
14	3.28	75.6	2.6	257.7	142.8	7.0	11.4	0.74	6.1	1.6	1.2	1.8	1.9
37	3.07	68.4	0.4	222.6	107.8	17.3	14.5	0.70	9.0	1.7	1.1	1.8	2.2
73	2.91	72.3	4.2	250.8	136.2	20.7	2.9	0.55	13.2	2.7	1.2	2.0	2.3
12	2.79	73.7	2.4	251.5	134.7	16.2	4.7	0.70	7.1	2.9	1.0	2.1	2.2
29	2.37	71.2	3.3	238.3	127.3	18.6	21.5	0.74	9.8	2.8	1.3	2.7	2.0
17	2.29	74.4	2.8	239.8	124.9	16.3	11.9	0.67	5.9	1.9	1.4	1.2	2.0
Mean	**4.07**	**70.6**	**1.4**	**238.5**	**122.4**	**10.3**	**6.1**	**0.84**	**7.6**	**2.5**	**1.1**	**1.8**	**2.1**
LSD	**0.68**	**0.9**	**0.9**	**10.1**	**8.0**	**9.2**	**11.0**	**0.11**	**4.4**	**0.9**	**0.5**	**0.9**	**0.4**
MSE	**1.04**	**2.9**	**2.1**	**285.0**	**163.4**	**129.1**	**60.6**	**0.02**	**35.0**	**0.5**	**0.1**	**0.2**	**0.1**
Min	**2.29**	**66.6**	**-0.4**	**191.4**	**91.6**	**0.7**	**-0.6**	**0.55**	**3.8**	**1.3**	**0.9**	**0.9**	**1.7**
Max	**5.62**	**75.6**	**4.2**	**267.9**	**147.0**	**24.7**	**21.5**	**1.00**	**15.5**	**4.3**	**1.9**	**3.0**	**2.5**

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; RL=root lodging; SL=stem lodging; EPP=ears per plant; ER=ear rot; GLS=grey leaf spot; RUST=common rust; ET=leaf blight turcicum; SEN=senescence LSD=least significant difference; MSE=mean square error; Min=minimum; Max=maximum.

Appendix 3 Performance of genotypes for grain yield and other agronomic traits across optimum sites in the 2009/10 and 2010/11 seasons

ENTRY	GYD t ha^{-1}	AD d	ASI d	PH cm	EH cm	RL %	SL %	EPP #	HC %	ER %	GLS 1-5	RUST 1-5	ET 1-5
74	7.86	73.1	0.6	296.5	148.5	4.9	5.0	0.97	22.4	4.5	1.5	1.3	2.2
68	6.79	67.8	0.1	257.1	120.6	10.0	0.6	0.95	5.2	7.3	1.8	1.0	1.5
57	6.63	70.0	0.5	262.5	142.5	11.1	6.7	1.00	11.1	2.7	1.6	1.0	2.6
48	6.63	69.2	0.5	291.1	151.6	6.0	3.7	1.08	8.7	9.2	3.9	1.4	1.1
61	6.62	67.5	-0.1	247.8	120.5	13.1	3.7	0.98	19.3	3.7	2.7	1.0	1.5
7	6.61	67.5	1.0	266.5	122.7	9.8	5.6	0.86	8.8	6.5	1.8	0.9	1.8
79	6.55	71.5	0.5	264.6	141.0	9.0	5.5	0.96	3.3	3.4	1.9	1.0	1.5
5	6.54	69.6	0.7	272.4	142.7	10.8	13.1	1.01	13.1	2.9	3.4	1.1	2.3
51	6.52	67.1	-0.9	265.6	116.9	8.0	7.6	0.98	16.0	7.3	3.6	1.0	1.6
52	6.45	68.5	0.2	261.9	125.6	6.0	1.8	0.95	24.8	4.4	3.1	1.3	2.0
45	6.34	67.3	0.6	259.6	124.8	5.7	7.9	0.94	22.4	1.4	2.7	1.0	1.5
4	6.30	67.5	0.8	233.3	107.2	17.2	3.5	1.07	15.8	2.6	2.7	1.3	2.1
60	6.17	64.3	-0.5	240.8	118.4	9.7	3.1	0.98	17.4	4.8	2.7	0.9	1.5
72	6.09	67.3	-0.4	247.8	116.9	8.4	3.1	1.02	21.5	3.0	1.5	1.0	1.5
47	6.06	68.5	0.6	267.1	123.6	2.7	7.6	0.96	21.5	7.7	4.3	1.5	2.1
10	6.05	66.3	0.2	262.1	122.6	4.3	2.5	0.91	14.6	7.8	3.3	1.0	2.2
8	6.05	67.4	1.0	257.7	131.4	8.1	7.0	0.96	6.3	3.0	4.2	0.9	1.7
38	6.02	66.0	1.8	264.1	119.9	6.4	7.1	0.92	18.3	3.9	3.4	1.1	2.0
49	6.00	68.6	0.7	265.1	131.2	7.7	9.1	1.16	17.8	5.6	3.4	1.0	1.9
54	5.98	67.3	1.4	259.7	137.6	8.7	4.6	0.95	6.3	1.2	2.1	0.9	1.8
77	5.95	69.5	0.6	257.4	124.4	12.3	-0.6	0.84	16.3	9.1	1.9	0.9	2.2
28	5.94	64.7	0.9	248.7	117.1	8.7	5.7	0.93	3.0	3.1	1.7	1.7	3.0
26	5.91	66.9	0.1	253.9	126.3	0.5	3.2	1.01	21.5	4.5	1.9	0.9	1.6
70	5.89	69.5	1.6	273.4	131.2	2.1	4.8	0.93	18.7	3.1	1.6	1.0	1.1
34	5.89	63.9	-0.4	233.9	108.2	8.1	15.7	0.99	15.7	5.9	3.0	1.0	2.1
58	5.87	66.1	0.7	243.3	117.6	10.1	5.7	1.03	15.2	1.9	2.2	1.0	1.9
62	5.81	64.5	-0.3	240.5	124.7	11.3	2.7	1.03	15.1	6.3	1.4	0.9	1.1
71	5.78	66.0	0.1	246.8	117.0	6.0	1.9	0.96	8.5	6.2	2.3	0.9	1.1
9	5.74	66.6	1.0	264.6	127.6	11.4	9.8	0.96	15.5	5.2	3.3	1.0	1.7
76	5.73	66.0	0.8	264.1	125.0	11.3	0.9	1.18	11.2	7.5	2.4	1.2	1.5
43	5.73	69.4	1.5	259.2	127.8	0.6	4.4	0.97	29.4	3.7	1.7	1.4	1.1
6	5.65	68.9	0.7	275.3	131.1	9.1	1.5	0.96	12.8	4.8	3.7	1.0	2.0
1	5.64	69.0	1.3	281.0	139.9	5.4	5.8	0.92	4.3	3.8	2.1	1.6	2.0
27	5.64	68.0	-1.1	260.5	127.1	10.9	3.3	0.89	10.3	4.1	2.5	1.4	2.0
80	5.56	67.0	0.3	250.6	117.2	5.7	2.6	0.93	4.2	1.9	1.7	1.0	2.0
67	5.55	70.6	0.8	282.6	136.2	11.2	1.1	1.04	15.4	7.1	2.3	1.1	1.9
63	5.53	67.6	0.2	251.2	122.5	25.2	6.7	0.97	5.4	2.4	1.3	1.0	1.6
40	5.52	68.7	1.3	270.5	129.5	17.0	3.3	0.91	25.1	6.1	2.3	0.9	1.0
16	5.52	69.1	0.1	266.2	128.1	6.9	14.5	0.98	14.2	4.8	2.6	0.9	2.1
59	5.50	66.5	-0.1	238.8	122.3	-0.5	8.4	0.85	19.5	4.5	1.4	0.9	2.3
78	5.48	69.1	0.9	263.8	121.3	4.9	4.9	0.94	17.5	4.1	2.0	1.0	2.5
31	5.44	68.8	0.4	268.7	136.2	17.0	5.1	0.89	25.6	9.9	2.5	1.5	2.2
11	5.40	66.9	1.6	264.3	134.6	30.7	16.9	0.87	9.7	3.5	1.8	1.3	1.6

Appendix 3 Performance of genotypes for grain yield and other agronomic traits across optimum sites in the 2009/10 and 2010/11 seasons

ENTRY	GYD t/ha	AD d	ASI d	PH cm	EH cm	RL %	SL %	EPP #	HC %	ER %	GLS 1-5	RUST 1-5	ET 1-5
3	5.38	67.5	1.4	268.2	124.9	13.8	7.7	0.94	5.3	5.7	3.9	1.2	1.5
53	5.28	67.2	-0.8	264.3	124.4	1.5	13.0	1.12	34.0	9.1	4.1	0.9	2.0
44	5.28	65.6	0.9	267.9	118.8	9.0	3.9	0.87	18.3	2.5	2.4	1.1	1.6
32	5.26	65.9	-0.4	250.5	107.9	11.4	2.2	0.94	12.0	4.8	3.2	1.1	2.7
66	5.22	69.4	1.4	264.0	119.7	4.9	0.1	0.89	9.3	5.0	1.8	1.0	2.0
46	5.17	70.0	1.0	281.5	142.7	13.3	11.0	0.93	17.7	7.4	2.8	1.1	2.4
75	5.09	69.3	0.7	272.1	127.0	10.6	8.1	1.08	3.4	3.7	2.3	1.5	1.3
65	5.05	72.0	2.3	281.2	143.0	14.6	5.8	0.90	7.0	2.7	2.0	1.1	2.5
15	5.03	67.4	0.1	258.8	129.7	6.3	0.4	0.97	9.5	5.7	2.0	1.1	1.7
42	4.99	65.2	0.8	250.8	109.8	11.1	1.7	0.94	18.1	2.8	2.5	1.3	0.9
35	4.98	66.6	0.0	246.6	108.8	16.9	6.2	0.93	14.6	3.8	2.3	1.2	1.9
2	4.96	68.7	1.9	278.4	140.9	18.5	9.9	0.81	7.1	9.9	3.7	1.6	2.7
36	4.91	63.8	-0.2	249.3	116.5	8.7	4.1	0.94	14.2	12.0	2.2	1.2	2.0
64	4.86	72.9	1.1	277.1	142.4	3.2	5.9	1.01	5.9	7.3	1.4	1.2	1.4
19	4.82	65.2	0.7	249.7	114.3	15.5	4.5	0.93	8.8	5.6	3.7	1.6	2.1
39	4.81	68.1	0.3	276.4	132.9	6.3	0.7	0.90	15.7	8.1	3.3	1.5	1.0
14	4.79	72.7	2.1	285.6	156.3	13.9	11.4	0.88	3.5	5.7	1.6	1.2	1.8
33	4.78	65.4	-1.0	237.0	105.9	8.8	4.0	0.85	7.6	6.2	2.2	1.0	2.8
41	4.77	68.4	1.4	268.2	125.0	7.9	8.5	0.85	9.1	8.9	2.9	0.9	1.8
21	4.75	65.5	0.3	252.7	115.8	7.3	4.0	0.84	2.9	5.0	1.3	1.0	1.4
50	4.73	67.2	0.6	255.8	118.9	7.6	3.3	0.85	16.6	7.7	3.1	1.3	1.6
69	4.71	68.7	0.3	209.7	96.2	8.3	20.8	0.87	3.5	4.3	2.6	1.0	1.0
24	4.71	63.9	0.1	248.8	119.3	3.9	4.8	0.96	4.5	4.3	2.7	1.0	1.7
20	4.64	66.6	0.9	249.8	122.8	7.7	2.2	0.88	19.4	4.9	2.4	0.9	1.4
55	4.63	67.4	0.2	254.1	131.5	6.3	2.9	0.97	13.0	2.2	2.6	1.0	2.2
56	4.56	68.0	1.0	242.5	110.2	3.6	9.0	0.97	3.5	3.4	3.0	1.0	1.5
25	4.52	65.7	-0.3	250.8	111.3	16.5	13.3	0.82	4.2	3.3	2.0	1.0	2.2
13	4.49	70.2	1.6	276.6	136.1	20.3	6.7	0.85	5.8	4.8	2.4	1.9	2.8
23	4.46	63.8	-0.1	249.9	119.5	4.5	9.0	0.92	16.1	12.9	2.1	1.3	2.3
30	4.44	65.4	-0.1	246.1	110.1	6.9	2.0	0.86	16.1	5.9	3.8	1.2	2.5
22	4.33	64.3	0.1	250.0	120.2	12.1	4.4	0.92	16.5	3.0	3.0	1.0	1.0
73	4.26	68.9	3.0	278.2	142.7	23.7	2.9	0.66	9.0	7.7	2.7	1.2	2.0
18	4.17	68.8	0.6	270.5	134.1	-1.1	6.6	0.98	22.1	6.8	2.3	1.4	1.9
37	3.67	65.3	0.1	240.9	111.6	25.2	14.5	0.87	7.9	6.7	1.7	1.1	1.8
12	3.54	70.4	0.9	282.9	137.1	17.3	4.7	0.78	3.7	4.1	2.9	1.0	2.1
17	3.10	71.2	1.6	270.4	125.2	14.0	11.9	0.90	19.5	8.2	1.9	1.4	1.2
29	2.91	67.8	2.1	257.7	129.1	17.9	21.5	0.81	7.0	6.1	2.8	1.3	2.7
Mean	**5.39**	**67.7**	**0.6**	**260.2**	**125.5**	**9.9**	**6.1**	**0.94**	**13.0**	**5.3**	**2.5**	**1.1**	**1.8**
LSD	**0.99**	**1.2**	**1.0**	**14.5**	**9.4**	**14.0**	**11.0**	**0.12**	**9.6**	**4.2**	**0.9**	**0.5**	**0.9**
MSE	**1.24**	**2.1**	**1.4**	**263.7**	**112.6**	**147.6**	**60.6**	**0.01**	**92.1**	**13.4**	**0.5**	**0.1**	**0.2**
Min	**2.91**	**63.8**	**-1.1**	**209.7**	**96.2**	**0.46**	**-1.1**	**-0.6**	**0.66**	**1.2**	**1.3**	**0.9**	**0.9**
Max	**7.86**	**73.1**	**3.0**	**296.5**	**156.3**	**0.59**	**30.7**	**21.5**	**1.18**	**12.9**	**4.3**	**1.9**	**3.0**

