

**An investigative study of the drought tolerance of F<sub>1</sub> maize (*Zea mays* L.) single crosses derived from PANNAR and CIMMYT inbred lines**

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## Thesis abstract

Drought is the most devastating abiotic stress limiting the production of maize (*Zea mays* L.) worldwide. The effect of drought stress is greatest in sub-Saharan Africa (SSA) where most small scale farmers rely on open pollinated varieties (OPVs); rather than certified hybrid seed grown under dryland<sup>1</sup> conditions. Small scale farmers perceive OPVs to be drought tolerant and yet the yields are as low as 1-2 t ha<sup>-1</sup>. In many parts of SSA, therefore, hybrid adoption rate is still below 20%. With the projected population growth in SSA, food insecurity is likely to worsen. However, the development of drought tolerant maize hybrids to help address declining food security is a relevant strategy in SSA. This is so because, drought tolerant hybrids will out yield OPVs. The main objective of this study was to investigate the drought tolerance of F<sub>1</sub> single cross maize hybrids developed from CIMMYT drought tolerant donor inbred lines and PANNAR elite inbred lines. The hybrids were tested under random and managed drought stress environments. The first set of F<sub>1</sub> hybrids was produced using a 12x12 North Carolina factorial mating design during winter (April-August 2012) and the second set of hybrids was produced in summer (November 2012-March 2013). Seventy F<sub>1</sub> hybrids were tested during the summer season (November 2012- April 2013) under random drought stress (RDS) environments in South Africa and Zimbabwe. One hundred hybrids were tested during the winter season (April – September 2013) in Zimbabwe at CIMMYT experimental stations in Save Valley and Chisumbanje under managed drought stress (MDS). The main traits measured included grain yield (GY), ear plant<sup>-1</sup> (EPP), anthesis-silking interval (ASI) and days to anthesis (DTA). Statistical analysis of collected data was conducted using GenStat 16<sup>th</sup> edition. Significant differences between hybrids and environments for grain yield and secondary traits were obtained. The genotype x environment (GxE) interactions was significant under both RDS and MDS environments indicating the differential performance of hybrids across environments. Using stability indices, the relatively stable hybrids were identified. General and specific Combining ability (GCA and SCA) analysis revealed lines that could be used as potential parents in development of hybrids. However, the presence of GCAXE and SCAXE interactions indicated that selection of inbred lines and their hybrid crosses should be done at target environments. Under RDS and MDS, EH79 and EH24 appeared to be the best performing hybrids, respectively, whereas inbred lines DT2, DT7 and UT8 had the best GCA and SCA under both RDS and MDS. Further efforts are required to develop potential drought tolerant hybrids and test for adaption in target environments.

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<sup>1</sup> Relying predominantly on rainfall

## Declaration

I declare that the thesis hereby submitted by me for the degree of Master of Science in Agriculture (Plant breeding) at the University of KwaZulu-Natal represents my own independent work and has not previously been submitted in any form for degree or diploma examination to another university.

Unless specifically acknowledged as being sourced from other researchers, all the pictures, data and graphs were original. Where other written sources have been quoted, then:

their words have been re-written but the general information attributed to them has been referenced; and

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

This dissertation does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the references sections.

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Caiphas Muyambo (Candidate)

As the candidate's supervisor I agree/do not agree to the submission of this thesis

.....  
Dr P E Shanahan (Supervisor)

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## **Dedication**

I dedicate this thesis to my late father Isaac Muyambo, my mother Rose Mashayamombe and my siblings.

## Table of contents

Thesis abstract.....	i
Declaration .....	ii
Acknowledgements .....	iii
Dedication .....	iv
Table of contents .....	v
List of figures .....	ix
List of tables.....	xii
List of acronyms .....	xv
General introduction.....	1
References .....	7
Chapter 1: Literature review .....	9
1.1 Introduction .....	9
1.2 Broad background and current understanding of drought .....	9
1.2.1 Drought stress .....	10
1.2.2 Drought resistance and tolerance .....	11
1.3 The test environment .....	12
1.4 Soil, water and plant relationships.....	13
1.4.1 Soil water potential and water content .....	13
1.4.2 Techniques for measuring soil water potential .....	15
1.4.3 Techniques for measuring soil water content.....	16
1.4.4 Comparison of techniques .....	16
1.5 Plant water status.....	18
1.5.1 The pressure chamber .....	1
1.5.2 The psychrometer .....	3
1.5.3 Relative water content .....	4
1.6 Genes associated with drought tolerance .....	5
1.7 Effects of drought stress and plant responses .....	5
1.7.1 Physiological responses .....	6
1.7.2 Biochemical responses.....	7
1.7.3 Morphological effects.....	8
1.8 Yield potential versus actual yield .....	11

<b>1.9</b>	<b>Breeding for drought stress environments .....</b>	<b>12</b>
1.9.1	Sources of genes for drought tolerance .....	13
1.9.2	Evaluation of genotypes in multi-environmental trials .....	15
1.9.3	Yield adaptation.....	17
1.9.4	Phenotypic stability .....	18
<b>1.10</b>	<b>Statistical methods of analysing trial data .....</b>	<b>19</b>
1.10.1	Analysis of variance .....	19
1.10.2	Joint regression analysis.....	21
1.10.3	Additive main effects and multiplicative interactions and GGE biplot analysis.....	21
<b>1.11</b>	<b>Future breeding methodologies .....</b>	<b>22</b>
1.11.1	Management of stress .....	22
1.11.2	Secondary traits and phenotypic correlations with yield .....	23
1.11.3	New phenotyping techniques .....	25
1.11.4	Molecular breeding and genetic engineering.....	26
1.10.5	Doubled haploid technology.....	27
<b>1.12</b>	<b>Conclusion.....</b>	<b>28</b>
<b>1.13</b>	<b>References.....</b>	<b>29</b>
<b>Chapter 2: Characterisation of F<sub>1</sub> maize (<i>Zea mays</i> L.) hybrids under random drought stress .....</b>		<b>40</b>
<b>Abstract .....</b>		<b>40</b>
<b>2.1</b>	<b>Introduction .....</b>	<b>41</b>
<b>2.2</b>	<b>Materials and methods.....</b>	<b>43</b>
2.2.1	Germplasm and mating design .....	43
2.2.2	Test environments and weather data.....	44
2.2.3	Trial management .....	45
2.2.4	Field trial design and data collection.....	45
2.2.5	Data analysis.....	46
<b>2.3</b>	<b>Results .....</b>	<b>49</b>
2.3.1	REML analysis within each environment .....	49
2.3.2	REML analysis across environments.....	54
2.3.3	Combining ability analysis for grain yield .....	58
2.3.4	Maturity groupings of hybrids.....	61
2.3.5	Heritability estimates for grain yield and secondary traits at each environment .....	63

2.3.6	Spearman's rank correlations between secondary traits and grain yield across four environments.....	63
<b>2.4</b>	<b>Discussion .....</b>	<b>65</b>
<b>2.5</b>	<b>Conclusion.....</b>	<b>71</b>
<b>2.6</b>	<b>References.....</b>	<b>73</b>
	<b>Appendices 2.....</b>	<b>76</b>
 <b>Chapter 3: Evaluation of the drought tolerance of F<sub>1</sub> maize (<i>Zea mays</i> L.) hybrids under managed drought stress.....</b>		
	<b>Abstract .....</b>	<b>78</b>
<b>3.1</b>	<b>Introduction .....</b>	<b>80</b>
<b>3.2</b>	<b>Materials and methods.....</b>	<b>82</b>
3.2.1	Germplasm.....	82
3.2.2	Environment information.....	82
3.2.3	Management and water stress induction .....	82
3.2.4	Experimental design and data collection .....	83
3.2.5	Data analysis.....	84
<b>3.3</b>	<b>Results .....</b>	<b>84</b>
3.3.1	Yield performance at each environment .....	84
3.3.2	Frequency distributions for selected traits under water stress and well watered conditions.....	86
3.3.3	Rank order of hybrids for grain yield .....	87
3.3.4	Yield under water stress relative to well watered conditions .....	89
3.3.5	Yield performance across environments.....	93
3.3.6	Combining ability analysis of nine traits of 72 F <sub>1</sub> hybrid crosses .....	97
3.3.7	Heritability estimates for grain yield and secondary traits .....	103
<b>3.4</b>	<b>Discussion .....</b>	<b>103</b>
<b>3.5</b>	<b>Conclusions.....</b>	<b>110</b>
<b>3.6</b>	<b>References.....</b>	<b>112</b>
	<b>Appendices 3.....</b>	<b>116</b>
 <b>Chapter 4: General thesis overview.....</b>		
<b>4.1</b>	<b>Introduction .....</b>	<b>119</b>
<b>4.2</b>	<b>Outputs from the study .....</b>	<b>120</b>
<b>4.3</b>	<b>Limitations for breeding for drought tolerance.....</b>	<b>121</b>



4.3.1	Selection of test environments.....	121
4.3.2	Induction of stress tolerance in maize.....	123
4.3.3	Techniques for evaluating plants for drought tolerance.....	123
4.3.4	Choice of parent lines.....	124
<b>4.4</b>	<b>Implications for breeding.....</b>	<b>124</b>
<b>4.5</b>	<b>References.....</b>	<b>125</b>
	<b>Presentations emanating from this thesis .....</b>	<b>126</b>

## List of figures

Fig. 1.1 Types of drought (Source: Rippey, 2014) .....	10
Fig. 1.2 Agricultural drought triangle (Adapted from Rippey, 2014) .....	10
Fig. 1.3 Relationship between matric potential and volumetric water content (Bilskie, 2001) .....	15
Fig. 1.4 Schematic of a Scholander pressure chamber (Lincoln and Eduardo, 2010) .....	2
Fig. 1.5 Portable plant water stress consoles (Soil Moisture, 2015) .....	3
Fig. 1.6 (a.) Isopiestic psychrometry to measure the water potential of a plant tissue and (b.) Movement of water between droplet solution and plant tissue (Lincoln and Eduardo, 2010) .....	4
Fig. 1.7 Absciscic acid signalling maize responses to drought (from Yang <i>et al.</i> , 2013) .....	7
Fig. 1.8 Relationship between yield loss per day of stress and growth stage in a maize hybrid bred in the 1960s. Outer lines show range of experimental results, middle line shows the average. Although experiments were not run to evaluate effects of stress occurring before 50 days after planting (Araus <i>et al.</i> , 2012) .....	10
Fig. 1.9 Yield gaps under different stress conditions (Dobermann <i>et al.</i> , 2003). T = Transpiration; pl = Plant; Ymax = Maximum yield; N = Nitrogen; P = Phosphorus; K = Pottassium; Y = Yield .....	12
Fig. 2.1 (a.) Location mean yield of 70 experimental single cross hybrids and 10 commercial checks; (b.) Mean yield of very early, early, medium and late maturity .....	49
Fig. 2.2 (a.) Open tips caused by drought stress; (b.) Ears filled to ear tip. Note: Open tips were measured only at the Greytown environment .....	51
Fig. 2.3 AMMI biplot of the mean performance ( $t\ ha^{-1}$ ) versus IPCA1 scores of 80 hybrids evaluated in four environments. The numbers from 1-10 were commercial hybrid checks and 11-80 were experimental hybrids .....	56