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; RL=root lodging; SL=stem lodging; EPP=ears per plant; HC=husk cover; ER=ear rot; GLS=grey leaf spot; RUST=common rust; ET=leaf blight turcicum; LSD=least significant difference; MSE=mean square error; Min=minimum; Max=maximum.

Appendix 4 Performance of genotypes for grain yield and other agronomic traits across managed drought sites in the 2009/10 and 2010/11 seasons

ENTRY	GYD t ha^{-1}	AD d	ASI d	PH cm	EH cm	RL %	EPP #	SEN 1-10
52	3.26	98.0	1.1	236.9	121.6	5.5	0.95	3.7
27	3.07	95.8	0.9	216.6	100.7	0.2	1.07	3.3
19	3.01	91.7	1.9	230.5	105.7	0.7	0.90	3.2
38	3.01	94.6	3.0	222.9	110.3	7.5	0.92	2.9
59	2.84	94.8	-0.1	210.4	102.3	3.5	0.91	3.8
36	2.79	90.6	-0.7	215.9	98.2	0.6	0.96	3.6
61	2.79	94.1	1.9	230.1	114.4	2.3	0.97	3.6
66	2.77	98.7	4.4	220.8	105.7	5.2	0.84	3.9
3	2.74	97.1	3.0	228.5	119.4	31.4	0.79	3.7
30	2.71	93.7	1.3	226.4	112.9	2.1	0.96	3.5
55	2.65	95.9	0.5	221.1	111.9	0.5	0.99	3.1
34	2.64	90.2	-0.4	195.6	83.5	3.4	1.08	3.8
63	2.62	92.5	0.9	201.7	100.4	-0.7	0.92	3.7
35	2.55	93.6	1.3	220.3	102.4	-0.5	1.07	4.5
60	2.51	89.4	0.8	196.0	93.3	-0.6	0.97	3.4
2	2.50	96.0	0.1	223.6	117.3	0.4	0.59	3.7
74	2.50	104.8	1.9	236.7	108.8	-0.5	0.42	3.3
79	2.49	104.7	0.7	233.5	121.2	7.8	0.72	3.1
11	2.47	95.6	-0.4	231.0	108.4	12.6	0.85	3.9
62	2.46	92.5	-0.7	215.3	116.2	-0.8	1.06	3.3
16	2.45	97.6	1.6	232.5	125.1	2.0	0.94	3.5
33	2.44	92.7	0.2	220.7	111.3	-1.8	0.99	3.4
58	2.43	92.7	2.8	206.7	96.7	-0.7	0.85	3.2
10	2.41	95.0	1.7	224.4	114.2	0.1	0.81	3.5
22	2.39	88.4	1.0	224.1	108.0	2.7	0.86	3.9
70	2.38	100.0	2.5	228.1	116.4	1.0	0.59	3.3
29	2.35	97.8	4.4	233.4	115.6	28.0	0.85	3.4
54	2.33	94.9	1.5	227.4	117.0	0.6	0.82	3.4
72	2.31	95.2	-2.8	211.7	100.7	13.6	1.04	3.6
40	2.28	98.8	2.6	222.0	97.1	-1.8	0.88	3.4
28	2.28	94.5	0.1	227.1	118.4	-1.4	0.94	3.7
32	2.24	93.3	2.9	222.0	97.4	-1.9	0.90	4.0
24	2.23	88.9	1.2	215.5	99.6	0.5	0.88	4.0
77	2.21	101.0	1.9	216.8	89.0	18.5	0.81	3.6
23	2.15	89.3	1.1	218.2	108.0	4.8	0.95	3.6
18	2.14	94.9	2.9	230.3	115.7	-1.6	0.76	4.0
31	2.09	98.7	1.0	219.6	108.4	1.8	0.86	3.4
21	2.05	93.5	2.1	220.8	108.1	7.4	0.81	3.6
9	2.04	96.0	1.3	224.7	119.7	0.1	0.73	4.0
25	2.04	90.0	0.9	216.8	96.3	3.3	1.06	4.1
41	2.04	98.5	1.1	227.8	120.1	1.3	0.62	3.2
26	2.04	95.9	2.1	213.0	93.5	12.5	0.90	3.5
5	2.03	101.5	2.4	241.3	129.8	10.7	0.38	3.4
67	2.03	100.7	2.3	238.7	117.4	0.6	0.73	4.0
48	1.99	102.5	0.8	260.3	148.3	6.5	0.97	3.1
76	1.99	93.3	2.3	217.5	101.3	7.8	0.85	3.4

Appendix 4 Performance of genotypes for grain yield and other agronomic traits across managed drought sites in the 2009/10 and 2010/11 seasons

ENTRY	GYD	AD	ASI	PH	EH	RL	EPP	SEN
	t ha^{-1}	d	d	cm	cm	%	#	1-10
46	1.99	100.9	2.0	239.9	114.6	4.7	0.86	4.0
6	1.98	101.4	1.9	235.5	126.3	7.6	0.49	3.8
15	1.95	94.7	1.7	226.4	107.4	2.9	0.92	4.2
47	1.95	97.5	1.7	230.7	114.0	4.3	0.69	3.1
75	1.94	97.7	4.6	232.8	105.1	0.6	0.66	3.5
45	1.92	96.9	1.3	230.7	116.7	3.3	0.73	3.5
44	1.91	92.7	2.8	214.9	107.2	-0.8	0.73	3.3
37	1.91	90.6	-2.4	206.5	97.6	4.9	0.68	3.7
12	1.87	97.3	3.8	229.6	114.2	9.5	0.55	3.8
39	1.84	99.1	-0.5	227.3	119.1	-0.4	1.49	2.9
4	1.83	95.2	1.7	209.9	91.7	1.3	0.63	3.7
1	1.83	99.8	3.0	230.2	121.2	-1.0	1.28	3.5
20	1.80	94.6	1.4	216.9	109.0	-0.9	0.93	4.0
50	1.79	96.5	2.1	228.9	114.5	16.0	0.58	3.8
49	1.78	101.4	1.4	234.8	117.3	10.9	0.40	3.8
78	1.78	101.1	1.5	238.0	119.0	3.2	0.51	3.4
57	1.78	97.9	2.1	212.5	101.4	1.2	0.72	3.2
42	1.77	91.0	3.8	200.5	82.7	-2.6	0.78	3.9
7	1.71	96.4	3.7	225.5	113.6	16.5	0.36	3.5
8	1.71	95.4	2.8	220.5	104.0	19.5	0.69	3.9
53	1.69	96.7	0.1	234.5	133.3	0.4	0.94	3.7
64	1.63	104.1	2.2	233.5	122.0	-0.2	0.57	3.6
71	1.47	94.2	0.5	231.5	94.5	0.4	0.88	3.8
80	1.42	96.1	1.7	214.9	100.2	1.4	0.67	3.0
17	1.40	102.3	4.5	214.2	115.9	19.4	0.30	3.5
68	1.40	96.8	4.0	219.3	102.1	-1.1	0.76	4.1
65	1.36	102.8	3.9	238.7	120.6	0.4	0.19	3.8
43	1.36	97.7	3.6	232.4	111.7	5.6	0.67	3.9
13	1.35	97.7	0.1	238.2	112.6	4.5	0.10	3.8
14	1.18	104.0	1.2	237.9	114.1	6.4	0.43	3.3
51	1.17	97.2	1.0	227.6	104.3	9.5	0.68	3.5
73	1.15	99.2	5.8	247.4	124.2	-0.7	0.21	4.0
56	1.04	99.3	2.8	209.3	96.1	23.3	0.44	3.7
69	0.59	95.6	2.9	166.9	66.4	-3.1	0.30	4.1
Mean	**2.09**	**96.4**	**1.7**	**223.7**	**109.3**	**4.5**	**0.77**	**3.6**
LSD	**0.92**	**2.6**	**2.3**	**20.9**	**23.0**	**15.7**	**0.45**	**0.73**
MSE	**0.42**	**3.3**	**2.8**	**220.0**	**267.5**	**62.5**	**0.05**	**0.3**
Min	**0.59**	**88.4**	**-2.8**	**166.9**	**66.4**	**-3.1**	**0.10**	**2.9**
Max	**3.26**	**104.8**	**5.8**	**260.3**	**148.3**	**31.4**	**1.49**	**4.5**

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; EH=ear height; RL=root lodging; EPP=ears per plant; SEN=senescence; LSD=least significant difference; MSE=mean square error; Min=minimum; Max=maximum.

Appendix 5 Performance of genotypes for grain yield and other agronomic traits across low nitrogen sites

ENTRY	GYD t ha^{-1}	AD d	ASI d	PH cm	RL %	EPP #	ER %
76	0.81	71.4	4.7	210.9	2.6	0.76	8.8
16	0.77	73.4	5.9	228.0	1.9	0.56	12.6
3	0.76	71.5	10.3	217.7	-2.0	0.76	10.1
1	0.75	73.4	4.6	222.3	-0.8	0.79	11.7
54	0.73	73.7	4.1	210.4	5.0	0.70	24.6
61	0.73	70.7	2.3	177.7	7.2	0.76	9.3
8	0.72	70.3	6.8	217.1	-1.0	0.78	12.5
78	0.71	74.1	7.9	192.3	6.3	0.65	27.9
57	0.68	73.6	8.6	221.9	-1.7	0.79	16.2
26	0.67	70.4	6.3	239.7	-3.1	0.71	26.0
39	0.67	73.8	4.4	238.9	-1.5	0.67	18.4
75	0.67	74.2	7.7	247.7	-5.8	0.70	15.7
59	0.65	70.6	1.2	192.1	-2.1	0.86	20.0
24	0.64	69.6	4.8	211.4	3.6	0.64	12.5
53	0.62	71.0	5.6	220.7	16.2	0.52	12.8
60	0.61	69.2	5.8	228.7	-2.2	0.94	7.1
34	0.60	69.5	7.6	195.0	0.3	0.79	14.1
47	0.60	73.2	3.4	214.5	-5.4	0.64	11.6
36	0.60	71.4	1.1	215.2	-0.4	0.77	16.7
7	0.59	71.7	8.9	222.6	0.8	0.82	25.1
14	0.59	76.6	10.6	211.8	-1.5	0.57	15.5
77	0.57	71.3	7.6	214.9	6.1	0.56	9.6
80	0.56	74.0	9.1	229.7	-1.9	0.77	11.5
4	0.55	71.4	7.3	204.4	5.9	0.82	27.3
42	0.54	71.4	6.9	205.8	-0.8	0.65	48.8
72	0.54	71.0	5.7	207.7	1.8	0.68	12.9
64	0.54	75.7	4.5	268.8	-1.4	0.62	10.1
55	0.54	72.4	3.3	206.4	1.1	0.87	15.9
62	0.53	71.7	2.9	227.4	-3.8	0.76	14.4
46	0.53	74.4	10.3	264.1	5.3	0.72	15.8
18	0.52	73.3	6.5	228.6	-0.3	0.70	17.4
48	0.52	77.3	8.7	251.5	5.4	0.78	8.8
23	0.51	70.6	2.9	189.8	-2.2	0.79	17.5
65	0.50	77.5	8.7	248.4	3.6	0.67	9.2
27	0.50	74.1	0.6	218.3	-1.0	0.58	8.6
17	0.50	76.7	8.4	200.5	17.9	0.50	15.8
79	0.50	78.5	4.5	228.2	-6.0	0.71	9.3
28	0.50	71.1	4.0	221.0	8.3	0.78	13.4
40	0.50	75.0	7.3	207.6	4.6	0.55	32.9
71	0.50	72.7	4.1	218.9	-0.3	0.70	9.8
68	0.49	72.7	8.1	226.9	7.9	0.73	9.0
21	0.49	70.1	6.4	204.9	10.6	0.71	7.4
19	0.48	71.3	3.9	198.0	12.3	0.68	10.9
58	0.48	73.0	6.9	223.0	-3.0	0.80	8.8
41	0.47	72.8	7.0	212.0	19.8	0.56	22.4
15	0.46	73.7	3.7	213.3	1.5	0.66	12.6

Appendix 5 Performance of genotypes for grain yield and other agronomic traits across low nitrogen sites

ENTRY	GYD t ha^{-1}	AD d	ASI d	PH cm	RL %	EPP #	ER %
74	0.46	75.1	9.8	266.9	5.3	0.68	16.3
66	0.46	71.7	8.6	205.1	9.6	0.68	14.2
13	0.43	74.8	9.7	212.9	35.7	0.54	19.6
20	0.43	70.7	6.7	217.9	33.3	0.62	29.9
63	0.42	70.7	8.0	191.5	25.4	0.61	13.9
38	0.41	70.8	6.2	207.2	2.7	0.77	14.4
35	0.39	70.6	10.0	221.1	1.0	0.65	19.7
49	0.39	73.7	8.5	240.8	-4.1	0.94	21.9
22	0.39	69.6	3.8	229.8	-5.5	0.76	13.9
67	0.38	77.2	7.7	229.4	-3.6	0.65	10.5
32	0.38	73.5	4.7	216.6	15.7	0.61	18.0
51	0.38	72.0	12.4	185.9	2.2	0.68	12.0
31	0.37	74.2	9.1	216.3	-2.3	0.63	22.3
52	0.37	71.9	8.3	238.2	1.7	0.74	47.3
29	0.36	75.2	9.7	203.9	3.1	0.57	23.7
12	0.36	77.0	10.3	219.5	6.0	0.68	14.5
9	0.35	74.5	7.8	204.3	6.4	0.70	29.0
44	0.35	71.7	9.6	228.1	0.3	0.62	14.9
6	0.34	70.9	10.2	246.3	-6.7	0.75	43.3
5	0.31	76.2	9.2	228.8	20.8	0.61	21.3
10	0.30	72.8	8.7	207.1	1.8	0.56	17.9
33	0.27	71.6	8.3	186.5	9.7	0.42	29.0
43	0.27	72.9	9.7	234.9	13.1	0.57	43.4
56	0.26	73.0	7.9	210.2	22.6	0.72	15.0
30	0.26	69.7	8.8	185.1	7.1	0.86	26.8
70	0.22	77.2	17.6	205.8	10.4	0.36	43.9
73	0.22	74.8	12.2	215.0	15.2	0.59	29.6
45	0.21	71.4	11.4	220.8	6.2	0.66	14.4
2	0.20	74.6	8.6	184.9	4.7	0.44	59.9
50	0.16	74.4	7.8	207.6	6.5	0.47	38.7
37	0.15	73.7	9.5	185.7	8.3	0.38	30.6
25	0.14	72.5	12.4	205.3	-0.3	0.44	21.3
11	0.10	71.9	12.6	234.5	31.6	0.67	16.8
69	0.08	75.5	8.8	208.3	6.0	0.51	24.3
Mean	**0.47**	**72.9**	**7.3**	**217.0**	**5.0**	**0.67**	**19.3**
LSD	**0.34**	**3.9**	**5.0**	**46.6**	**22.3**	**0.22**	**21.8**
MSE	**0.03**	**7.5**	**6.3**	**546.9**	**125.0**	**0.02**	**119.6**
DF	**79**						

GYD=grain yield; AD=anthesis days; ASI=anthesis silking interval; PH=plant height; RL=root lodging; EPP=ears per plant; ER=ear rot; LSD=least significant difference; MSE=mean square error; DF=degrees of freedom.

Appendix 6 Line general combining ability effects for grain yield across different environments

Line	Across	Optimum	Drought	Low N
K64r	-0.120	-0.377	0.372	-0.074
N3.2.3.3	0.172	0.593	-0.076	0.141
RS61P	0.717	0.635	0.369	0.582
NAW5885	0.131	0.355	0.119	0.213
2N3d	-0.900	-0.924	-0.215	-0.702
2Kba	-0.267	-0.699	-0.152	-0.122
SC5522	-0.953	-1.319	-0.537	-0.639
RA214P	0.180	0.373	-0.264	-0.208
LSD	**0.007**	**0.024**	**0.013**	**0.08**

LSD=least significant difference; Low N=low nitrogen.

Appendix 7 Tester general combining ability effects for grain yield across different environments

Tester	Across	Optimum	Drought	Low N
1	-0.92	-1.19	-0.12	-0.68
2	-0.64	-0.97	0.28	-0.46
3	0.16	0.22	-0.13	0.30
4	0.19	-0.02	0.59	0.27
5	0.24	0.16	-0.04	0.18
6	0.07	0.02	-0.03	-0.21
7	0.01	0.10	-0.16	-0.07
8	0.13	0.25	-0.10	-0.01
9	-0.11	0.01	-0.42	0.11
10	-0.84	-1.34	0.27	-0.64
11	0.34	0.48	0.39	0.09
12	-0.92	-1.10	0.01	-0.64
LSD	**0.007**	**0.024**	**0.013**	**0.08**

LSD=least significant difference; Low N=low nitrogen.

Appendix 8 Mean grain yield (t ha^{-1}) for 80 genotypes across seven environments

Genotype	E1	E2	E3	E4	E5	E6	E7	Mean t ha^{-1}
G1	7.22	1.44	4.69	1.83	4.40	2.63	3.31	**3.65**
G2	6.40	1.39	3.98	2.50	4.00	3.05	3.50	**3.55**
G3	7.18	1.72	4.01	2.74	4.04	2.05	3.35	**3.58**
G4	8.37	2.36	5.32	1.83	3.71	4.00	2.95	**4.08**
G5	8.23	1.67	5.27	2.03	4.73	3.14	2.92	**4.00**
G6	7.09	1.96	4.07	1.98	4.77	3.78	4.07	**3.96**
G7	7.82	1.66	5.24	1.71	5.23	2.73	3.31	**3.96**
G8	7.25	2.01	5.60	1.71	4.51	3.15	4.31	**4.08**
G9	6.04	1.51	5.49	2.04	4.38	2.80	2.64	**3.56**
G10	7.49	1.37	5.00	2.41	4.69	3.31	4.70	**4.14**
G11	7.32	1.37	3.55	2.47	4.18	3.56	3.54	**3.71**
G12	5.25	1.44	3.06	1.87	2.48	3.05	2.88	**2.86**
G13	5.33	1.59	3.45	1.35	3.92	3.78	4.12	**3.36**
G14	5.61	1.74	3.85	1.18	4.39	3.73	3.09	**3.37**
G15	6.74	1.48	3.94	1.95	4.06	2.72	5.09	**3.71**
G16	6.78	1.14	4.58	2.45	4.08	2.55	3.40	**3.57**
G17	4.42	0.60	2.33	1.40	2.97	2.10	2.34	**2.31**
G18	6.05	1.50	2.63	2.14	3.37	3.13	4.27	**3.30**
G19	5.53	1.36	4.52	3.01	3.71	2.99	4.09	**3.60**
G20	5.95	2.02	3.25	1.80	4.13	3.27	5.96	**3.77**
G21	5.71	1.19	4.33	2.05	3.37	3.27	4.54	**3.49**
G22	5.91	1.87	3.44	2.39	3.27	3.70	5.83	**3.77**
G23	5.75	1.00	4.13	2.15	2.95	3.11	4.11	**3.31**
G24	5.65	1.22	4.35	2.23	3.55	3.35	3.16	**3.36**
G25	4.95	1.04	4.80	2.04	2.85	3.40	3.81	**3.27**
G26	8.31	1.73	4.84	2.04	3.84	3.47	3.73	**3.99**
G27	7.50	1.57	4.59	3.07	3.70	3.20	3.07	**3.82**
G28	7.11	1.56	5.54	2.28	3.89	3.23	3.26	**3.84**
G29	4.19	0.89	2.44	2.35	2.70	1.98	2.21	**2.39**
G30	4.99	2.05	4.63	2.71	3.09	3.02	4.44	**3.56**
G31	6.83	1.51	4.77	2.09	3.70	2.87	4.71	**3.78**
G32	6.58	1.65	4.73	2.24	3.41	3.62	4.32	**3.79**
G33	5.19	0.95	4.75	2.44	3.20	3.71	4.18	**3.49**
G34	7.07	1.68	5.98	2.64	3.60	3.65	4.30	**4.13**
G35	5.35	1.74	5.38	2.55	3.54	2.64	4.55	**3.68**
G36	6.09	1.35	4.57	2.79	3.54	2.93	4.01	**3.61**
G37	4.74	0.64	3.24	1.91	2.57	2.90	3.59	**2.80**
G38	6.99	1.88	5.82	3.01	3.98	4.16	3.89	**4.25**
G39	6.98	1.89	3.10	1.84	3.29	2.11	2.53	**3.11**
G40	7.27	1.10	4.39	2.28	3.78	2.40	2.62	**3.41**
G41	6.44	1.06	3.10	2.04	4.38	3.08	3.54	**3.38**
G42	5.75	1.50	4.68	1.77	4.15	2.58	2.86	**3.33**
G43	7.83	1.62	4.69	1.36	3.36	2.84	2.40	**3.44**
G44	6.45	1.51	4.52	1.91	3.93	3.26	3.04	**3.52**
G45	7.51	1.97	6.03	1.92	4.21	3.00	4.59	**4.18**
G46	6.35	2.40	3.71	1.99	4.42	2.99	3.89	**3.68**
G47	7.39	1.28	5.11	1.95	4.58	3.47	3.97	**3.96**
G48	8.37	1.63	4.60	1.99	5.39	3.23	4.61	**4.26**
G49	7.47	2.12	4.53	1.78	4.88	1.91	2.48	**3.60**