Fig. 2.4 AMMI biplot of IPCA1 versus IPCA2 scores for 80 genotypes tested in four environments. The numbers from 1-10 were commercial hybrid checks and 11-80 were experimental hybrids.....	57
Fig. 2.5 Mean ( $t\ ha^{-1}$ ) general combining ability across four environments of seven female lines of the 7x10 factorial mating design for grain yield ( $t\ ha^{-1}$ ) .....	60
Fig. 2.6 Mean ( $t\ ha^{-1}$ ) general combining ability across four environments of seven male lines of the 7x10 factorial mating design for grain yield ( $t\ ha^{-1}$ ) .....	60
Fig. 2.7 Single cross hybrids with significantly positive SCA effects for grain yield ( $t\ ha^{-1}$ ) under random drought stress environments .....	61
Fig. 2.8 Single cross hybrids with significantly negative SCA effects for grain yield ( $t\ ha^{-1}$ ) under random drought stress environments .....	61
Fig. 3.1 Frequency distributions of grain yield ( $t\ ha^{-1}$ ) of 110 $F_1$ hybrids evaluated under (a.) water stressed (mean of Chisumbanje-WS and Save Valley WS) and (b.) well watered (Chisumbanje-WW) conditions.....	86
Fig. 3.2 Frequency distributions of ears per plant of 110 $F_1$ hybrids evaluated under (a.) water stressed (mean of Chisumbanje-WS and Save Valley WS) and (b.) well watered (Chisumbanje-WW) conditions.....	87
Fig. 3.3 Frequency distributions of anthesis-silking interval ( $t\ ha^{-1}$ ) of 110 $F_1$ hybrids evaluated under (a.) water stressed (mean of Chisumbanje-WS and Save Valley WS) and (b.) well watered (Chisumbanje-WW) conditions .....	87
Fig. 3.4 Mean yields of the two water stressed (WS) environments relative to mean yield of the well watered (WW) environment. The relative yield percentages indicates mean of both experimental hybrids and commercial checks .....	89
Fig. 3.5 Best experimental and commercial hybrids versus worst experimental and commercial hybrid checks at each environment and mean of the best hybrid across all three environments versus mean of the worst hybrid across three environments. CH = commercial hybrid; EH = experimental hybrid; CHIS-WW = Chisumbanje well watered; CHIS-WS = Chisumbanje water stress; SAVE-WS = Save water stress .....	91

Fig. 3.6 AMMI1 biplot of 110 $F_1$ single cross maize hybrids evaluated in three environments for grain yield .....	95
Fig. 3.7 AMMI2 biplot of 110 $F_1$ single cross maize evaluated in three environments for grain yield ( $t\ ha^{-1}$ ) .....	96
Fig. 3.8 General combining ability effects for grain yield ( $t\ ha^{-1}$ ) of (a.) eight female lines and (b.) nine male lines under well watered conditions. UT = Unknown tolerance; DT = Drought tolerance .....	98
Fig. 3.9 Specific combining ability for grain yield ( $t\ ha^{-1}$ ) of (a.) 21 of 72 hybrids with significant positive and (b.) 18 of 72 hybrids with significant negative effects under well watered conditions. EH = Experimental hybrids.....	99
Fig. 3.10 General combining ability effects for grain yield ( $t\ ha^{-1}$ ) of (a.) eight female and (b.) nine male lines evaluated at two water stress environments. UT = Unknown tolerance; DT = Drought tolerance.....	102
Fig. 3.11 Specific combining ability for grain yield ( $t\ ha^{-1}$ ) of (a.) 16 of 72 hybrids with significant positive and (b.) 18 of 72 hybrids with negative SCA effects at two water stress environment. EH = Experimental hybrids.....	102

## List of tables

Table 1.1 Some of the main factors influencing plant water status .....	1
Table 2.1 Commercial hybrid checks and CIMMYT lines used in the experiment.....	43
Table 2.2 Average weekly rainfalls (mm) per test environment measured from planting to harvesting (December 2012 to May 2013), location altitude and GPS coordinates .....	44
Table 2.3 Traits measured or derived from the 2012/2013 RDS environments.....	45
Table 2.4 Summary statistics for four traits measured at each of the four random drought environments .....	50
Table 2.5 Rank order for grain yield of the top 20 hybrids for each of the four random drought stress environments.....	52
Table 2.6 Spearman's rank correlation coefficients for 70 hybrids plus 10 checks at four random drought stress environments .....	52
Table 2.7 Mean yield of the top 25 experimental hybrids and commercial hybrid checks at the LP (Greytown and ART) and the HP environments (Cedara and Devonla). The LP yield as a percentage of the HP yield is ranked from highest to lowest. The ranks of each hybrid in the LP and HP environments are in parenthesis.....	53
Table 2.8 REML Wald statistics for fixed effects of grain yield ( $\text{t ha}^{-1}$ ) hybrids, environments and GxE interaction .....	54
Table 2.9 AMMI ANOVA for grain yield ( $\text{t ha}^{-1}$ ) evaluated across four environments under random drought stress. ....	54
Table 2.10 Cultivar superiority index ( $P_i$ ) across the four environments, IPCA1 score and regression coefficient ( $b_i$ ), with hybrids ranked from the most stable to the least based on $P_i$ . The $b_i$ coefficient corresponds to hybrids ranked from the top yielding to the lowest in the top 30....	58
Table 2.11 REML analysis of general and specific combining ability for grain yield ( $\text{t ha}^{-1}$ ) across four environments .....	59

Table 2.12 Standard correlation coefficients between GCA effects of female and male parents and the mean performance of the experimental hybrids for grain yield ( $\text{t ha}^{-1}$ ) in each of four environments .....	60
Table 2.13 Standard correlation coefficients between SCA effects and the mean performance of each corresponding experimental hybrid for grain yield ( $\text{t ha}^{-1}$ ) in each of four environments ....	61
Table 2.14 Experimental hybrids placed in four maturity groups based on average days to anthesis across all locations. CH1, CH3, CH4 and CH7 were used as the benchmark commercial hybrids to group the experimental hybrids.....	62
Table 2.15 Narrow sense heritability ( $h^2$ ) and standard error estimates for yield and secondary traits of 80 hybrids including 10 checks evaluated at each of four environments. ....	63
Table 2.16 Spearman's rank correlation coefficients between grain yield and secondary traits (in which they were measured) in the LP environment .....	64
Table 2.17 Spearman's rank correlation coefficients between grain yield and secondary traits (in which they were measured) in the HP environment .....	65
Table 3.1 Weekly rainfall data for Chisumbanje and Save Valley research stations recorded from June-December 2013.....	83
Table 3.2 Agronomic traits measured or derived from the winter trials conducted in Chisumbanje and Save Valley in Zimbabwe in 2013 .....	84
Table 3.3 Mean squares of four traits at the well watered environment in Chisumbanje.....	85
Table 3.4 Mean squares of four traits at the water stress environment in Chisumbanje .....	85
Table 3.5 Mean squares of four traits at the water stress environment in Save Valley .....	85
Table 3.6 Summary statistics for GY ( $\text{t ha}^{-1}$ ) at each of the three environments .....	85
Table 3.7 Rank order of the top 20 hybrids for grain yield ( $\text{t ha}^{-1}$ ) at each of the three managed random drought stress environments .....	88
Table 3.8 Spearman's rank correlation coefficients between the three managed drought stress environments for grain yield ( $\text{t ha}^{-1}$ ) .....	88

Table 3.9 Rank orders of top 20 experimental hybrids for percentage grain yield under water stressed conditions relative to well watered conditions. Hybrid ranking under well watered conditions in parenthesis.....	90
Table 3.10 Comparison of best experimental hybrids relative to commercial hybrids expressed as a percentage at each environment, mean of the two water stress environments and across the three environments .....	91
Table 3.11 Phenotypic correlation coefficients between measured and derived traits averaged across all experimental and commercial hybrids and across the two water stressed environments .....	92
Table 3.12 Phenotypic correlation coefficients between measured and derived traits averaged across all experimental and commercial hybrids under well watered conditions.....	93
Table 3.13 WALD statistics for the REML analysis across the three managed drought stressed environments, CHIS-WW, CHIS-WS and SAVE-WS for four selected traits .....	93
Table 3.14 AMMI ANOVA for grain yield ( $\text{t ha}^{-1}$ ) of 110 hybrids evaluated in three managed drought stress environments during winter season in Zimbabwe .....	94
Table 3.15 Ranking of the first four AMMI selections of 110 hybrids (commercial and experimental) evaluated in water stressed and well watered environments.....	96
Table 3.16 REML WALD statistics for general (GCA) and specific (SCA) combining ability for grain yield and secondary traits under the well watered conditions of Chisumbanje.....	98
Table 3.17 Combining ability analysis for grain yield ( $\text{t ha}^{-1}$ ) and secondary traits across two water stress environments .....	101
Table 3.18 Narrow-sense heritability estimates of measured (and derived) traits under WW and WS conditions. ....	103

## List of acronyms

Acronym	Definition
ABA	Absciscic Acid
AMMI	Additive Main effects and Multiplicative Interaction
ANOVA	Analysis of variance
ART	Agriculture Research Trust
ASI	Anthesis silking interval
AWS	Automatic Weather Station
B	Biomass
CIMMYT	International Maize and Wheat Improvement Centre
CMI	Crop moisture index
CML	CIMMYT Maize Lines
CV	Coefficient of Variation
CWP	Crop water productivity
CWU	Crop water use
DAP	Days after planting
DH	Double haploid
DS	Drought stress
DT	Drought tolerance
DTA	Days to anthesis
DTMA	Drought Tolerant Maize for Africa
E	Environment
EH	Ear height
EI	Environmental index
LSEN	Leaf senescence
EP	Ear position
EPP	Ears per plant
ET	Evapotranspiration
EUW	Efficient use of water
FAO	Food And Agriculture Organization
GCA	General combining ability
GDD	Growing degree days
G	Genotype



<b>Acronym</b>	<b>Definition</b>
GLS	Grey leaf spot
GM	Grain moisture
GY	Grain yield
HG	Heterotic group
ha	Hectares
HYP	Hybrid yield potential
IITA	International Institute of Tropical Agriculture
IPCA	Interaction principal component axis
LAI	Leaf Area Index
LP	Low potential
LSD	Least Significant Difference
LSEN	Leaf senescence
MAS	Marker assisted selection
MDS	Managed drought stress
MET	Multi-environmental trials
MS	Mean square
NDVI	Normalized Difference Vegetation Index
NIR	Near Infrared
OA	Osmotic adjustment
PDSI	Palmer Drought Severity Index
PH	Plant height (cm)
PM	Population mean
P-value	Probability value
RDS	Random drought stress
REML	Restricted maximum likelihood
ROS	Reactive Oxygen Species
RUE	Radiation use efficiency
SAPBA	South African Plant Breeders Association
SAWC	Soil available water content
SCA	Specific combining ability
SCWB	Soil-crop water balance
SNP	Single-Nucleotide Polymorphism
SREG	Sites Regression

Acronym	Definition
SS	Sum of squares
SSA	Sub-Saharan Africa
T	Metric tonnes
TE	Transpiration efficiency
UKZN	University of KwaZulu-Natal
UT	Unknown tolerance
USA	United States of America
WEMA	Water Efficient Maize for Africa
WS	Water stress
WU	Water use
WUE	Water use efficiency
WW	Well watered
YP	Yield potential

## **General introduction**

### **History and production of maize**

Maize (*Zea mays* L.), understood to have originated from teosinte<sup>1</sup> (*Zea* spp.) (Beadle, 1939; Wet and Harlan, 1972; Tiana *et al.*, 2009), has evolved to become the third most widely grown cereal crop after *Triticum aestivum* (wheat) and *Oryza sativa* (rice). Worldwide, about  $785 \times 10^6$  t of maize grains are produced of which sub-Saharan Africa (SSA) accounts for only 6.5%. On average,  $8.0 \text{ t ha}^{-1}$  are produced in developed temperate regions versus  $3.5 \text{ t ha}^{-1}$  in the less developed SSA countries. Yet in SSA, maize is the most important staple food crop for more than 1.2 billion people (Edmeades, 2013). With the rising population and growing food insecurity, there is a rising need to boost maize productivity.