Appendix 8 Mean grain yield (t ha^{-1}) for 80 genotypes across seven environments

Genotype	E1	E2	E3	E4	E5	E6	E7	Mean t ha^{-1}
G50	5.50	1.29	4.53	1.79	3.43	2.75	2.80	**3.16**
G51	7.64	1.38	6.02	1.17	4.70	3.16	3.82	**3.99**
G52	8.91	2.35	5.04	**3.26**	4.04	3.06	3.26	**4.27**
G53	7.41	0.88	3.73	1.69	4.01	2.35	3.62	**3.38**
G54	7.72	1.54	4.26	2.33	4.90	3.63	6.12	**4.36**
G55	5.76	1.77	3.96	2.65	3.61	3.29	5.55	**3.80**
G56	5.60	1.82	3.44	1.04	3.99	2.46	4.31	**3.24**
G57	8.20	2.44	6.42	1.78	4.29	4.26	5.19	**4.66**
G58	7.22	1.96	5.51	2.43	3.89	3.93	5.02	**4.28**
G59	6.78	2.08	5.00	2.84	3.89	3.35	5.15	**4.16**
G60	6.69	2.83	6.37	2.51	4.18	3.91	4.31	**4.40**
G61	8.33	2.31	**6.68**	2.79	3.65	3.65	5.76	**4.74**
G62	7.84	1.49	4.67	2.46	4.33	3.53	4.18	**4.07**
G63	6.87	1.30	5.40	2.62	3.23	4.08	**6.51**	**4.29**
G64	8.10	1.28	2.59	1.63	3.39	2.94	3.75	**3.38**
G65	6.42	2.02	4.00	1.36	4.26	3.09	4.53	**3.67**
G66	6.68	1.31	3.95	2.77	4.13	3.70	3.30	**3.69**
G67	6.94	1.19	4.55	2.03	4.44	**4.45**	4.23	**3.98**
G68	9.99	1.70	4.82	1.40	4.22	3.77	3.72	**4.23**
G69	5.90	0.94	3.84	0.79	3.19	3.18	3.86	**3.10**
G70	8.02	1.03	4.62	2.38	4.05	3.80	4.90	**4.11**
G71	7.56	1.22	4.31	1.47	4.43	3.73	4.01	**3.82**
G72	7.46	1.51	5.66	2.31	4.18	3.93	4.16	**4.17**
G73	5.50	1.29	2.91	1.15	3.46	2.00	2.24	**2.65**
G74	**10.43**	**4.08**	5.29	2.50	**6.16**	2.50	4.85	**5.12**
G75	7.96	1.64	3.10	1.94	4.21	3.43	3.56	**3.69**
G76	7.25	2.28	4.51	1.99	4.56	3.16	2.53	**3.75**
G77	6.83	2.11	5.13	2.21	4.31	2.79	3.08	**3.78**
G78	6.96	1.94	4.30	1.78	4.20	3.33	5.12	**3.95**
G79	8.39	1.28	5.51	2.49	4.51	3.82	3.56	**4.22**
G80	6.93	2.02	4.56	1.42	4.12	2.30	4.00	**3.62**
MEAN	**6.83**	**1.62**	**4.50**	**2.09**	**3.96**	**3.17**	**3.92**	

E1=Agricultural Research Trust farm; E2=Harare low N; E3=Kadoma; E4=Chiredzi winter; E5=Rattray Arnold Research Station; E6=Chiredzi summer; E7=Chisumbanje; Underlined and bold values= highest yielder in the given environment.

Appendix 9 Minor allele frequency and corresponding number of single nucleotide polymorphism markers

Minor allele frequency	Number of SNPs
0.50	30
0.48	27
0.47	18
0.46	2
0.45	46
0.44	9
0.43	32
0.42	11
0.41	13
0.40	15
0.39	20
0.38	19
0.37	11
0.36	27
0.35	14
0.34	17
0.33	33
0.32	15
0.31	15
0.30	22
0.29	22
0.28	19
0.27	13
0.26	21
0.25	24
0.24	40
0.23	22
0.22	4
0.21	21
0.20	27
0.19	11
0.18	42
0.17	32
0.16	20
0.15	18
0.14	33
0.13	29
0.12	16
0.11	33
0.10	34
0.09	33
0.08	16
0.07	43
0.06	3
0.05	50
0.04	30
0.03	13
0.02	39
0.00	25
Total	**1129**

SNP=single nucleotide polymorphism.

Appendix 10 Polymorphic information content values and corresponding number of single nucleotide polymorphism markers

PIC	Number of SNPs
0.38	30
0.37	164
0.36	69
0.35	71
0.34	37
0.33	32
0.32	47
0.31	21
0.30	64
0.29	24
0.28	20
0.27	29
0.26	16
0.25	44
0.24	25
0.23	20
0.22	18
0.21	33
0.20	14
0.19	31
0.18	13
0.17	20
0.16	34
0.15	33
0.13	16
0.12	32
0.11	11
0.10	3
0.09	33
0.08	47
0.06	1
0.05	19
0.04	32
0.00	26
Total	**1129**

PIC=polymorphic information content; SNP=single nucleotide polymorphism.

Appendix 11 F_1 mean grain yield (t ha^{-1}), specific combining ability, mid- and high-parent heterosis and genetic distance under optimum conditions

Hybrid	GYD	MPH	HPH	GD	SCA
L2/T10	5.64	161.86	138.10	0.35	0.34
L2/T10	4.96	92.97	54.98	0.35	0.38
L2/T4	5.38	121.69	84.74	0.36	0.94
L2/T9	6.54	187.98	151.43	0.33	-0.33
L2/T5	5.65	121.51	78.75	0.35	0.38
L2/T8	6.61	168.56	121.69	0.34	-0.97
L2/T3	6.05	170.08	138.18	0.36	0.13
L2/T11	5.74	97.42	48.19	0.36	-0.37
L2/T7	6.05	143.13	99.14	0.33	0.62
L2/T6	5.40	143.84	116.91	0.32	0.42
L7/T10	3.54	136.19	49.48	0.36	-0.05
L7/T1	4.49	134.68	40.44	0.34	0.34
L7/T9	4.79	196.74	84.32	0.28	-0.28
L7/T7	5.03	174.04	65.41	0.24	0.16
L7/T6	5.52	253.53	121.49	0.31	-0.07
L6/T9	3.10	49.51	19.32	0.33	-0.35
L6/T1	4.17	75.63	30.35	0.34	-0.55
L6/T4	4.82	115.98	65.51	0.35	0.37
L6/T5	4.64	97.21	46.97	0.35	0.51
L6/T8	4.75	109.70	59.39	0.34	0.67
L6/T3	4.33	111.91	70.61	0.35	-0.15
L6/T7	4.46	94.35	46.72	0.36	-0.48
L6/T12	4.71	133.05	89.06	0.33	-0.13
L6/T6	4.52	123.93	81.66	0.34	-0.01
L1/T2	5.91	194.14	162.77	0.32	-1.10
L1/T10	5.64	172.30	137.83	0.37	-0.05
L1/T12	5.94	178.73	138.43	0.33	-0.14
L1/T1	2.91	16.91	-9.21	0.23	0.12
L1/T4	4.44	89.80	52.62	0.27	-0.49
L1/T9	5.44	148.92	109.19	0.33	0.99
L1/T5	5.26	113.26	66.36	0.31	0.12
L1/T8	4.78	101.13	60.30	0.31	-0.44
L1/T3	5.89	173.19	131.78	0.33	0.57
L1/T11	4.98	76.43	28.56	0.30	-0.18
L1/T7	4.91	104.06	61.43	0.36	0.70
L1/T6	3.67	72.36	47.44	0.33	-0.26
L4/T4	6.02	163.02	106.98	0.32	-0.57
L4/T9	4.81	125.11	84.85	0.29	-0.68

Appendix 11 F_1 mean grain yield (t ha^{-1}), specific combining ability, mid- and high-parent heterosis and genetic distance under optimum conditions

Hybrid	GYD	MPH	HPH	GD	SCA
L4/T5	5.52	128.46	74.60	0.34	-0.36
L4/T8	4.77	105.21	60.11	0.31	0.99
L4/T3	4.99	136.99	96.40	0.29	0.35
L4/T11	5.73	175.41	130.06	0.29	0.42
L4/T7	5.28	124.19	73.67	0.32	-0.26
L4/T6	6.34	204.87	154.67	0.29	-0.71
L5/T1	5.17	76.68	61.49	0.36	-0.18
L5/T4	6.06	117.89	108.16	0.34	-0.06
L5/T9	6.63	152.70	155.13	0.29	-0.55
L5/T5	6.00	106.68	90.00	0.20	0.26
L5/T8	4.73	67.99	58.69	0.33	-0.19
L5/T3	6.52	151.37	156.82	0.34	0.47
L5/T11	6.45	97.91	66.71	0.35	0.11
L5/T7	5.28	85.74	73.83	0.33	0.00
L3/T1	5.98	83.45	86.89	0.33	-0.07
L3/T4	4.63	48.63	59.10	0.30	-0.17
L3/T9	6.63	124.14	155.18	0.27	-0.86
L3/T5	5.87	81.27	85.86	0.34	0.72
L3/T8	5.50	74.50	84.45	0.29	0.74
L3/T3	6.17	110.52	142.84	0.30	0.83
L3/T11	6.62	84.23	71.14	0.29	0.25
L3/T7	5.81	82.81	91.22	0.32	0.36
L3/T6	5.53	90.23	121.94	0.27	0.13
L8/T9	4.86	97.79	86.76	0.28	0.84
L8/T1	5.05	83.36	57.86	0.33	-0.30
L8/T4	5.22	99.83	79.23	0.28	-1.05
L8/T5	5.55	102.91	75.62	0.34	-0.13
L8/T8	6.79	156.75	127.89	0.26	-0.19
L8/T3	4.71	94.20	85.41	0.32	0.61
L8/T11	5.89	90.58	52.17	0.32	0.13
L8/T7	5.78	116.00	90.07	0.33	0.76
L8/T6	6.09	153.83	144.65	0.29	-0.54
Mean	**5.19**	**117.39**	**85.29**	**0.31**	**0.03**
Min	**2.91**	**16.91**	**-9.21**	**0.20**	**-1.10**
Max	**6.79**	**253.53**	**162.77**	**0.37**	**0.99**

GYD=grain yield; MPH=mid-parent heterosis; HPH=high-parent heterosis; GD=genetic distance; SCA=specific combining ability; Min=minimum; Max=maximum.