### **Food security in Africa**

About 33% of the population in SSA is undernourished and it is in this region where hunger is projected to worsen over the next two decades unless some drastic measures are taken (IPCC, 2007). This is so because production of the most consumed cereal crops, most importantly maize, lags behind population growth rate. Consequently, local food reserves are not enough to meet demand. To satisfy the rising demand for food, most SSA countries are relying increasingly on maize imports (25% of maize is currently imported) and food handouts (IPCC, 2007). However, access to maize is still limited by many factors that hamper effective distribution of imported (or donated) food; for example, higher prices, poor roads, absence of communication lines, poor government policies, etc. These challenges imply that overdependence on food imports and handouts is unsustainable and increased local maize production could be a better solution to address food insecurity. The need to increase local production was underpinned by a Tanzanian farmer who was quoted in CIMMYT's 2009 newsletter as saying, "No maize, no life". However, local maize production in SSA is hampered by a multitude of problems that reduce maize yields (FAO, 2005).

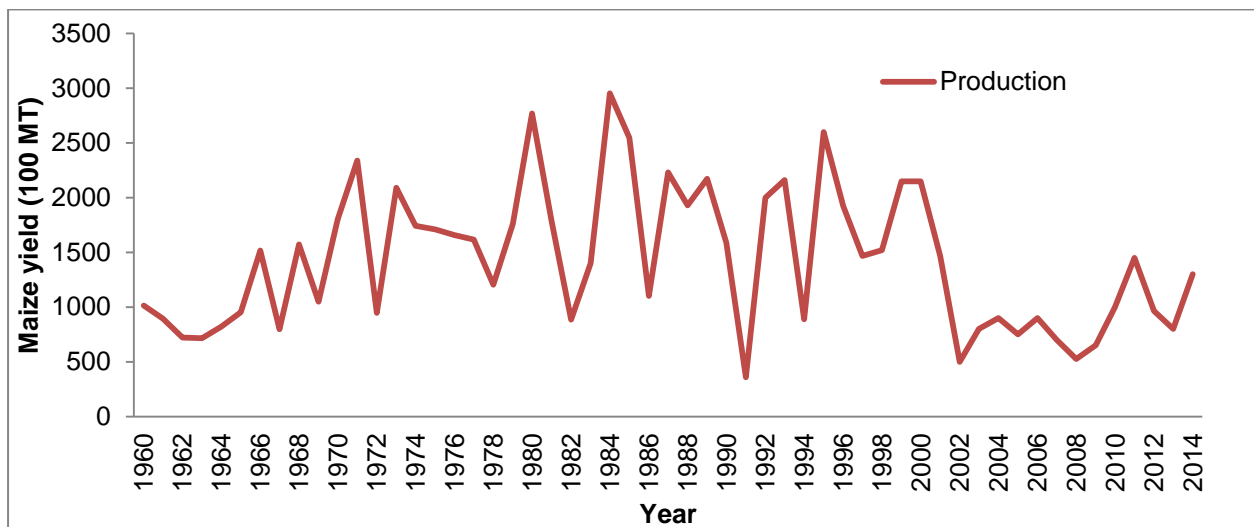
### **Drought an increasing problem in Africa**

Even though maize is adaptable to diverse climatic and edaphic soil conditions, productivity is still constrained by a wide range of abiotic (for example, drought and acidity) and biotic (for example; pests and diseases) factors. Most studies have widely reported drought to be by far the most important constraint in the water limited areas of SSA, with yield losses as high as 50% depending on the severity of drought (Bänziger *et al.*, 2000; Magorokosho *et al.*,

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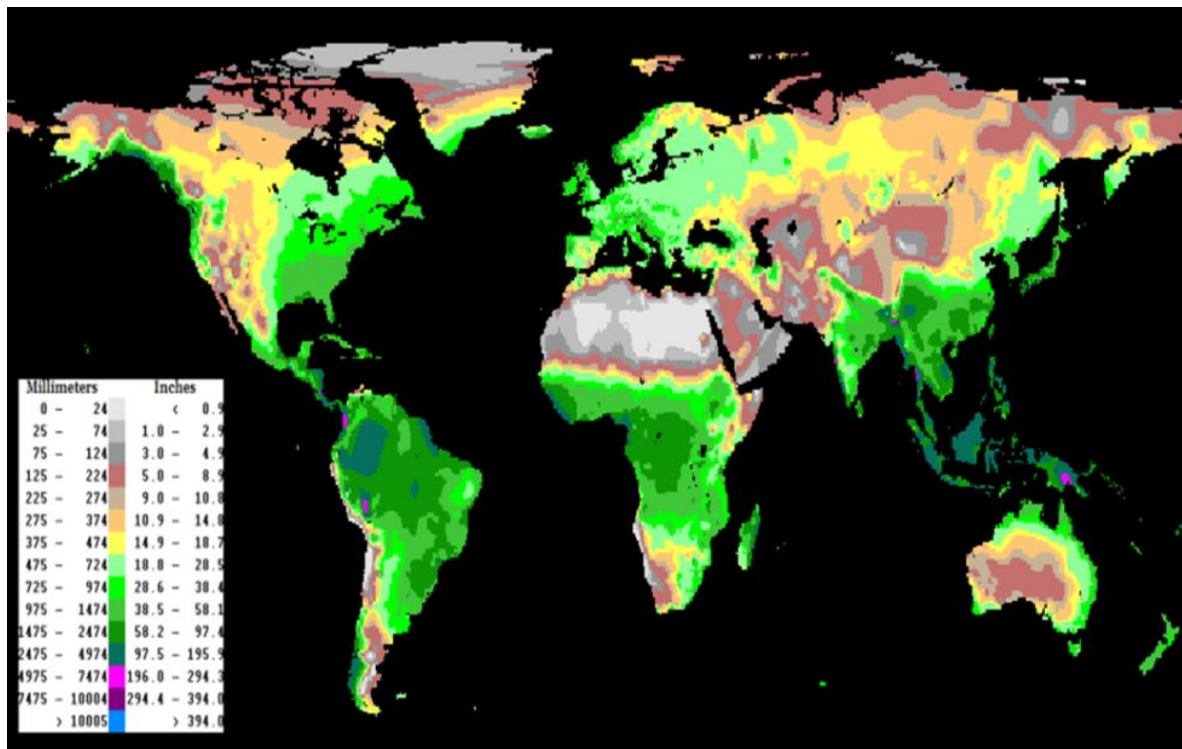
<sup>1</sup> A primitive ancestor of maize

2003; Bänziger *et al.*, 2004; Barker *et al.*, 2004; Campos *et al.*, 2004; Forster, 2004; Bänziger *et al.*, 2006; Lu *et al.*, 2011). A case study of Zimbabwe (Fig.1) (Richardson, 2005) indicated that a shift from predominantly commercial farming to small scale (subsistence) farming caused a significant reduction in maize production (USDA, 2009, 2015). Among other reasons for reduced yields is that, the crops grown by the small scale farmers are more prone to drought because of inadequate irrigation facilities.

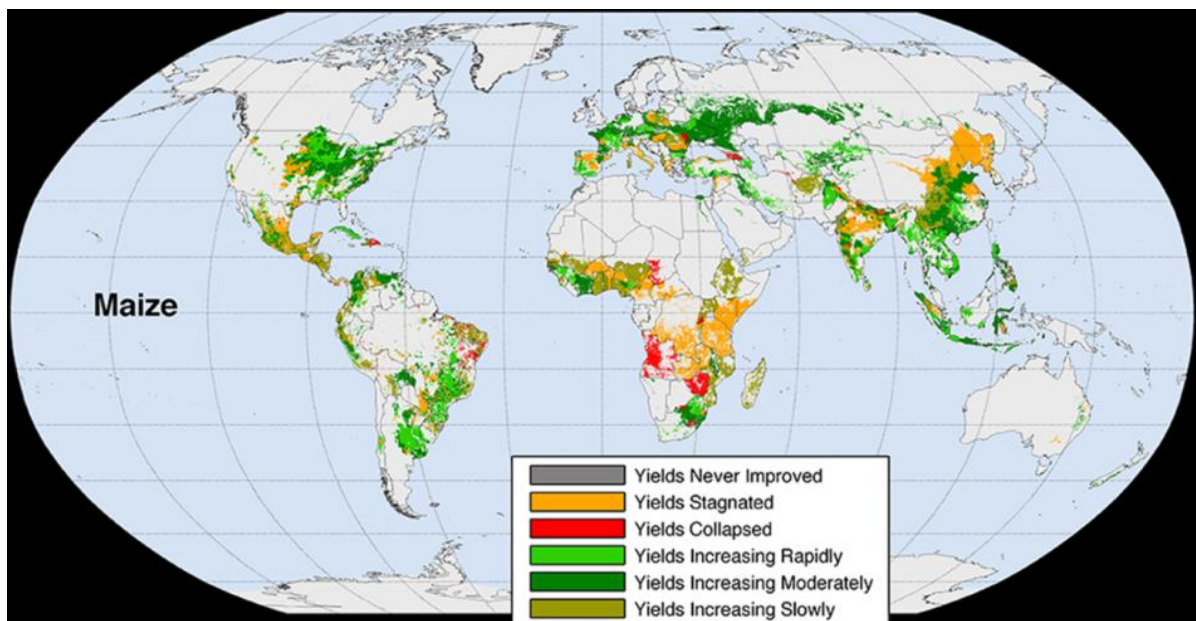


**Fig. 1 Decrease in yield in Zimbabwe from 1960 to 2014 due to land reform program instituted by the government in 2000 (USDA, 2015)**

Global rainfall distribution presented in Fig. 2 provides the ranges of rainfall received in different parts of SSA. Grey coloured regions (such as the northern and south western regions of Africa) indicate the driest regions that receive between 0-124 mm rainfall per annum. Green coloured regions (such as the Democratic Republic of Congo) receive from 474 to 2474 mm of rain per annum. There is an association between drought prone areas (Fig. 2) and poor maize production areas (Fig. 3). In some areas, maize yields have either collapsed or stagnated (such as Zimbabwe), or are moderately or rapidly increasing (for example, in South Africa) (Fig. 3).



**Fig. 2 Global rainfall distribution** (Source: <http://www.climate-charts.com/world-climate-maps.html>)



**Fig. 3 Global maize production** (Source: <http://phys.org/news/2012-12-yields-plateaued-world-important-crops.html>)

The effects of drought stress cannot be sustainably alleviated by providing supplemental water in these drought stricken areas where water is already in short supply and where it is a finite and reducing resource (FAO, 2013). Also, in the midst of a possible climate change (Abraha and Savage, 2006; IPCC, 2007) there is an obvious need to develop maize hybrids

with tolerance to drought (Blum, 1988; Campos *et al.*, 2004; Cairns *et al.*, 2012) to improve maize yields under water limited conditions.

### **The imperative to improve maize yield**

There has been a substantial increase in average global maize yields worldwide as a result of conventional breeding procedures (Bänziger *et al.*, 2006). Nonetheless the average yield ( $8.2 \text{ t ha}^{-1}$ ) of grain in temperate regions is not matched by the  $3.5 \text{ t ha}^{-1}$  on average produced in the less developed SSA countries of the tropical regions (Edmeades, 2008). The high yields in the developed regions are reportedly due to the use of improved cultivars and good agronomic practices. And yet, in SSA, the adoption rate for hybrid cultivars is still as low as 20% in many countries (Ragasa *et al.*, 2013). In Mozambique, the medium maturing open pollinated variety (OPV), Matuba with a yield ranging from  $1\text{-}2.0 \text{ t ha}^{-1}$  is still commonly cultivated (Jewell *et al.*, 1995; Esipisu, 2014). The total area under hybrid maize cultivation in Mozambique is below 4% (Esipisu, 2014). In Ghana, of the 60% land under maize cultivation, only 15% is planted to hybrid cultivars (Ragasa *et al.*, 2013). Perhaps, either farmers are still using traditional OPVs because there are no alternative varieties that can adapt to local drought conditions or there is inadequate education on the importance of adopting hybrid cultivars. There is not only the need for drought tolerant hybrids *per se*, but also hybrids with improved performance that can out-yield traditional, undeveloped varieties that are being planted and recycled in many parts of Africa. The adoption of improved drought tolerant hybrid cultivars could provide a solution to increase maize yield without increasing the land area allocated to the crop. This is so because arable land is a finite resource and is in fact rapidly declining (Campos *et al.*, 2004). Improved agronomic practices in conjunction with using improved hybrid cultivars would also improve yield without increasing land area. With drought tolerant hybrid cultivars, the mechanisms to cope with drought stress are “built into” the genetics of the seed distributed to farmers (Campos *et al.*, 2004).

Currently, the two public organisations, the International Maize and Wheat Improvement Centre (CIMMYT) and the International Institute of Tropical Agriculture (IITA) are in partnership to produce drought tolerant (DT) inbred lines and hybrid cultivars. The CIMMYT-IITA partnership, through the Drought Tolerance Maize for Africa (DTMA) project, released a number of drought tolerant donor inbred lines into the public domain (<http://dtma.cimmyt.org/index.php/varieties/dt-donors>). Even though DT inbred lines are available, large private seed companies seem to be slow in adopting and commercialising drought tolerant cultivars as the emphasis thus far has been breeding for favourable environments. As a result, the Water Efficient Maize for Africa (WEMA) project which seeks

to develop royalty-free drought tolerant African maize cultivars using conventional breeding, marker-assisted breeding, and biotechnology was initiated (AATF, 2010). With only five countries (Tanzania, Kenya, Uganda, Mozambique and South Africa) participating in the project, it will prove challenging to develop drought tolerant cultivars for the whole of the maize producing regions in Africa. Also, apart from the private breeding company, Monsanto, there is no other seed company actively participating in the WEMA project. This means that there is no effective introgression of the CIMMYT-IITA drought tolerant inbred lines with private sector germplasm (AATF, 2010). Even so, with concerted efforts, if maize breeders utilize the already identified CIMMYT-IITA DT lines, it is most probable that hybrids with some level of drought tolerance could be released.

It is apparent from the literature that much remains to be done to develop maize hybrids with improved drought tolerance (Bänziger *et al.*, 2000, 2006). The integration of the available CIMMYT-IITA DT inbred lines with private sector inbred lines has been slow. The main challenge associated with breeding for drought environments is the complexity of the drought tolerance trait itself (Cattivelli *et al.*, 2008; Blum, 2011). There is an obvious need for greater efforts and development of appropriate technologies in breeding for drought tolerance. This will also involve the wide testing of newly developed hybrids to identify the most stable performing hybrids.

## **Research objective**

The main objective of this study was to develop F<sub>1</sub> single cross maize hybrids from CIMMYT drought tolerant inbred lines and PANNAR elite lines, and test the performance of hybrid progenies under random drought stress (RDS) and managed drought stress (MDS) conditions.

## **Specific objectives**

The specific objectives of the study were to:

- i. To determine the performance of F<sub>1</sub> maize hybrids under RDS environments in South Africa and Zimbabwe;
- ii. To evaluate the performance of F<sub>1</sub> maize hybrids under MDS in Zimbabwe;
- iii. To establish the genetic association between yield and secondary traits of experimental hybrids under both RDS and MDS environments ;
- iv. Investigate genotype by environment (GxE) interactions and yield stability of F<sub>1</sub> maize hybrids under both RDS and MDS environments; and

- v. To investigate the general combining ability (GCA) and specific combining ability (SCA) of parental inbred lines.

## **Study hypotheses**

- i. There is genetic variation for drought tolerance in existing elite germplasm which can be exploited to produce drought tolerant genotypes.
- ii. Existing genetic variation for drought tolerance is heritable and therefore can be transferred to susceptible genotypes.
- iii. Experimental hybrids exhibit stable yield performance across all environments
- iv. The GCA and SCA of parental lines can be used to determine performance of inbred lines in hybrid combinations

## **Thesis structure**

**Chapter 1:** Literature review

**Chapter 2:** Characterisation of F<sub>1</sub> maize (*Zea mays* L.) hybrids under random drought stress

**Chapter 3:** Evaluation of the drought tolerance of F<sub>1</sub> maize (*Zea mays* L.) hybrids under managed drought stress

**Chapter 4:** General thesis overview



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# Chapter 1

## Literature review

### 1.1 Introduction

The literature relevant to the subject of drought tolerance in maize is reviewed in this chapter. The aspects of drought tolerance that are considered of importance for this study are: (i) historical and current understanding of drought stress; (ii) testing environments and their attributes; (iii) plant water relations; (iv) breeding strategies and new phenotyping techniques; (v) limitations of methodologies for evaluating drought tolerance; (vi) yield potential versus actual yield; (vii) yield adaptation and stability; and (viii) statistical methods for analysing data.

### 1.2 Broad background and current understanding of drought

An understanding of drought and its effects on crops is fundamental for efficient breeding programmes in drought prone environments (Moser, 2004). The subject of drought tolerance in maize has largely remained academic owing to the complexity of its effects on agriculture and socio-economics with dire consequences for agricultural productivity (Boyer, 1982; Jaleel *et al.*, 2009). All of the definitions provided for drought in the literature can be grouped into four main categories, i.e., meteorological, agricultural, hydrological and socio-economic drought (Wilhite and Glantz, 1985).

Precipitation and evapotranspiration are the most important environmental factors of drought. They affect the amount of water that infiltrates the soil and reaches underground water reserves and the amount of moisture lost. Excessive water loss leads to soil water deficiency, resulting in reduced uptake of water by plants. This causes agricultural drought which adversely affects crop yield. All the types of drought (Fig. 1.1) either directly or indirectly lead to undesirable socio-economic effects (Rippey, 2015). In this study, agricultural drought is central, while the other two types, hydrological and meteorological, can be regarded as peripheral factors that directly and indirectly intensify the magnitude of agricultural drought. For instance, below average precipitation and high evaporation rates can diminish water reserves and water available for plant uptake, causing plant stress. From this viewpoint, an agricultural drought triangle can be constructed (Fig. 1.2) where both meteorological and hydrological droughts lead to agricultural drought.

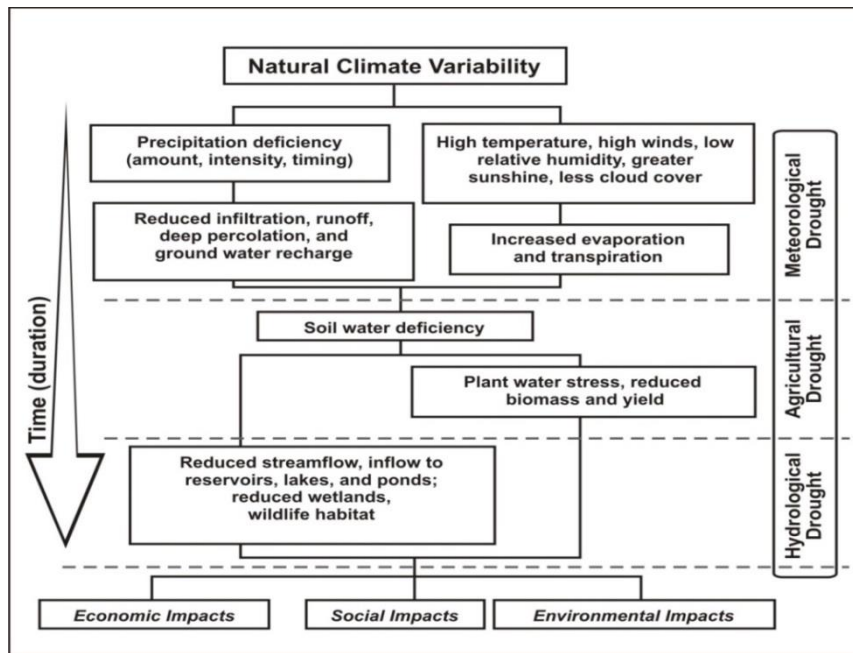


Fig. 1.1 Types of drought (Source: Rippey, 2014)

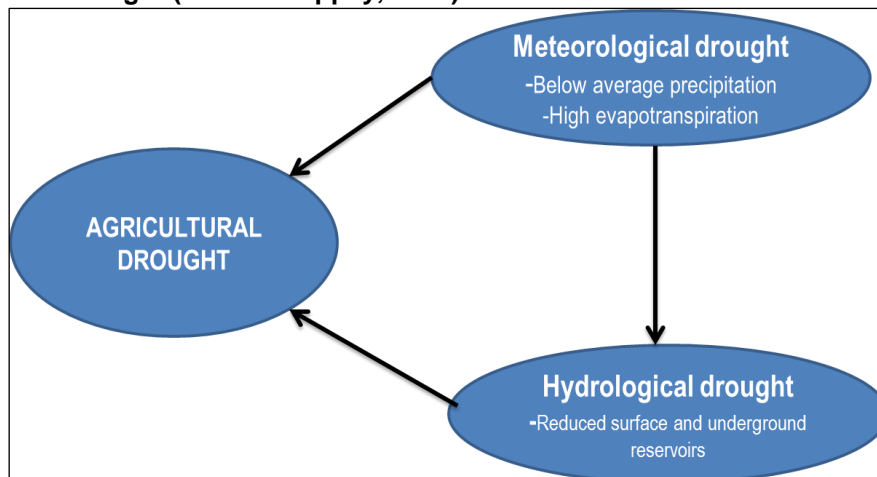


Fig. 1.2 Agricultural drought triangle (Adapted from Rippey, 2014)

### 1.2.1 Drought stress

Plant “stress” refers to any physiological condition caused by factors that disrupt normal metabolic patterns bringing about injury, disease or aberrant physiology (Bänziger *et al.*, 2000). Stress in plants is caused by a number of factors such as drought, low temperature, salt, flooding, heat, oxidative stress and heavy metal toxicity. Drought is reportedly the chief cause of plant stress in the tropics (Blum, 1996) a region that frequently receives unpredictable, irregular and sometimes below average precipitation (Bänziger *et al.*, 2000). In this review drought stress is defined as a condition when soil available water content (SAWC) is reduced to a level where the plant cannot take up sufficient amounts of water (Campos *et al.*, 2004). This occurs because atmospheric conditions cause excessive loss of water through evapotranspiration (ET) with little replacement from the soil. According to

Campos *et al.* (2004), agricultural drought stress occurs when SAWC is reduced to below 65%. The soil's water holding capacity depends largely on the soil properties such as texture, which also determines plant water uptake. This is the reason why in some areas (e.g. with lateritic soils), plants show signs of water stress even when there is above average precipitation. For purposes of this review, the plant is portrayed as a biological system that functions between two opposing physical systems (i.e. the soil withholds moisture from the plant while the atmosphere removes water from the plant through evaporation). Drought stress occurs when conditions within these two physical systems prompt the loss of water from the plant or restrict water uptake to a level where the plant's bio-physiological processes are negatively affected, as a result, drought stress symptoms are observed (Rippey, 2014).