Appendix 12 F_1 mean grain yield (t ha^{-1}), specific combining ability, mid- and high-parent heterosis and genetic distance under low nitrogen conditions

Hybrid	GYD	MPH	HPH	GD	SCA
L2/T10	2.50	1.81	37.61	0.35	-0.05
L2/T10	2.68	-4.93	34.05	0.35	0.07
L2/T4	3.06	-1.09	20.05	0.36	-0.11
L2/T9	4.45	12.84	59.92	0.33	0.73
L2/T5	3.76	-5.49	2.74	0.35	0.13
L2/T8	3.02	46.20	106.45	0.34	-0.39
L2/T3	3.66	-10.26	-2.44	0.36	0.18
L2/T11	2.85	14.00	31.63	0.36	-0.30
L2/T7	2.60	-10.25	5.15	0.33	0.26
L2/T6	2.69	-41.13	-49.88	0.32	0.04
L7/T10	2.69	7.22	47.57	0.36	0.09
L7/T1	2.97	132.01	110.26	0.34	-0.01
L7/T9	3.19	95.24	48.38	0.28	-0.50
L7/T7	2.73	54.27	3.01	0.24	0.10
L7/T6	1.90	-12.29	-47.34	0.31	-0.15
L6/T9	0.94	57.33	38.38	0.33	0.43
L6/T1	2.74	-59.76	-69.63	0.34	-0.13
L6/T4	2.47	52.94	36.89	0.35	-0.07
L6/T5	3.83	148.86	155.50	0.35	-0.06
L6/T8	2.14	19.85	-2.95	0.34	0.07
L6/T3	3.55	-8.61	-31.01	0.35	0.06
L6/T7	1.74	62.79	27.66	0.36	0.03
L6/T12	2.13	-48.53	-66.42	0.33	-0.28
L6/T6	2.02	22.20	11.91	0.34	0.66
L1/T2	3.12	273.74	47.49	0.32	-0.55
L1/T10	2.89	207.31	138.11	0.37	-0.41
L1/T12	2.87	189.25	125.97	0.33	-0.45
L1/T1	1.60	119.17	51.11	0.23	-0.28
L1/T4	3.97	17.57	-20.06	0.27	0.26
L1/T9	2.83	142.85	55.71	0.33	0.49
L1/T5	3.12	48.01	-8.81	0.31	0.10
L1/T8	1.76	180.81	107.80	0.31	-0.15
L1/T3	3.06	-8.03	-43.34	0.33	0.05
L1/T11	3.29	75.08	10.21	0.3	0.45
L1/T7	2.41	91.95	21.47	0.36	0.23
L1/T6	1.20	-18.33	-53.49	0.33	-0.56
L4/T4	3.55	15.00	-12.28	0.32	0.06
L4/T9	3.44	104.50	39.14	0.29	-0.16
L4/T5	1.95	70.97	10.85	0.34	0.74

Appendix 12 F_1 mean grain yield (t ha^{-1}), specific combining ability, mid- and high-parent heterosis and genetic distance under low nitrogen conditions

Hybrid	GYD	MPH	HPH	GD	SCA
L4/T8	1.89	61.25	30.07	0.31	-0.41
L4/T3	2.73	-6.15	-39.15	0.29	-0.12
L4/T11	3.10	47.56	-1.80	0.29	-0.16
L4/T7	2.85	170.81	126.34	0.32	0.34
L4/T6	3.84	-6.40	-44.89	0.29	-0.31
L5/T1	4.53	235.21	180.15	0.36	-0.07
L5/T4	2.26	66.63	126.62	0.34	-0.19
L5/T9	3.01	-24.52	-11.35	0.29	0.15
L5/T5	4.05	-8.05	-3.00	0.2	0.16
L5/T8	2.50	63.88	135.34	0.33	-0.09
L5/T3	2.57	-23.54	-19.35	0.34	0.41
L5/T11	4.51	-17.48	-7.68	0.35	-0.08
L5/T7	1.45	46.72	66.49	0.33	-0.12
L3/T1	2.72	-66.25	-71.92	0.33	0.21
L3/T4	3.27	12.38	35.99	0.3	-0.53
L3/T9	4.54	21.25	28.15	0.27	-0.18
L3/T5	3.68	6.21	37.45	0.34	0.20
L3/T8	3.83	52.87	46.45	0.29	0.67
L3/T3	5.35	69.78	145.61	0.3	0.41
L3/T11	4.27	29.09	23.67	0.29	0.08
L3/T7	2.71	90.32	92.37	0.32	-0.14
L3/T6	2.39	53.71	57.39	0.27	0.47
L8/T9	2.28	-32.50	-47.75	0.28	0.30
L8/T1	3.80	13.51	68.26	0.33	-0.64
L8/T4	2.39	-41.13	-26.32	0.28	-0.11
L8/T5	2.20	14.02	62.95	0.34	-0.09
L8/T8	3.16	-33.63	-6.17	0.26	0.18
L8/T3	1.76	-28.59	-5.60	0.32	-0.01
L8/T11	1.95	-18.45	2.07	0.32	0.43
L8/T7	2.19	-52.81	-36.86	0.33	-0.10
L8/T6	2.74	-47.17	-28.16	0.29	-0.12
Mean	**2.90**	**33.09**	**25.73**	**0.31**	**0.01**
Min	**0.94**	**-66.25**	**-71.92**	**0.20**	**-0.64**
Max	**5.35**	**273.74**	**155.50**	**0.37**	**0.74**

GYD=grain yield; MPH=mid-parent heterosis; HPH=high-parent heterosis; GD=genetic distance; SCA=specific combining ability; Min=minimum; Max=maximum.

Appendix 13 Mean performance of three-way hybrids for grain yield and other agronomic traits under managed drought in the 2011 winter season

ENTRY	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER	SEN	TEX	EA
SC7//L5	2.89	63.3	2.8	178.3	78.0	0.43	10.6	19.8	0.76	5.4	0.5	1.5	2.4
SC8//L10	2.89	63.9	1.2	174.7	101.0	0.53	11.1	5.1	0.65	16.0	0.5	2.7	3.3
SC3//L2	2.88	66.4	1.6	167.7	82.7	0.45	15.8	22.0	0.65	30.8	0.6	3.5	3.6
SC2//L5	2.87	64.9	1.5	170.5	77.4	0.46	11.7	13.1	0.75	6.3	0.6	1.0	2.7
SC10//L6	2.86	66.4	1.0	177.1	88.9	0.52	-1.5	12.1	0.69	16.3	0.5	2.7	3.0
SC3//L1	2.63	65.2	5.4	176.3	81.3	0.41	8.0	12.2	0.56	21.9	0.5	3.0	3.0
SC2//L9	2.61	67.9	1.6	180.3	96.7	0.51	11.7	10.8	0.69	26.0	0.6	3.0	3.8
SC10//L5	2.59	63.7	0.8	178.4	90.4	0.47	10.4	29.4	0.76	4.9	0.6	3.0	3.2
CZH0616	2.57	64.7	4.0	174.8	85.8	0.50	6.3	9.9	0.69	11.4	0.5	3.0	2.9
023WH31	2.54	69.4	1.8	185.9	100.9	0.55	11.8	11.4	0.67	23.3	0.5	2.8	3.2
SC635	2.54	62.9	6.6	133.1	93.0	0.59	10.8	24.5	0.61	34.9	0.6	3.5	3.6
CZH0837	2.53	65.1	1.9	197.6	89.8	0.48	13.4	12.4	0.69	14.4	0.6	2.9	3.5
SC4//L10	2.51	63.0	1.5	170.3	99.4	0.55	33.0	-1.1	0.73	22.5	0.6	3.4	3.5
SC11//L2	2.50	66.6	1.7	182.7	91.3	0.44	9.4	13.9	0.67	24.0	0.5	3.1	2.9
SC3//L10	2.44	61.8	1.0	165.5	90.8	0.49	13.3	10.3	0.83	14.1	0.6	2.7	3.4
SC2//L8	2.42	64.7	-1.1	185.3	90.1	0.53	34.4	13.3	0.79	33.4	0.6	3.2	3.6
SC7//L10	2.42	62.3	3.1	168.6	66.5	0.37	17.2	16.0	0.74	6.8	0.5	2.3	3.3
SC1//L5	2.42	66.2	1.4	166.1	82.6	0.46	14.7	32.0	0.71	3.9	0.5	1.8	2.4
SC8//L8	2.34	70.6	1.2	169.1	104.6	0.56	-2.3	5.8	0.57	32.9	0.5	2.8	4.0
SC3//L9	2.33	67.3	1.1	167.1	103.6	0.55	21.7	22.5	0.54	19.3	0.6	2.8	3.1
013WH29	2.31	66.3	1.5	172.6	93.4	0.51	18.3	0.7	0.66	13.2	0.5	2.4	3.3
SC7//L7	2.28	65.7	3.7	170.6	81.9	0.48	2.7	11.1	0.62	32.9	0.5	2.3	3.5
SC1//L2	2.25	67.8	3.5	171.5	91.3	0.50	3.7	26.5	0.62	9.2	0.5	3.3	3.3
SC3//L8	2.25	62.7	0.3	165.4	81.9	0.48	6.7	25.2	0.53	180.9	0.6	2.7	3.8
SC7//L9	2.24	67.6	1.6	183.8	99.3	0.52	10.3	8.6	0.54	21.4	0.5	2.3	3.2
SC5//L10	2.23	66.3	1.7	188.2	105.7	0.53	28.9	9.6	0.62	13.3	0.6	3.6	3.6
SC1//L6	2.23	67.7	1.3	165.5	91.8	0.50	4.8	2.5	0.61	24.2	0.6	2.9	3.4
SC5//L8	2.22	66.4	1.3	173.1	99.7	0.54	8.7	15.6	0.65	34.2	0.5	3.1	4.0
SC2//L4	2.21	65.7	1.9	180.9	95.2	0.52	22.5	14.0	0.56	27.3	0.6	3.1	3.4
SC2//L10	2.19	62.2	0.9	166.1	92.4	0.54	50.7	9.7	0.65	14.4	0.6	2.6	3.3
SC11//L5	2.18	63.8	0.9	170.7	94.5	0.47	12.6	28.7	0.65	19.5	0.6	1.6	2.7
SC3//L11	2.14	64.0	0.7	156.6	91.8	0.47	23.1	33.6	0.70	26.5	0.6	2.7	2.9
SC4//L11	2.14	65.4	2.5	174.4	100.7	0.55	-0.4	14.5	0.63	19.6	0.5	3.3	4.1
SC3//L7	2.12	65.4	0.1	161.3	83.6	0.50	14.7	24.5	0.77	2.3	0.6	2.8	2.8
SC10//L8	2.11	64.0	1.8	163.6	95.6	0.53	12.1	6.0	0.72	23.8	0.5	3.6	4.0

Appendix 13 Mean performance of three-way hybrids for grain yield and other agronomic traits under managed drought in the 2011 winter season