### **1.2.2 Drought resistance and tolerance**

The two commonly used terms in the literature are “drought resistance” and “drought tolerance”. Drought resistance is the ability of a plant to maintain favourable water balance and turgidity even when exposed to drought conditions whereas drought tolerance refers to the degree to which a plant can survive under drought conditions (Turner, 1979). According to Levitt (1972;1985) drought resistance is equal to “tolerance”, and/or “avoidance”. Most of the literature uses resistance and tolerance almost indistinguishably, as applied to the plant's response to drought (Oliver *et al.*, 2010). However, in plant pathology, resistance has been used to refer to physical or chemical barriers that inhibit infection or limit its extent while tolerance refers to traits that neither reduce nor eliminate infection, but instead reduce the consequences or degree of damage (Ballini *et al.*, 2008). This separation of tolerance versus resistance is undoubtedly difficult to make in the practical world. It can be appreciated that plants use both mechanisms, even though at a given point, one could be more important than the other.

The strongest support of Levitt's terminology and definition of drought resistance was given by Blum (2005), a world renowned author on the subject of plant stress and water relations, when he wrote that: “*Drought resistance and its components are almost constantly being ‘redefined’, whereas newcomers to this discipline often express outstanding inventive capacity for terminology. For their benefit they are reminded that the original terminology and definitions of Levitt (1972) still hold very well. This is not nostalgia; it is a matter of scientific correctness*”. Accordingly, Levitt's definition and terminology are not entirely refuted, but there is another viewpoint in relation to the terminology. It is important to note that resistance, by its mechanism, is similar to drought avoidance. They both are accomplished by the organism's physical or chemical barriers that prevent infection (in case of diseases) or

water loss (Tollenaar *et al.*, 1994). This could mean that resistance is not an umbrella term for both avoidance and tolerance as posited by Levitt (1972). In his same review, Blum (2005) referring to drought tolerance wrote that: “... *this is sometimes seen as the second defence line after dehydration avoidance*”. In essence, therefore, the plant naturally possesses both resistance and tolerance traits but the degree of expression of each could vary with genotypes. Assuming that the plant has both traits, it can be hypothesized that when resistance mechanisms are broken, the plant survives by tolerating drought stress.

In practice, do plants express drought resistance and/or drought tolerance? Every living organism requires water to survive. Whenever there is a deviation from optimum levels of water, the effect is always translated to yield loss in crop plants. Complete resistance or immunity to diseases has been reported in the plant pathology field where researchers have claimed that some genotypes are not susceptible to certain diseases meaning they can survive and yield under maximum disease pressure without showing any symptoms (Ballini *et al.*, 2008). But it is almost inconceivable that there could be complete resistance to drought as implied by some authors, e.g., Moser (2004), who wrote about the “drought resistance myth”. The myth states that genotypes that show better growth and yield under limited water supply are drought resistant. The same terminology was used by Blum (2005). Here, the use of the term drought tolerance will be solely adopted. A genotype that yields better than others under a defined level of drought stress and compared to its yield potential under optimum conditions (or yield of control hybrids) will be regarded as relatively drought tolerant.

Another term drought escape, which is merely another form of drought avoidance, will be adopted to refer to two concepts: (i) plants that escape late season drought by maturing sufficiently early; and (ii) plants exposed to stress that can escape drought by shutting down the normal process of growth and divert resources towards early maturity (Fussell *et al.*, 1991; Bänziger *et al.*, 2000). Symptoms observed in concept (ii) are, among others, early flowering and leaf senescence. This way of escaping drought is associated with a high yield penalty.

### **1.3 The test environment**

“Environment” is an umbrella term that encompasses locations and seasons (Blum, 1988). A location is simply a trial site or test site, which is a subset of a larger bracket called environment (E). Blum (1988) considered an environment as being defined by three main components: (i) moisture; (ii) temperature; and (iii) soil-physical and chemical properties. The moisture of an environment is largely influenced by precipitation and subsequent soil

crop water balance. Soil crop water balance (SCWB) is a function of soil-plant water gain (through irrigation or precipitation), loss through evapotranspiration ( $E_o$ ) and crop water use (CWU) (Ines *et al.*, 2001). Temperature is another component of the environment that influences mainly the plant's phenology, developmental phases, growth rates, yield components and final grain yield. It is largely affected by seasonal changes, altitude and other weather attributes that lower or elevate temperatures. The most important effect of temperature on maize growth is its effect on growing degree days (GDD). The GDD is the total amount of heat units required for the plant to reach specific growth stages (McMaster and Wilhelm, 1997). Lastly, the soil-chemical component determines the amount and availability of toxic minerals and essential plant nutrients. Aluminium toxicity has been regarded as the most important mineral related problem in the soil environment (Yang *et al.*, 2013).

In Blum's (1988) definition of the test environment, the biotic component was not mentioned. The environment can also be defined by the predominant living organisms such as weeds, pests, pathogens, etc. Biometricians have defined the genotype's environment in terms of the performance of tested genotypes for the trait/s in question. This is important because it establishes the value of an environment using the genotype's response. The set of environments also establishes the value of different traits expressed by a genotype. The value depends on the range of genotypes and traits being measured. For example, an environment could be defined by its capability to discriminate between susceptible and resistant genotypes to certain diseases such as *Cercospora zeae-maydis* (Grey leaf spot; GLS). With regards to drought stress, the most important categorization of environments should largely depend on amount of rainfall, heat units and crop yield. Selection of a test environment should depend on how these attributes affect genotype performance and/or how genotypes consistently respond over time (Tolessa *et al.*, 2013).

## **1.4 Soil, water and plant relationships**

Even though water is abundant, covering about 71% of the earth's space, its availability in the soil and to plants is restricted (Chavarria and dos Santos, 2012). All plant species require from the soil an amount of water that can satisfy all metabolic processes.

### **1.4.1 Soil water potential and water content**

The status of water in the soil is either described in terms of the amount (water content) in the soil matrix or the energy (water potential) associated with holding water within the soil matrix (Decagon Devices, 2011). Soil water content is defined as the amount of water contained in the soil pores expressed on a gravimetric or volumetric basis. It gives an

indication of how much water is available per given soil or soil profile (Bilskie, 2001). Soil water content is influenced by the soil's physical characteristics such as its texture and structure, and also environmental variables such as evaporation and rainfall. The maximum available water is termed field capacity (FC) and the minimum soil water content is called the permanent wilting point (PWP). Field Capacity (reached after 2-3 days after rain or irrigation) is the amount of soil moisture or water content held in the soil after excess water has drained away. Water at FC is available to the plant. Permanent wilting point refers to when the soil has dried off and plant cannot draw water from the soil. The difference between FC and PWP constitutes plant available water (PAW) (Cepuder *et al.*, 2008). To avoid plant water deficit, the soil should be kept within the range of PAW.

Another soil water content point described by Cepuder *et al.* (2008) is called the “refill point” which is intermediate between FC and PWP. This is important for irrigation scheduling. It indicates the soil water content at which plant production starts to dwindle as the plant becomes water stressed (Kirkham, 2005). When a plant is approaching the refill point, it shows signs of wilting late in the day, particularly in hot dry conditions. This indicates that the soil adjacent to the root zone has dried or is at a water potential that is too low for plants to draw up (Shock *et al.*, 2003). Under field conditions, this zone can be refilled overnight as the soil redistributes its water. Consequently, the wilting may not be visible in the morning. Many times in crop farming, soil water is refilled through irrigation and the amount of water used for refilling will vary depending on soil type, evaporation conditions, crop, and management practices used (Cepuder *et al.*, 2008). In a pot environment, for example, where the root zone is limited by the depth and width of the pot, water is rapidly lost through evaporation and there is little water redistribution at night. It is, therefore, recommended to add water at night to keep the plant just above wilting point (Shock *et al.*, 2003; Cepuder *et al.*, 2008).

Total soil water potential ( $\Psi_T$ ) is the energy contained per unit amount of soil water, relative to pure free water at the soil surface (Cepuder *et al.*, 2008). Total soil water potential ( $\Psi_T$ ) is affected mainly by osmotic ( $\Psi_O$ ) and matric potential ( $\Psi_M$ ) of the soil. If there were no forces acting on soil water, then all water in the soil would be readily available to plants. Essentially, there are three forces acting on soil water and these are gravitational, matric, and osmotic (Sojka, 1985; Livingston and Topp, 2006). Water molecules have potential energy just as all matter has potential energy by virtue of their position in the gravitational field. The soil osmotic potential results from the interaction between water and other electrostatic poles in the soil such as salts whilst the matrix arrangement of soil solid particles results in capillary and electrostatic forces which determine the soil matric water potential (Bilskie, 2001). The magnitude of matric forces is determined by textural, physical and chemical properties of the



soil solid matter which affect the capillary and absorptive nature of the soil (Shock *et al.*, 2003). Soil water potential is used primarily for determining the direction and rate of water flow between locations with differing potentials. There is always water flux within the soil profile; water moves between soil and plant roots, and between the soil and atmosphere. In all natural systems, movement of materials is generally dependent on energy gradients, that is, materials move from areas of high energy levels to low energy levels (Bilskie, 2001). This is explained by the second 'Law of Thermodynamics' which states that connected systems with differing energy levels will move towards an energy state of equilibrium (Decagon Devices, 2011).

The relationship between water content and matric potential can be established using soil water characteristic curves (Fig. 1.3) (Bilskie, 2001). At the same matric potential, the three different soil types do not have the same volumetric water content because of differences in water holding capacities. Conversely, at the same volumetric water content, clay holds water at the highest matric potential of the three soil types.

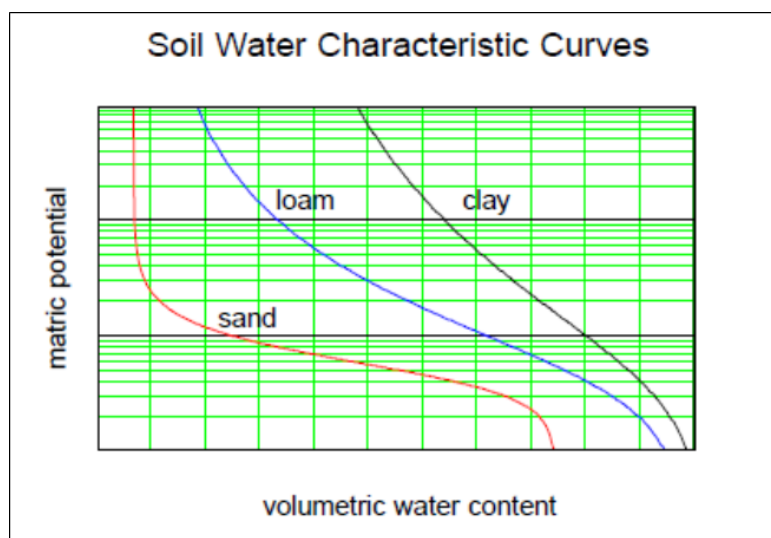


Fig. 1.3 Relationship between matric potential and volumetric water content (Bilskie, 2001)

#### 1.4.2 Techniques for measuring soil water potential

The various instruments and techniques used to measure soil water potential do not measure the whole range of water potentials in the soil. They are mostly sensitive only to matric potential making the results less representative especially when the contribution of other forces is greater (Bilskie, 2001; Livingston and Topp, 2006) and very few of these techniques are practical (Savage and Gebregiorgis, 2006). Soil water potential can be measured directly by determining water pressure or water surface elevation relative to a datum. Indirect measurement involves techniques that measure a surrogate<sup>1</sup> property that

<sup>1</sup> A parameter that indirectly provides a measure of soil water potential

correlates with water potential such as electrical resistance or conductivity, water vapour pressure, water content or plant xylem potential (Livingston and Topp, 2006). Examples of methods used to measure soil water potential include tensiometry, thermocouple psychrometric, electrical conduction (generically Buoyucous blocks), heat dissipation such as the Campbell Scientific sensor model 229 (Bilskie, 2001) and MPS-2 Dielectric Water Potential (Decagon Devices, 2011). Tensiometry is the most common technique used to measure soil water potential.

### **1.4.3 Techniques for measuring soil water content**

Techniques used to measure soil water content should be able to effectively quantify FC and permanent wilting point (PWP). Early farmers used to measure soil water content through its effects on plants, a wilting plant implied that water was needed (Cepuder *et al.*, 2008). As agricultural science developed, more sophisticated techniques to measure soil water content were developed. The most common techniques are the gravimetric method, neutron probe, time domain reflectometry (TDR), frequency domain reflectometry (FDR) and capacitance sensors (Cepuder *et al.*, 2008). All of these, to some degree, involve soil disturbances. The gravimetric method is more reliable and regarded as the most accurate (Noborio, 2001) and can be used for comparison and calibration of the other techniques (Chanasyk and Naeth, 1996; van Overmeeren *et al.*, 1997).

### **1.4.4 Comparison of techniques**

As far as plant water stress is concerned, techniques that quantify water potential or water tension such as tensiometers, MPS-2 dielectric water potential sensors and resistance blocks, are more relevant because soil water potential is directly related to the ability of plants to extract water from the soil, regardless of how much water is available (Charlesworth, 2000). But none of these techniques can measure soil water potential up to -1500 kPa (permanent wilting point). Tensiometers only measure water potential between 0-75 kPa (gauge-type tensiometers can measure up to 80 kPa) and the readings are difficult to convert to soil water content. Resistance blocks can measure water potential from 0-1000 kPa while MPS-2 dielectric water potential sensors measure from 5 to 500 kPa (Charlesworth, 2000). Most plants, however, show signs of water stress at soil water potential of 50 kPa and hence all these techniques are in the range of practical interest (Livingston and Topp, 2006; Cepuder *et al.*, 2008).

High-frequency electromagnetic techniques are the most promising to measure soil water content (Huisman *et al.*, 2003). A well-established and more precise electromagnetic technique has been shown in literature to be TDRs which have an added advantage of

measuring both dielectric permittivity and bulk soil conductivity (Dalton *et al.*, 1984). All other water content techniques were described by Seyfried and Murdock (2004) as alternatives to TDRs. Apart from the standard gravimetric method, the accuracy and precision of these alternative techniques can be established by comparing with TDR measurements (Dalton *et al.*, 1984; Robinson *et al.*, 1999; Huisman *et al.*, 2001; Evett *et al.*, 2002; Seyfried and Murdock, 2004). However, all these electromagnetic techniques have a limitation of taking point measurements (Seyfried and Murdock, 2004).

Remote sensing with either passive microwave radiometry or active radar instruments such as Ground Penetrating Radar (GPR) is also a more promising technique to measure soil water content over large scales (Jackson *et al.*, 1996; Huisman *et al.*, 2001; Huisman *et al.*, 2003). However, measurements of soil water content in the upper 0.05 m of the soil profile using remote sensing requires vegetation cover to be at a minimum (Jackson *et al.*, 1996). In addition, the technique is not applicable to all soil conditions (Huisman *et al.*, 2003). Comparatively, the TDR technique is more popular in agriculture than GPR (Huisman *et al.*, 2003). This is probably because of the complicated behaviour of unguided waves used in GPR compared with waves guided by a TDR sensor. This perhaps affects the travel time of electromagnetic waves and determination of soil water content.

For effective measurement of soil water content at different depths in the soil profile, the EnviroSMART or EasyAGII (Campbell® Scientific) which uses capacitance sensors are robust (Charlesworth, 2000; Evett *et al.*, 2002). The devices can effectively measure infiltration rate, drainage and root activity. Crop water-use can easily be interpreted. However, the disadvantage of the technique is that the sensors are fixed into access tubes and they are not portable. The Diviner 2000® (Sentek Technologies) which uses the same calibration as the EnviroSMART is portable. It can be moved conveniently from one site to another like the neutron probe (Corbeels *et al.*, 1999). The only limitation is its inability to measure water content at various depths because it has only one sensor mounted at the one end of a probe 1 m or 1.6 m in length (Charlesworth, 2000).

All dielectric, capacitance sensors and the neutron probe techniques require some form of calibration and computer software which add to the costs (Evett *et al.*, 2002). Even though the devices come with general soil calibrations, soil specific calibrations need to be done to obtain accurate results (Charlesworth, 2000). In comparison to all other techniques, the use of the neutron probe technique has dwindled because of the danger associated with the electromagnetic radiation released by the probes (Chanasyk and Naeth, 1996; Corbeels *et al.*, 1999).

## 1.5 Plant water status

Plant water potential indicates the status of the water within a plant (Cleary *et al.*, 2009). It is an integrated measure of the soil water tension in the rooting zone (the water supply), the resistance to water movement within the plant, and the demand for transpiration which is imposed by the atmospheric factors such as temperature, humidity, wind, etc. (Reicosky and Lambert, 1978). Plant water deficit develops when excess water is lost from the plant through transpiration without replacement from the soil (Blum, 2011). This causes a reduction in leaf turgor pressure leading to cell collapse. At this point, leaves begin to wilt. Stomata generally respond to reduced turgor by closing down in order to reduce water loss through transpiration (Sojka, 1985). Plant water stress indicates the plant's water status and how below and above ground environment influences it. These factors are always changing and hence plant water status always changes. For example plant water requirement is higher during midday and least just before sunrise. Pre-sunrise plant water status values will usually reflect average soil water tension if the soil is uniformly irrigated. Midday plant water status values reflect the tension experienced by the plant as it pulls water from the soil to satisfy atmospheric demand (O'Toole and Tomar, 1982; Cleary *et al.*, 2009).

Plant water stress develops when there is insufficient soil water to support the plant's demand for water. When this occurs, physiological processes, such as photosynthesis are negatively affected, subsequently reducing plant growth and/or ultimately resulting in plant death. Data that give information on plant water status can be used to evaluate the plant's need for water or how well it is adapted to its environment (Cleary *et al.*, 2009). There are three basic aspects in understanding plant water stress. These are atmospheric demand on the plant, plant regulation or plant reaction to water stress and soil supply or the composition of the soil (Table 1.1; Lincoln and Eduardo, 2010).

**Table 1.1 Some of the main factors influencing plant water status**

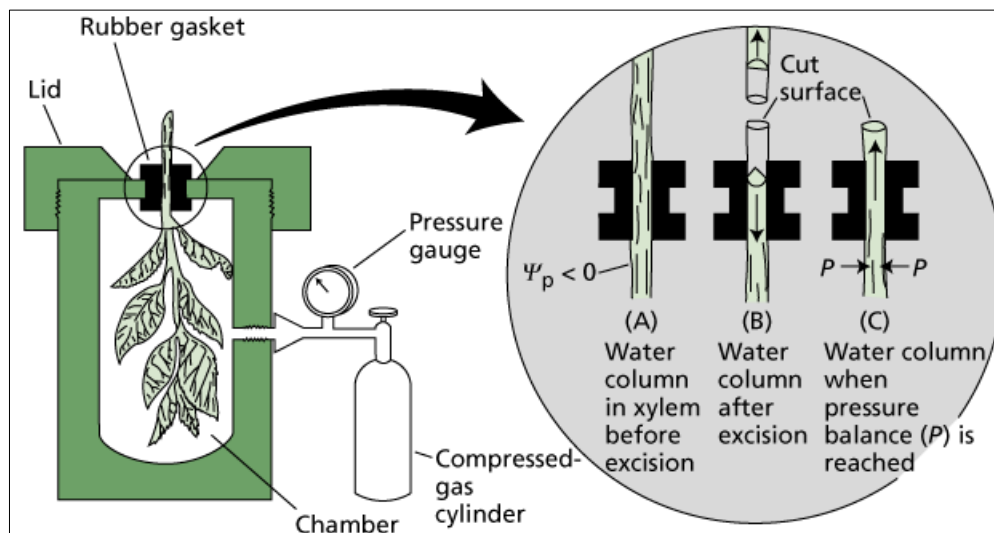
Determinants of plant water status	Main factors
Atmospheric demand	Radiation, Air temperature Humidity, Wind
Plant regulation	Leaf rolling, Leaf necrosis Stomatal conductance Osmotic adjustment Leaf flagging, Leaf abscission
Soil water supply	Water content Soil temperature Soil depth, Soil texture, Soil structure

It is vital to measure plant water status as it indicates the ability of the plant to grow and function in a given environment. Such information can be used as a guide for managing the plants' moisture environment so as to improve growth and crop yield. The neutron probe, tensiometer, infrared thermometer and pan evaporimeter are techniques commonly used, but these do not give a direct response of the plant to its environment which is the soil and atmosphere (Cleary *et al.*, 2009). In 1965 the Scholander pressure chamber was introduced to allow direct measurement of plant water status (Scholander *et al.*, 1965). Apart from the pressure chamber, Lincoln and Eduardo (2010) suggested the use of psychrometers, cryoscopic osmometer and pressure probes for direct measurements of plant water stress. A pressure probe is the only instrument that can measure water status of a single cell (Lincoln and Eduardo, 2010).

### **1.5.1 The pressure chamber**

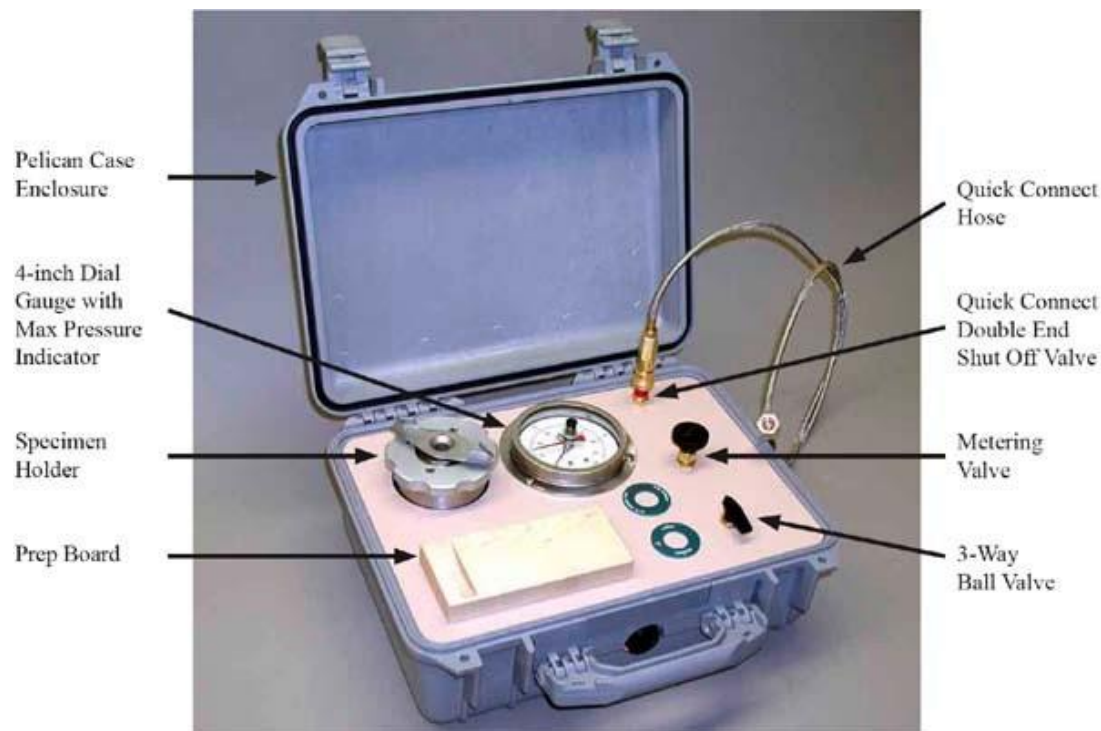
The pressure chamber technique was introduced by Dr Scholander and his associates in the 1960s (Scholander *et al.*, 1965). In its simplest terms, the pressure chamber can be thought of as measuring the "blood pressure" of a plant, except that for plants it is water rather than blood and the water is pulled through the plant by the "suction" force of transpiration rather than pumped by a heart (Shackle, 2012). Water within a plant moves through xylem vessels, which are a network of pipes carrying water from the roots to the leaves. Water in the xylem is always under tension, and as the soil dries due to evaporation through the leaves, the tension increases. Under these conditions it could be said that the plant is beginning to experience "high blood pressure" (Shackle, 2012).