ENTRY	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER	SEN	TEX	EA
SC10//L7	2.08	66.9	1.7	161.4	83.7	0.49	19.2	25.4	0.69	13.0	0.5	2.7	3.2
SC11/L8	2.08	62.8	1.3	177.1	95.3	0.51	15.0	9.0	0.82	21.4	0.5	2.2	3.2
SC11//L6	2.05	65.9	1.1	167.3	85.9	0.49	-1.9	10.1	0.68	9.6	0.5	2.6	3.5
SC4//L12	2.04	63.0	1.8	167.2	100.8	0.59	31.8	11.6	0.71	45.6	0.6	4.1	3.6
SC1//L8	2.01	64.6	0.7	178.8	92.6	0.48	20.9	9.7	0.75	32.3	0.6	2.8	3.3
SC3//L3	1.99	63.6	2.7	188.0	92.6	0.48	20.6	14.8	0.57	27.8	0.6	3.0	3.5
SC6//L12	1.99	62.7	1.8	182.6	100.2	0.48	17.0	20.3	0.66	22.5	0.6	3.6	3.8
SC8//L9	1.99	68.5	3.9	178.2	103.5	0.67	2.2	1.8	0.50	26.8	0.5	2.6	4.1
SC10//L2	1.98	68.0	3.3	179.2	95.6	0.56	9.8	6.4	0.53	37.6	0.5	3.4	3.7
SC10//L1	1.96	66.5	5.7	187.3	98.9	0.55	12.2	6.9	0.56	40.2	0.5	3.0	4.1
SC10//L3	1.94	67.7	5.8	180.5	96.8	0.52	10.3	5.5	0.58	17.6	0.5	3.4	3.4
SC2//L6	1.94	64.9	0.1	157.8	79.9	0.48	7.6	24.3	0.65	6.9	0.6	2.6	3.8
SC9/L12	1.94	64.8	1.1	174.0	98.9	0.53	17.7	20.2	0.66	27.8	0.6	4.0	3.4
SC2//L2	1.90	66.0	1.1	171.9	94.6	0.54	17.8	19.3	0.53	48.5	0.6	3.8	4.4
SC6//L10	1.89	63.9	3.1	169.6	97.2	0.56	26.3	18.5	0.59	34.0	0.6	3.5	3.9
SC9//L9	1.86	69.3	3.8	169.0	113.7	0.60	16.4	10.6	0.54	18.1	0.5	2.9	3.5
SC2//L11	1.85	66.0	1.7	169.7	92.8	0.51	12.9	11.8	0.69	42.7	0.6	2.7	3.9
SC513	1.82	65.6	2.8	189.8	82.1	0.43	41.9	8.0	0.58	28.2	0.5	1.9	3.4
SC2//L7	1.79	66.1	2.0	169.8	88.3	0.48	29.3	29.3	0.61	21.3	0.6	3.5	3.1
SC1//L3	1.79	67.6	3.0	175.2	93.8	0.50	16.9	13.6	0.53	37.5	0.5	3.1	3.0
SC11//L7	1.78	65.4	2.2	164.6	90.5	0.53	0.7	32.3	0.68	7.9	0.6	1.7	3.1
SC8//L5	1.75	63.4	1.9	171.6	98.0	0.53	5.7	10.5	0.57	32.1	0.5	2.8	3.8
SC8//L12	1.75	66.2	0.0	172.1	89.3	0.52	8.1	14.7	0.63	36.6	0.6	3.3	4.5
SC5//L1	1.74	68.4	5.8	177.1	107.3	0.57	14.2	7.1	0.34	16.9	0.5	3.7	3.2
SC9//L10	1.71	63.4	3.3	181.5	100.2	0.54	16.1	13.3	0.61	10.4	0.5	3.3	3.4
SC6//L11	1.67	67.5	3.3	186.3	107.1	0.58	7.8	11.8	0.52	43.0	0.5	3.5	4.6
SC2//L4	1.64	66.4	2.7	176.5	95.5	0.51	17.8	12.1	0.50	41.9	0.6	3.8	3.2
SC7//L6	1.64	66.8	2.0	175.7	83.0	0.46	0.0	12.1	0.58	31.7	0.6	2.7	3.9
SC3//L6	1.63	66.0	0.8	175.6	78.4	0.47	10.5	16.9	0.57	12.9	0.6	2.9	3.3
SC7//L2	1.60	66.2	5.1	188.6	99.3	0.50	14.3	9.7	0.47	18.1	0.5	2.9	3.4
SC3//L4	1.60	64.5	1.4	184.3	97.5	0.51	19.3	26.0	0.56	29.3	0.7	3.5	3.2
SC1//L4	1.59	66.6	4.1	191.1	96.2	0.50	1.6	10.1	0.52	31.9	0.6	3.3	3.2
SC5//L3	1.55	67.7	3.5	185.4	104.3	0.54	16.6	-0.3	0.42	29.1	0.5	3.1	3.5
SC5//L7	1.52	67.7	2.1	170.7	95.1	0.52	12.0	20.6	0.53	45.7	0.6	3.5	4.3
SC9//L11	1.51	67.3	3.6	160.9	102.2	0.70	9.9	7.7	0.48	25.5	0.6	2.8	3.5
SC2//L1	1.48	66.7	6.8	185.6	94.9	0.50	19.8	7.4	0.44	39.0	0.6	3.4	4.1
013WH01	1.45	68.5	5.2	198.5	109.2	0.54	7.9	4.6	0.33	40.3	0.6	2.5	3.2

Appendix 13 Mean performance of three-way hybrids for grain yield and other agronomic traits under managed drought in the 2011 winter season

ENTRY	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER	SEN	TEX	EA
SC1//L1	1.44	66.9	5.2	176.7	83.0	0.47	7.7	14.3	0.41	24.0	0.5	3.0	3.4
SC1//L7	1.41	68.1	2.0	168.3	88.5	0.48	8.1	26.7	0.51	12.5	0.6	3.0	3.5
SC5//L6	1.34	69.2	1.1	174.1	100.2	0.54	2.8	6.9	0.42	30.2	0.6	3.3	4.0
SC4//L9	1.27	67.6	3.0	165.9	108.2	0.60	1.7	8.6	0.42	40.6	0.5	2.9	4.2
SC7//L3	1.26	65.9	4.7	184.5	95.1	0.51	8.5	1.6	0.42	18.4	0.5	2.9	3.7
SC8/L11	1.22	67.6	3.6	179.6	102.3	0.54	6.4	14.6	0.36	31.1	0.6	2.7	3.5
SC6/L9	1.19	68.2	2.9	181.4	102.5	0.55	7.9	12.3	0.49	21.5	0.5	3.3	4.2
SC7//L4	1.18	65.7	5.9	195.1	103.0	0.52	9.8	5.6	0.39	34.1	0.5	2.5	3.5
SC5//L2	1.15	69.6	6.3	187.5	98.1	0.51	8.7	9.0	0.41	27.0	0.5	4.1	4.1
SC5//L11	0.83	68.9	2.6	168.5	117.7	0.65	0.2	7.3	0.37	26.8	0.6	3.3	4.1
SC10//L4	0.83	68.1	7.4	190.5	107.3	0.56	6.8	11.9	0.26	48.0	0.5	3.4	4.2
SC11//L4	0.80	66.1	3.4	178.6	102.2	0.51	22.3	16.8	0.51	22.9	0.5	3.0	3.3
013WH63	0.68	66.9	5.1	190.2	97.0	0.53	17.2	6.7	0.40	40.6	0.6	3.9	4.3
Mean	**1.95**	**66.0**	**2.6**	**175.5**	**94.5**	**0.52**	**13.1**	**13.7**	**0.59**	**26.6**	**0.6**	**3.0**	**3.5**
LSD (0.05)	**1.03**	**2.0**	**2.4**	**17.8**	**13.1**	**0.10**	**20.3**	**13.0**	**0.16**	**46.7**	**0.1**	**0.9**	**0.9**
MSE	**0.53**	**2.1**	**4.4**	**80.5**	**130.6**	**0.00**	**104.5**	**128.9**	**0.02**	**1651.1**	**0.0**	**0.2**	**0.4**
Min	**0.68**	**61.8**	**-1.1**	**133.1**	**66.5**	**0.37**	**-2.3**	**-1.1**	**0.26**	**2.3**	**0.5**	**1.0**	**2.4**
Max	**2.89**	**70.6**	**7.4**	**198.5**	**117.7**	**0.70**	**50.7**	**33.6**	**0.83**	**180.9**	**0.7**	**4.1**	**4.6**

GYD=grain yield (t ha^{-1}); AD=anthesis days; ASI=anthesis silking interval (days); PH=plant height (cm); EH=ear height (cm); EPO=ear position (0-1); RL=root lodging (%); SL=stem lodging (%); EPP=ears per plant (#); ER=ear rot (%); SEN=senescence (1-10); TEX=texture (1-5); EA=ear aspect (1-5); LSD=least significant difference; MSE=mean square error; Min=minimum; Max=maximum.

Appendix 14 Mean performance of three-way hybrids for grain yield and other agronomic traits under optimum conditions in the 2011 winter season

ENTRY	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER	TEX	EA
SC9//L10	4.79	69.9	2.1	162.9	90.9	0.57	34.6	17.8	0.66	15.4	3.6	4.1
SC10//L5	4.65	71.2	2.9	174.0	90.7	0.54	-5.2	16.5	0.94	7.8	3.1	3.7
SC4//L10	4.64	70.8	1.4	171.2	103.1	0.60	12.0	9.6	0.75	8.6	3.7	3.9
SC6//L10	4.64	71.2	2.0	176.5	98.3	0.56	30.4	7.6	0.83	8.6	3.8	3.8
SC8//L10	4.63	71.6	1.8	179.6	98.5	0.55	2.2	6.9	0.79	11.0	3.6	4.1
SC3//L8	4.63	69.7	4.0	157.6	82.2	0.52	0.9	20.2	0.84	2.8	3.3	3.8
SC11//L5	4.60	69.8	4.0	156.8	89.7	0.58	2.5	22.3	0.91	3.3	2.7	3.5
SC6//L9	4.58	73.6	0.9	187.0	104.2	0.56	-9.7	9.8	0.69	12.1	3.7	3.3
SC9//L9	4.52	75.1	0.7	177.7	99.6	0.57	-2.3	43.4	1.25	6.5	4.8	2.6
SC1//L6	4.50	71.6	2.1	172.6	89.1	0.53	9.4	11.2	0.79	3.2	3.3	3.6
SC10//L6	4.50	71.5	1.5	166.9	92.5	0.55	-6.9	9.2	0.75	6.1	3.7	3.9
SC8//L9	4.46	74.6	0.3	183.4	105.5	0.57	-2.8	2.3	0.80	6.0	3.4	3.5
SC10//L7	4.42	72.1	5.5	163.1	90.2	0.55	0.6	35.4	0.88	7.6	3.2	3.4
SC2//L9	4.40	72.7	1.4	181.8	95.9	0.55	-11.5	15.8	0.77	11.8	3.3	3.8
SC1//L2	4.36	73.8	1.1	174.9	88.3	0.51	-5.4	15.1	0.66	18.8	3.7	3.7
SC8//L5	4.36	70.9	2.4	174.8	94.8	0.54	6.4	6.7	0.80	8.9	2.9	3.2
013WH29	4.33	71.2	2.5	162.1	96.6	0.61	-5.2	12.8	0.80	19.6	3.3	3.6
SC10//L8	4.33	71.2	1.8	172.5	100.1	0.58	-11.1	15.5	0.79	9.8	3.4	3.9
SC8//L8	4.27	74.3	0.7	181.6	101.7	0.56	11.2	10.7	0.74	11.5	3.4	3.7
SC11//L8	4.22	70.4	1.4	171.3	103.0	0.60	7.8	10.4	0.86	7.3	2.9	3.6
SC5//L6	4.11	74.0	-0.1	182.6	86.8	0.49	-4.1	13.0	0.82	3.2	3.5	3.9
CZH0616	4.01	70.6	3.7	152.9	86.5	0.58	-6.4	22.8	0.89	13.5	3.1	3.7
SC10//L1	4.00	71.5	5.6	176.6	94.2	0.54	7.0	18.4	0.84	8.4	3.5	4.1
SC11//L6	3.99	72.8	-0.1	165.9	91.3	0.55	3.5	18.3	0.84	10.0	3.0	3.3
SC3//L10	3.99	68.6	5.7	180.3	94.6	0.53	5.1	11.6	0.85	5.8	3.1	3.7
SC5//L11	3.99	72.8	1.6	184.1	116.4	0.63	-7.4	15.3	0.69	15.4	3.3	4.4
SC2//L10	3.92	69.9	3.7	160.7	92.6	0.58	30.3	3.2	0.80	17.6	3.4	4.3
SC5//L10	3.87	72.2	0.9	177.3	108.8	0.62	14.2	9.1	0.70	11.0	2.9	3.4
SC6//L11	3.86	72.7	1.5	182.7	102.6	0.56	22.7	14.2	0.72	11.4	3.3	3.6
SC8//L11	3.83	73.6	-0.1	186.1	109.1	0.59	-3.8	24.3	0.75	14.7	3.2	3.9
SC7//L9	3.81	73.2	1.0	180.8	102.1	0.57	14.8	22.1	0.82	9.8	2.8	3.9
SC2//L11	3.81	70.2	3.8	167.6	94.3	0.57	8.0	21.3	0.77	11.5	3.6	3.7
SC7//L10	3.80	70.4	2.6	165.3	85.0	0.53	15.5	15.8	0.81	9.8	3.1	3.5
SC7//L6	3.78	71.9	1.4	176.3	85.4	0.49	-8.5	20.4	0.70	7.8	3.2	3.5