The pressure chamber is a device that applies pressure to a leaf (or a shoot) (Fig. 1.4). The amount of pressure that it takes to cause water to appear at the cut surface of the petiole (for leaves) indicates the amount of pressure necessary to force water out of the leaf cells into the xylem of the petiole and is a function of the water potential of the leaf cells (Boyer, 1967). A high value of pressure means a high value of tension and a high degree of water stress (Cepuder *et al.*, 2008; Shackle, 2012).



**Fig. 1.4 Schematic of a Scholander pressure chamber (Lincoln and Eduardo, 2010)**

The applicability of the pressure chamber to measuring leaf water potential under field conditions has been possible through the development of portable water stress consoles in 1974 by Soil Moisture Equipment Company in USA (Fig. 1.5). The latest 3115 (Soil Moisture Equipment Corp., Santa. Barbara, CA, USA) series is economical, field potable and can be mounted at the back of a truck while taking measurements across the field and the 3005HGPL (Soil Moisture Equipment Corp., Santa. Barbara, CA, USA) series is capable of taking numerous readings quickly, accurately and with precision (Soil Moisture, 2015).



**Fig. 1.5 Portable plant water stress consoles (Soil Moisture, 2015)**

### 1.5.2 The psychrometer

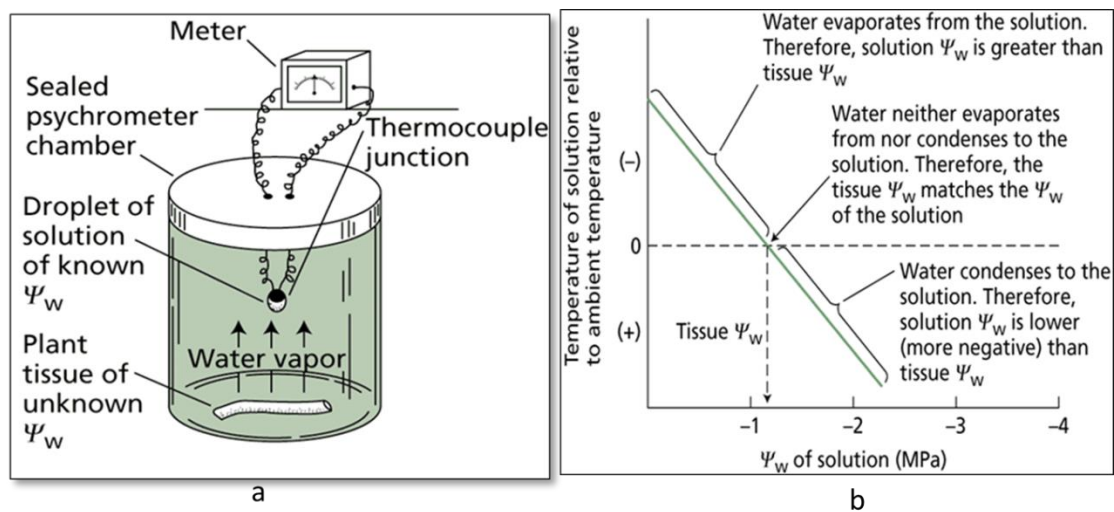
The principle of psychrometry is based on the fact that the vapour pressure of water is lowered as its water potential is reduced (Daniel, 1981; Lincoln and Eduardo, 2010; Martinez *et al.*, 2011). Thermocouple psychrometers measure the water vapour pressure of a solution or plant sample on the basis that evaporation of water from a surface cools that surface. The isopiestic psychrometer (Fig. 1.6), has been used extensively since 1965 (Lincoln and Eduardo, 2010) and was shown to effectively measure leaf water potential (Boyer, 1967). Measurements are done by placing a piece of plant tissue inside a small chamber that contains a temperature sensor (a thermocouple in this case). The thermocouple is placed in contact with a small droplet of a standard solution of known solute concentration ( $\psi_s$ ) and thus known  $\psi_w$ . If the plant tissue has lower water potential than the droplet, then water evaporates from the droplet through the air and is absorbed by the plant tissue. The opposite occurs if the droplet has low water potential (Fig. 1.6). The surface of the droplet from which the water evaporates is cooled down whereas the surface which absorbs water is warmed (Lincoln and Eduardo, 2010). The change in temperature can be calibrated to provide a measurement of the plant tissue  $\psi_w$  (Martinez *et al.*, 2011).

Psychrometers can be used to measure water potential of both excised and intact tissue. Measuring intact tissue gives the plants'  $\psi_w$  and crushing the tissue provides the  $\psi_s$  and the difference between the two values can be used to calculate turgor pressure (Lincoln and Eduardo, 2010):

$$\text{Turgor pressure } (\Psi_p) = \Psi_w - \Psi_s$$

Eqn 1.1

Thermocouple psychrometers can be used to verify the accuracy of a pressure chamber; however, the difference in the potential which probably exists between water in the leaf tissue (simplastic and apoplastic) and xylem complicates comparison between these two techniques (Boyer, 1967). More recently, there has been an increase in the use of PSY1 Stem Psychrometers for plant water potential measurements (Daniel, 1981; Martinez *et al.*, 2011). Stem psychrometers were released in 2010 by the ICT international company in Australia (Lincoln and Eduardo, 2010).



**Fig. 1.6 (a.) Isopiestic psychrometry to measure the water potential of a plant tissue and (b.) Movement of water between droplet solution and plant tissue (Lincoln and Eduardo, 2010)**

### 1.5.3 Relative water content

Relative water content (RWC) is another technique used to measure plant water status or rather leaf water status. It was previously known as relative turgidity (Smart and Bingham, 1974). The similarity with the pressure chamber and psychrometric techniques is that leaf tissue is commonly used. Leaf water status is linked to several leaf physiological variables that affect plant performance, such as leaf turgor, growth, stomatal conductance, transpiration, photosynthesis and respiration (Yamasaki and Dillenburg, 1999). This technique does not measure the energy status of leaf water, but it is a more useful parameter in water balance analysis and provides a relationship between plant physiological traits and level of drought stress (Quilambo, 2004). The RWC procedure involves measuring fresh mass of leaf discs followed by flotation in water for up to 4 hours. Thereafter, the turgid mass is measured. The leaf tissue is then oven dried to a constant mass at 85°C. RWC is calculated as follows:

$$\text{Relative water content (RWC)} = (\text{fresh mass-dry mass}) / (\text{turgid mass-dry mass})$$

Eqn 1.2



Thus, RWC is the ratio of the amount of water in the leaf tissue at sampling to that present when tissues are fully turgid. Another indirect method introduced by Smart and Bingham (1974) is called relative tissue mass which is simply the ratio between fresh mass to turgid mass. Maintenance of high leaf turgor which is the case when plant cells have relatively high water content is ideal for plant growth. Apart from stomatal closure, osmotic adjustment (OA) has been attributed to serve the same purpose of maintaining positive and high turgor potential during water stress (Quilambo, 2004; Blum, 2011). Osmotic adjustment involves the accumulation of solutes in plant cells in response to decreases in plant water potential, consequently lowering osmotic potential. This adaptive mechanism will attract water into plant cells, maintaining turgor pressure (Babu *et al.*, 1999) causing smaller changes in plant RWC (Quilambo, 2004) and improves stomatal conductance, photosynthesis and growth (Gebre and Tschapliski, 2000).

## **1.6 Genes associated with drought tolerance**

Understanding the molecular mechanisms of plant response to drought is important for improvement of drought tolerance using molecular techniques (Nakashima *et al.*, 2014). Gene expression is affected by changes in available water and may indicate some of the pathways important for adaptive responses to stress. For example, some dehydration regulated genes are reported to be affected by abscisic acid (ABA) whereas other drought induced genes act independently from ABA pathways (Nakashima *et al.*, 2014). This suggests the complexity of the drought response phenomenon. In *Arabidopsis*, research has uncovered the potential existence of four ABA-independent pathways and one ABA dependent pathways that control stress-inducible gene expression (Liu *et al.*, 2013). The ABA-responsive element (ABRE) has been reported to be the major *cis*-element for ABA-responsive gene expression (Nakashima *et al.*, 2014) whereas, the Dehydration Responsive Element Binding proteins (DREBs) are thought to be the major transcription factors (TFs) that control stress-inducible gene expression in the ABA-independent pathway (Liu *et al.*, 2013). In maize, two DREB genes (ZmDREB1A and ZmDREB2A) belonging to the DREB1 and DREB2 subgroups, respectively, were demonstrated to be up-regulated in response to plant water stress. The understanding of genes associated with drought tolerance is a vital step towards marker assisted breeding.

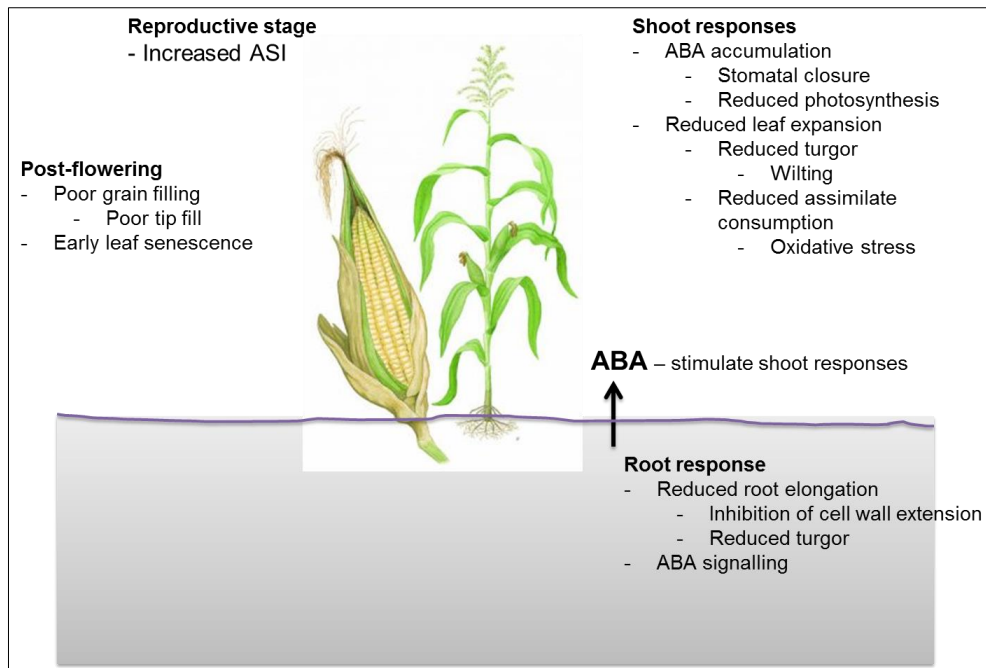
## **1.7 Effects of drought stress and plant responses**

The effect of drought on gene expression is observed in the physiological and morphological changes at whole plant level. According to Blum (1996), drought is a multi-dimensional stress that affects some key physiological and biochemical processes at cellular level.