Appendix 14 Mean performance of three-way hybrids for grain yield and other agronomic traits under optimum conditions in the 2011 winter season

ENTRY	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER	TEX	EA
SC8//L12	3.74	72.3	1.1	171.0	90.9	0.54	0.6	7.3	0.85	15.5	3.7	4.3
023WH31	3.74	72.6	1.4	175.1	96.9	0.57	-3.3	8.2	0.62	7.0	3.1	3.9
SC2//L6	3.74	71.4	2.9	161.8	88.2	0.56	-4.4	22.7	0.75	-1.1	3.7	3.8
SC7//L5	3.72	69.7	3.5	163.6	79.9	0.51	-10.0	22.0	0.87	17.2	2.6	4.0
SC3//L9	3.60	72.6	2.3	165.7	92.3	0.58	-10.1	4.2	0.82	11.2	3.3	4.1
SC2//L4	3.55	72.6	-0.6	176.6	89.4	0.53	0.2	14.2	0.83	7.5	3.5	4.3
SC1//L8	3.51	70.6	2.7	162.2	104.2	0.66	-2.0	16.7	0.76	12.3	3.4	4.1
SC10//L2	3.50	74.2	1.4	179.5	93.8	0.53	-2.9	7.0	0.78	7.6	3.9	3.6
SC3//L11	3.50	68.5	6.9	159.9	82.2	0.53	-1.7	11.6	0.82	11.4	3.2	3.3
SC635	3.48	71.8	-0.5	153.1	82.4	0.55	57.8	13.0	0.51	10.0	3.6	4.0
SC10//L3	3.43	73.0	2.9	185.7	95.3	0.53	-3.7	23.2	0.73	27.1	3.7	4.3
SC9//L11	3.41	72.2	1.0	179.1	101.7	0.58	8.1	28.2	0.82	15.7	3.3	4.4
SC10//L4	3.33	75.2	-0.1	189.9	112.1	0.61	12.9	20.7	0.67	5.1	3.6	3.9
SC513	3.30	71.2	0.7	178.9	87.2	0.50	-2.3	22.8	0.81	15.7	3.1	3.6
SC5//L8	3.28	71.7	1.3	176.8	99.5	0.56	-6.6	11.5	0.77	15.1	3.3	4.4
SC5//L2	3.28	73.3	0.8	179.6	89.8	0.51	-5.8	22.9	0.58	17.6	4.0	4.1
SC3//L2	3.22	72.5	5.7	171.1	81.5	0.48	-1.9	11.5	0.77	13.0	4.0	3.4
SC2//L2	3.19	72.6	1.6	166.8	83.5	0.51	-2.4	18.8	0.83	20.5	3.6	4.3
SC4//L9	3.18	74.4	0.0	173.6	96.2	0.54	12.8	12.6	0.65	17.3	3.5	3.2
SC5//L11	3.18	73.6	1.4	187.4	105.9	0.58	2.9	12.6	0.71	15.9	3.8	4.5
SC4//L12	3.13	69.6	2.2	173.2	93.2	0.54	-3.0	13.9	0.76	22.1	4.0	4.6
SC7//L3	3.12	71.3	5.3	176.3	85.2	0.49	14.8	10.6	0.63	22.3	3.4	4.2
SC1//L3	3.12	73.0	2.8	177.1	85.9	0.50	14.3	22.5	0.83	14.6	3.7	3.7
SC7//L2	3.12	72.0	3.7	176.0	87.4	0.51	3.1	22.4	0.82	8.2	3.5	3.6
SC1//L1	3.07	71.0	6.1	182.3	91.8	0.50	-4.2	13.9	0.83	15.4	4.1	4.3
SC11//L7	3.07	70.9	4.7	162.1	81.7	0.51	7.6	20.6	0.80	8.9	2.9	3.4
SC6//L12	3.04	70.5	3.1	167.7	87.5	0.52	-2.8	25.1	0.81	20.9	4.1	4.6
SC3//L4	3.03	71.3	3.2	169.7	80.9	0.50	-3.5	7.1	0.74	8.6	3.6	4.6
SC11//L2	3.03	72.6	1.2	177.7	103.5	0.60	23.7	14.0	0.76	5.0	3.7	3.9
SC11//L4	3.03	72.9	1.3	186.4	106.0	0.59	54.0	22.3	0.63	9.8	3.7	3.9
SC3//L3	3.00	69.8	2.9	177.0	91.2	0.52	-2.8	18.2	0.82	11.8	3.8	3.9
SC5//L7	3.00	73.3	1.4	173.4	100.7	0.59	21.6	18.1	0.76	2.3	3.4	4.7
SC2//L8	2.92	70.5	2.6	165.1	94.2	0.56	-3.5	22.2	0.80	9.1	3.4	4.4
SC5//L1	2.90	72.5	4.9	190.7	93.7	0.48	-4.1	15.0	0.77	16.0	3.9	4.6
CZH0837	2.89	70.9	3.8	176.6	91.4	0.53	-5.2	23.9	0.76	22.6	3.5	4.1
SC7//L7	2.88	71.2	4.4	168.3	87.5	0.53	15.3	22.1	0.82	15.4	3.1	4.3

Appendix 14 Mean performance of three-way hybrids for grain yield and other agronomic traits under optimum conditions in the 2011 winter season

ENTRY	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER	TEX	EA
SC2//L5	2.73	71.0	1.6	153.9	78.3	0.54	5.0	21.9	0.88	15.3	3.2	4.6
SC2//L1	2.71	70.6	6.3	179.5	88.4	0.52	4.7	11.4	0.75	24.5	3.6	4.0
SC4//L11	2.71	71.7	2.1	170.9	107.3	0.63	1.3	17.8	0.73	21.8	4.0	4.3
SC1//L7	2.70	71.3	3.8	165.3	83.6	0.52	-3.5	25.1	0.76	12.8	3.4	4.3
SC2//L7	2.61	70.7	4.2	159.3	74.4	0.50	15.3	23.6	0.79	18.7	3.3	4.0
SC9//L12	2.61	72.0	1.0	165.2	80.5	0.49	14.0	16.3	0.79	25.2	3.8	4.4
013WH63	2.56	72.4	-0.2	171.0	93.0	0.54	24.2	18.9	0.60	5.8	3.8	4.6
SC3//L6	2.54	70.8	3.3	164.3	83.3	0.52	2.2	12.3	0.77	8.4	3.8	4.0
SC3//L7	2.43	70.2	3.3	149.5	69.3	0.48	1.1	25.9	0.81	2.9	3.2	3.9
SC2//L4	2.37	71.6	1.8	174.2	83.6	0.49	-0.2	21.9	0.85	18.6	3.9	4.0
SC7//L4	2.36	73.2	0.3	185.2	104.5	0.58	38.7	20.3	0.65	9.7	4.0	4.2
SC3//L1	2.24	69.7	7.0	171.0	88.0	0.52	-3.7	20.4	0.84	21.6	3.9	4.6
SC1//L5	2.20	71.3	3.8	158.3	81.3	0.52	-5.6	19.4	0.90	10.2	3.0	3.3
SC1//L4	1.99	72.7	0.0	178.1	100.3	0.57	26.4	16.6	0.66	18.3	4.2	4.6
013WH01	1.95	74.7	0.5	183.8	106.6	0.59	31.1	10.6	0.65	7.6	3.6	4.1
Mean	**3.51**	**71.8**	**2.4**	**172.6**	**93.0**	**0.55**	**5.4**	**16.6**	**0.78**	**12.2**	**3.5**	**3.9**
LSD (0.05)	**1.33**	**1.6**	**2.6**	**13.0**	**11.6**	**0.06**	**29.4**	**17.5**	**0.21**	**12.8**	**0.7**	**0.8**
MSE	**0.90**	**2.0**	**1.7**	**128.8**	**101.5**	**0.00**	**218.8**	**154.1**	**0.02**	**41.3**	**0.3**	**0.4**
Min	**1.95**	**68.5**	**-0.6**	**149.5**	**69.3**	**0.48**	**-11.5**	**2.3**	**0.51**	**-1.1**	**2.6**	**2.6**
Max	**4.79**	**75.2**	**7.0**	**190.7**	**116.4**	**0.66**	**57.8**	**43.4**	**1.25**	**27.1**	**4.8**	**4.7**

GYD=grain yield (t ha^{-1}); AD=anthesis days; ASI=anthesis silking interval (days); PH=plant height (cm); EH=ear height (cm); EPO=ear position (0-1); RL=root lodging (%); SL=stem lodging (%); EPP=ears per plant (#); ER=ear rot (%); SEN=senescence (1-10); TEX=texture (1-5); EA=ear aspect (1-5); LSD=least significant difference; MSE=mean square error; Min=minimum; Max=maximum.

Appendix 15 Mean performance of three-way hybrids for grain yield and other agronomic traits in combined analysis in the 2011 winter season

ENTRY	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER	SEN	TEX	EA
SC8//L10	3.76	68.5	1.3	178.3	99.8	0.54	6.6	5.8	0.70	14.8	0.5	3.4	3.7
SC10//L6	3.68	69.5	1.1	169.5	90.7	0.54	-4.2	10.9	0.71	13.8	0.5	3.5	3.5
SC10//L5	3.62	68.2	1.3	175.1	90.6	0.51	2.6	24.2	0.83	5.6	0.6	3.1	3.4
SC4//L10	3.58	67.7	1.4	170.9	101.3	0.58	22.5	3.2	0.74	19.1	0.6	3.7	3.7
SC2//L9	3.50	70.7	1.5	181.5	96.3	0.54	0.1	12.8	0.72	22.5	0.6	3.2	3.8
SC3//L8	3.44	66.9	1.2	159.6	82.0	0.51	3.8	23.2	0.66	136.4	0.6	3.1	3.8
SC11//L5	3.39	67.4	1.6	160.3	92.1	0.53	7.5	26.1	0.75	15.5	0.6	2.4	3.1
SC1//L6	3.36	70.1	1.5	170.8	90.4	0.52	7.1	6.0	0.68	19.0	0.6	3.2	3.5
013WH29	3.32	69.2	1.8	164.7	95.0	0.57	6.6	5.5	0.72	14.8	0.5	3.1	3.5
SC3//L1	3.31	67.1	2.9	167.3	78.9	0.48	0.3	20.6	0.80	8.4	0.5	2.3	3.2
SC1//L2	3.31	71.4	2.9	174.1	89.8	0.51	-0.9	21.9	0.64	11.6	0.5	3.6	3.5
SC8//L8	3.30	72.8	1.0	178.5	103.2	0.56	4.5	7.7	0.63	27.5	0.5	3.2	3.9
CZH0616	3.29	68.2	3.9	158.4	86.2	0.55	0.0	15.0	0.77	11.9	0.5	3.1	3.3
SC6//L10	3.26	68.3	2.8	174.7	97.7	0.56	28.4	14.1	0.69	27.7	0.6	3.7	3.8
SC10//L7	3.25	70.0	2.7	162.7	87.0	0.52	9.9	29.4	0.77	11.6	0.5	3.1	3.3
SC9//L10	3.25	67.3	3.0	167.5	95.5	0.56	25.3	15.1	0.63	11.6	0.5	3.5	3.7
SC8//L9	3.22	72.2	3.0	182.1	104.5	0.61	-0.3	2.0	0.62	21.6	0.5	3.2	3.8
SC10//L8	3.22	68.3	1.8	170.3	97.9	0.56	0.5	9.8	0.75	20.3	0.5	3.4	4.0
SC3//L10	3.22	65.9	2.2	176.6	92.7	0.51	9.2	10.8	0.84	12.0	0.6	3.0	3.5
SC9//L9	3.19	72.8	3.0	175.5	106.7	0.58	7.0	23.8	0.82	15.2	0.5	4.3	3.1
SC11//L8	3.15	67.4	1.3	172.8	99.2	0.56	11.4	9.6	0.83	17.9	0.5	2.7	3.4
023WH31	3.14	71.4	1.7	177.8	98.9	0.56	4.2	10.1	0.65	19.2	0.5	3.1	3.6
SC7//L10	3.11	67.2	3.0	166.1	75.8	0.46	16.3	15.9	0.77	7.6	0.5	2.9	3.4
SC8//L5	3.06	67.9	2.0	174.0	96.4	0.54	6.0	9.0	0.66	26.3	0.5	2.9	3.5
SC2//L10	3.06	66.8	1.6	162.1	92.5	0.56	40.5	7.1	0.71	15.2	0.6	3.2	3.8
SC3//L2	3.05	70.0	2.6	170.3	82.1	0.47	6.9	17.8	0.70	26.3	0.6	3.9	3.5
SC5//L10	3.05	69.8	1.5	180.0	107.2	0.59	21.6	9.4	0.65	12.7	0.6	3.1	3.5
SC7//L9	3.03	70.9	1.4	181.5	100.7	0.55	12.5	14.0	0.65	18.5	0.5	2.7	3.6
SC11//L6	3.02	70.0	0.8	166.3	88.6	0.53	0.8	13.4	0.75	9.7	0.5	2.9	3.4
SC635	3.01	68.2	4.8	148.1	87.7	0.57	34.3	19.9	0.57	28.7	0.6	3.6	3.8
SC10//L1	2.98	69.5	5.7	179.3	96.6	0.54	9.6	11.5	0.67	32.2	0.5	3.4	4.1
SC3//L9	2.96	70.5	1.4	166.0	97.9	0.57	5.8	15.2	0.65	17.3	0.6	3.1	3.6
SC6//L9	2.89	71.4	2.4	185.6	103.3	0.56	-0.9	11.3	0.57	19.1	0.5	3.6	3.7
SC2//L6	2.84	68.8	0.8	160.8	84.1	0.53	1.6	23.7	0.69	4.9	0.6	3.4	3.8

Appendix 15 Mean performance of three-way hybrids for grain yield and other agronomic traits in combined analysis in the 2011 winter season

ENTRY	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER	SEN	TEX	EA
SC2//L11	2.83	68.5	2.2	168.1	93.5	0.55	10.5	15.6	0.72	34.9	0.6	3.4	3.8
SC3//L11	2.82	66.7	2.2	159.1	87.0	0.51	10.7	24.8	0.75	22.7	0.6	3.1	3.1
SC2//L6	2.80	68.6	1.5	158.0	77.9	0.51	8.4	16.6	0.80	8.6	0.6	2.6	3.6
SC6//L11	2.77	70.6	2.9	183.6	104.9	0.57	15.3	12.7	0.60	35.1	0.5	3.3	4.1
SC11//L2	2.76	70.2	1.6	178.9	97.4	0.54	16.6	13.9	0.71	19.3	0.5	3.6	3.4
SC1//L8	2.76	68.2	1.2	166.4	98.4	0.59	9.5	12.5	0.76	27.3	0.6	3.2	3.7
SC5//L8	2.75	69.6	1.3	175.8	99.6	0.55	1.0	13.9	0.69	29.4	0.5	3.3	4.2
SC8//L12	2.75	69.9	0.3	171.3	90.1	0.53	4.4	11.7	0.72	31.4	0.6	3.6	4.4
SC10//L2	2.74	71.7	2.8	179.4	94.7	0.54	3.5	6.6	0.63	30.1	0.5	3.8	3.7
SC5//L6	2.73	72.1	0.8	180.5	93.5	0.51	-0.6	9.3	0.58	23.5	0.6	3.5	4.0
CZH0837	2.71	68.5	2.4	181.8	90.6	0.51	4.1	17.0	0.72	16.5	0.6	3.4	3.8
SC7//L6	2.71	69.9	1.9	176.2	84.2	0.48	-4.2	15.4	0.62	25.7	0.6	3.1	3.7
SC10//L3	2.69	70.9	5.1	184.4	96.1	0.52	3.3	12.5	0.64	20.0	0.5	3.6	3.9
SC2//L8	2.67	68.2	-0.2	170.2	92.2	0.55	15.5	16.9	0.79	27.3	0.6	3.4	4.0
SC2//L4	2.59	70.1	1.9	176.6	92.5	0.52	9.0	13.0	0.63	33.3	0.6	3.6	3.8
SC4//L12	2.58	66.9	1.9	171.7	97.0	0.56	14.4	12.5	0.73	39.7	0.6	4.0	4.1
SC7//L7	2.58	69.0	3.8	168.9	84.7	0.51	9.0	15.5	0.70	28.5	0.5	2.9	3.9
SC513	2.56	68.9	2.3	181.6	84.7	0.47	19.8	13.9	0.67	25.1	0.5	2.8	3.5
SC2//L2	2.55	69.9	1.2	168.0	89.1	0.52	7.7	19.1	0.65	41.5	0.6	3.7	4.3
SC8//L11	2.53	71.2	2.7	184.5	105.7	0.57	1.3	18.5	0.51	27.0	0.6	3.1	3.7
SC6//L12	2.51	67.4	2.1	171.4	93.8	0.51	7.1	22.2	0.72	22.1	0.6	3.9	4.2
SC3//L3	2.50	67.3	2.8	179.7	91.9	0.50	8.9	16.1	0.67	23.8	0.6	3.6	3.7
SC9//L11	2.46	70.2	2.9	174.6	102.0	0.63	9.0	15.9	0.61	23.0	0.6	3.1	3.9
SC1//L3	2.46	70.8	3.0	176.6	89.9	0.50	15.6	17.2	0.65	31.8	0.5	3.6	3.4
SC3//L1	2.44	67.9	5.8	172.3	84.6	0.48	2.2	15.5	0.67	21.9	0.5	3.6	3.8
SC11//L7	2.43	68.7	2.8	162.7	86.1	0.52	4.1	27.6	0.73	8.2	0.6	2.6	3.2
SC4//L11	2.42	69.2	2.4	171.8	104.0	0.60	0.5	15.8	0.67	20.1	0.5	3.8	4.2
SC5//L11	2.41	71.2	2.4	180.2	117.0	0.64	-3.6	10.5	0.50	23.9	0.6	3.3	4.2
SC5//L3	2.36	71.3	3.0	186.9	105.1	0.56	9.8	4.9	0.54	25.8	0.5	3.6	4.0
SC7//L2	2.36	69.7	4.7	179.2	93.3	0.50	8.7	14.8	0.61	15.6	0.5	3.3	3.5
SC5//L1	2.32	70.9	5.6	187.3	100.5	0.52	5.0	10.2	0.51	16.7	0.5	3.8	3.9
SC3//L4	2.31	68.6	1.9	173.3	89.2	0.50	7.9	18.4	0.64	24.2	0.7	3.6	3.9
SC1//L5	2.31	69.3	2.0	160.2	82.0	0.50	4.5	27.0	0.78	5.5	0.5	2.7	2.9
SC2//L4	2.29	69.3	1.9	175.9	89.4	0.50	11.1	17.1	0.68	25.1	0.6	3.7	3.7
SC3//L7	2.27	68.3	0.9	152.5	76.4	0.48	7.9	25.1	0.79	2.4	0.6	3.1	3.3
SC9//L12	2.27	69.1	1.1	167.4	89.7	0.50	15.8	18.6	0.71	27.1	0.6	3.9	3.9

Appendix 15 Mean performance of three-way hybrids for grain yield and other agronomic traits in combined analysis in the 2011 winter season

ENTRY	GYD	AD	ASI	PH	EH	EPO	RL	SL	EPP	ER	SEN	TEX	EA
SC5//L7	2.26	71.0	1.9	172.8	97.9	0.56	16.8	19.6	0.62	34.9	0.6	3.4	4.5
SC1//L1	2.26	69.4	5.4	180.9	87.4	0.49	1.7	14.2	0.58	21.8	0.5	3.9	3.8
SC4//L9	2.22	71.7	2.3	171.7	102.2	0.57	7.2	10.2	0.52	34.8	0.5	3.4	3.7
SC5//L2	2.22	71.8	4.9	181.6	93.9	0.51	1.4	14.5	0.48	24.6	0.5	4.1	4.1
SC2//L7	2.20	68.8	2.6	161.9	81.4	0.49	22.3	27.0	0.68	20.6	0.6	3.3	3.5
SC7//L3	2.19	69.1	4.8	178.4	90.2	0.50	11.7	5.2	0.51	19.4	0.5	3.2	4.0
SC2//L1	2.09	69.0	6.6	181.0	91.6	0.51	12.3	9.0	0.56	35.4	0.6	3.6	4.1
SC3//L6	2.08	68.9	1.5	167.1	80.9	0.50	6.4	15.0	0.65	11.8	0.6	3.5	3.7
SC10//L4	2.08	72.4	5.5	190.1	109.7	0.59	9.9	15.5	0.43	37.3	0.5	3.6	4.0
SC1//L7	2.05	70.0	2.5	166.0	86.1	0.50	2.3	26.0	0.61	12.6	0.6	3.3	3.9
SC11//L4	1.91	70.2	2.9	184.4	104.1	0.56	38.1	19.0	0.56	19.6	0.5	3.5	3.6
SC1//L4	1.79	70.2	3.1	181.3	98.2	0.54	14.0	12.7	0.58	28.5	0.6	3.9	3.9
SC7//L4	1.77	70.2	4.5	187.7	103.7	0.56	24.2	11.4	0.50	28.0	0.5	3.6	3.9
013WH01	1.70	72.2	4.0	187.5	107.9	0.57	19.5	7.0	0.46	32.1	0.6	3.3	3.6
013WH63	1.62	70.2	3.8	175.8	95.0	0.54	20.7	11.6	0.48	31.9	0.6	3.9	4.4
Mean	**2.73**	**69.5**	**2.5**	**173.3**	**93.7**	**0.53**	**9.3**	**14.9**	**0.66**	**23.0**	**0.6**	**3.4**	**3.7**
LSD (0.05)	**0.84**	**1.3**	**1.9**	**10.7**	**8.7**	**0.05**	**17.9**	**10.5**	**0.13**	**35.1**	**0.1**	**0.5**	**0.6**
MSE	**0.72**	**2.0**	**3.8**	**116.7**	**116.1**	**0.00**	**161.6**	**139.0**	**0.02**	**1248.7**	**0.0**	**0.3**	**0.4**
Min	**1.62**	**65.9**	**-0.2**	**148.1**	**75.8**	**0.46**	**-4.2**	**2.0**	**0.43**	**2.4**	**0.5**	**2.3**	**2.9**
Max	**3.76**	**72.8**	**6.6**	**190.1**	**117.0**	**0.64**	**40.5**	**29.4**	**0.84**	**136.4**	**0.7**	**4.3**	**4.5**

GYD=grain yield (t ha^{-1}); AD=anthesis days; ASI=anthesis silking interval (days); PH=plant height (cm); EH=ear height (cm); EPO=ear position (0-1); RL=root lodging (%); SL=stem lodging (%); EPP=ears per plant (#); ER=ear rot (%); SEN=senescence (1-10); TEX=texture (1-5); EA=ear aspect (1-5); LSD=least significant difference; MSE=mean square error; Min=minimum; Max=maximum.