Symptoms observed at whole plant level are manifestations of complex reactions underlying the plant's cellular system. The set of responses observed depend on three main characteristics of drought stress i.e. severity, duration and intensity (Barker *et al.*, 2004), interacting with the plant's underlying genetic make-up and plant developmental stages (Bray, 1993; Pandey *et al.*, 2000).

### **1.7.1 Physiological responses**

At the onset, drought stress stimulates the release of abscisic acid (ABA) from the roots to the leaves and a simultaneous increase in synthesis of ABA in leaves. Accumulation of ABA is the major driver of drought stress responses as postulated by Setter (1996). It triggers a complex series of events and plant behaviour such as leaf rolling; stomatal closure; reduced leaf expansion in favour of root growth; accelerated leaf senescence; kernel abortion; increased root:shoot ratio (because of reduced shoot sink strength); leaf area; delay in silk development (Tardieu and Davies, 1992; Setter, 1996), etc. These are also referred to as drought escape responses. Drought escape responses force the plant into early maturity, that is, speeding up reproductive and grain filling phases. Each of the plant responses (Fig. 1.7) directly or indirectly affects other plant functions. For example, stomatal closure reduces transpiration resulting in increase in leaf temperature. Leaf temperature increases because the cooling effect of transpiration is curtailed (Blum 1988). Most importantly, water deficit disrupts all major components of photosynthesis, subsequently reducing biomass accumulation (Tezara *et al.*, 1999; Allen and Ort, 2001; Bruce *et al.*, 2002). Photosynthetic capacity may be reduced by several factors linked to the morphological changes explained above. For example, stomatal closure also reduces CO<sub>2</sub> availability (Bruce *et al.*, 2002; Jaleel *et al.*, 2009) which is vital for photosynthesis.



**Fig. 1.7 Absciscic acid signalling maize responses to drought (from Yang *et al.*, 2013)**

Ordinarily, ABA is released as a regulatory hormone to control and reduce water loss and allow the plant to survive under drought conditions (Bänziger *et al.*, 2000), but this comes with a yield penalty when it is released excessively. That is why the suggestion by Setter (1996) to select for genotypes that accumulate very high levels of ABA as a selection criterion is not viable physiologically.

Most species are able to accumulate osmotically active substances (e.g. sucrose and other non-reducing sugars, proline, and glycine betaine) in the cytoplasm and vacuole, which causes a fall in cellular water potential (Bray, 1993) and allows the plant to take up more soil water (Babu *et al.*, 1999). The accumulation of these solutes prevents cellular dehydration and the phenomenon is called osmotic adjustment (OA), which is stimulated by ABA (Setter, 1996). Osmotic adjustment maintains cell turgor and normal cell function for a longer period under drought stress (Bray, 1993; Setter, 1996; Bänziger *et al.*, 2000; Barker *et al.*, 2004). In the event of cell turgor loss, accumulation of proline apparently protects protein structures from deformation (Bänziger *et al.*, 2000). Even though OA is recognized as an effective component for preventing dehydration in many species such as *Triticum aestivum* (wheat), *Sorghum bicolor* (sorghum), *Oryza sativa* (rice) (Bänziger *et al.*, 2000), *Cicer arietinum* (chickpea), *Pisum sativum* (field pea), *Helianthus annuus* (sunflower) and turf grasses (Babu *et al.*, 1999), its function in maize is reportedly minimal (Bänziger *et al.*, 2000).

### 1.7.2 Biochemical responses

Water deficit directly affects Photosystem II (where water is split into oxygen and hydrogen ions) of the photosynthetic pathway by uncoupling it, resulting in free high energy electrons

in the leaf (Munné-Bosch and Cela, 2006). The high energy electrons cause photo-oxidation of chlorophyll and loss of photosynthetic capacity. In addition, water stress induces excessive release of reactive oxygen species (ROS) that are harmful to plant cells and normal enzyme activity (Andjelkovic *et al.*, 2008; Farooq *et al.*, 2009). The production of ROS in plants, known as the oxidative burst, was reported by Anjum *et al.* (2011) to be an early defence response to water-stress. The ROS act as secondary messenger to trigger subsequent defence reactions in plants. Being reactive, the ROS can be extremely damaging to plants by increasing lipid peroxidation, protein degradation, DNA fragmentation and ultimately cell death (Mittler, 2002; Apel and Hirt, 2004).

### **1.7.3 Morphological effects**

Maize growth stages can be grouped into germination, emergence, vegetative, flowering (anthesis and silking), grain filling and senescence phases (du Plessis, 2003). All maize growth stages are susceptible to drought stress but the degree of damage is variable

The initial phases of plant growth, i.e., imbibition, germination, emergence, seedling growth (Blum, 1996; Achakzai, 2009), growth expansion and stand establishment are negatively affected (Bänziger *et al.*, 2000; Jaleel *et al.*, 2009), causing a poor establishment of the plant that can be translated to succeeding stages.

During the vegetative phase, reduced cell division (mainly under severe drought stress) and expansion causes a reduction in leaf area index (LAI), root, stem expansion and subsequently ear yield is reduced (Eck, 1986; Blum, 1996; Bänziger *et al.*, 2000; Jaleel *et al.*, 2009). Retarded leaf growth and accelerated early leaf senescence (which typically start from lower leaves) results in incomplete canopy and reduced radiation capture. Another phenomenon called leaf firing where leaves start to dry up and senesce from the top can be observed. This, together with stomatal closure, photo-oxidation and enzyme damage cause a decline in total photosynthesis and respiration rates (Bänziger *et al.*, 2000).

Prolonged drought stress during the vegetative phase reduces elongation of stem internodes as a result of reduced cell development, causing stunted growth. This diminishes the capacity of stems to store assimilates. Studies have revealed that assimilates stored in the stems are a major source of carbohydrates during the grain filling period under drought stress when photosynthesis is limited (Blum, 1996), hence availability of water during the vegetative phase is as important, but not as critical as at flowering.

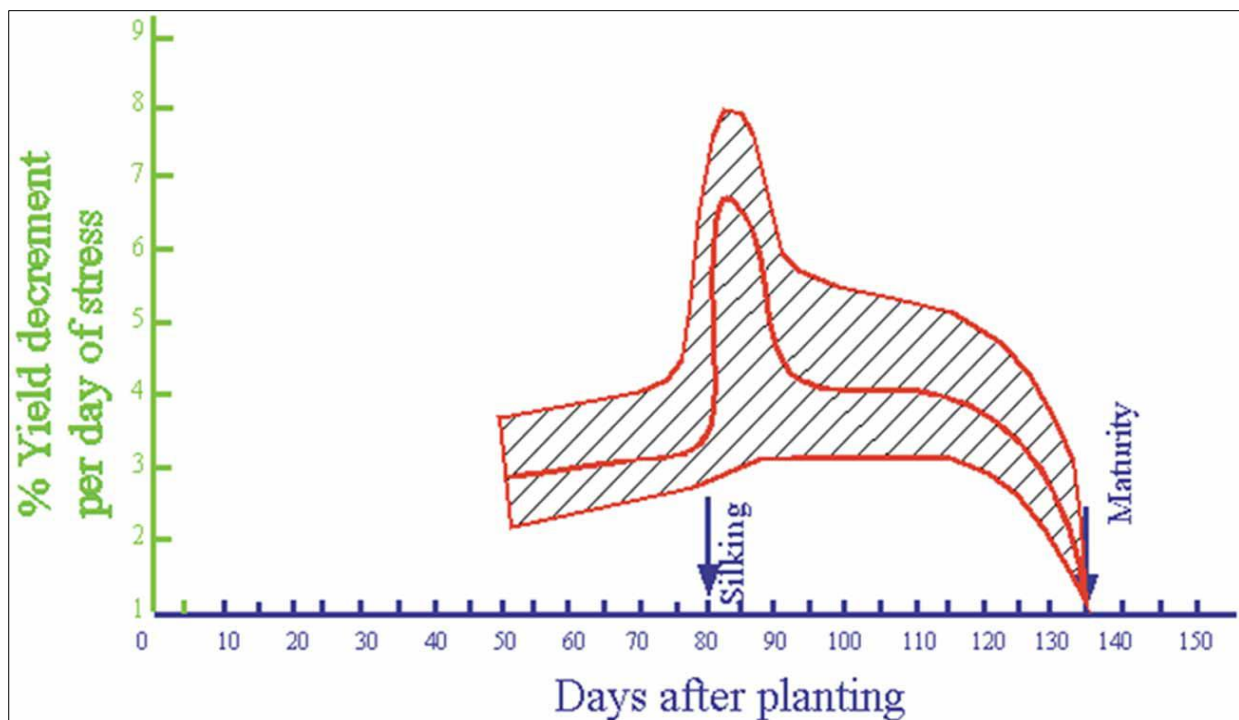
Numerous studies have revealed that the maize crop is most susceptible to drought stress during the period bracketing flowering or the reproductive stage (Frey, 1981; Eck, 1986; Westgate and Debra, 1989; Bolaños and Edmeades, 1996; Bänziger and Lafitte, 1997;

Bänziger *et al.*, 2000; Moser, 2004; Moser *et al.*, 2006; Monneveux *et al.*, 2008; Cairns *et al.*, 2012). This includes anthesis, silk development, pollination and grain filling period. Days to silking are commonly increased while anthesis *per se* is less affected (Blum, 1996; Vasal *et al.*, 1996; Barker *et al.*, 2004; Munyiri *et al.*, 2010; Mhike *et al.*, 2012). Hence pollination may occur but embryo development is severely hindered (Westgate and Boyer, 1986). Frey (1981) noted that the most critical period for yield determination in the life cycle of maize begins approximately two weeks prior to silking until 2-3 weeks after silking. This was confirmed by a number of authors including Grant *et al.* (1989), Bänziger *et al.* (2000) and Boyer and Westgate, 2004. Major stresses before silking typically cause ovule abortion and inhibition of ear development. If ears or silks develop, further stress after pollination leads to kernel abortion and reduced kernel numbers (Frey, 1981; Blum, 1996). This is caused by inadequate assimilates from storage reserves and/or current photosynthesis (Bänziger *et al.*, 2000). Silk and kernel development are dependent on the availability of photosynthates (Schussler and Westgate, 1995).

Even though a maize plant can potentially produce a large number of silks, ears and pollen per tassel, Blum (1996) reported that many of them degenerate and die due to drought stress thus reducing the total sink strength and the most needed reproductive carbon demand to draw assimilates towards the developing ears. It is almost undisputable, therefore, that the risk of losing a crop can be reduced by making sure that the period bracketing the reproductive stage is not exposed to severe stress (Bänziger *et al.*, 2000).

A strong correlation between drought stress (timing, severity and duration) and anthesis-silking interval (ASI; the difference between days to 50% anthesis and days to 50% silking on the same plant) has been extensively reported (Bänziger *et al.*, 2000; Magorokosho *et al.*, 2003). Anthesis-silking interval and ears per plant (EPP) are the two major traits that are reported to be strongly correlated to grain yield (Vasal *et al.*, 1996; Mhike *et al.*, 2012) with ASI being the most important. This differential response to drought stress of the maize reproductive parts is a result of the biology of the plant itself which bears separate staminate and pistillate flowers on the same plant. Delay in silking, which is also linked to accumulation of ABA in the plant, causes pollen to be shed before the stigmas are mature, resulting in unsuccessful pollinations. A study by Bassetti and Westgate (1994) revealed that both protandry (pollen shed before stigmas are ready) and protogyny (silks ready before pollen shed) affect kernel development. Perfect kernel set was observed on ears that silked within one day of pollen shed. Ears that had silks emerging more than one day after anthesis showed a progressive loss of kernels starting from the tip to the base. In the same study, it was observed that plants produced viable pollen within seven days after the commencement of pollen shed. It was therefore concluded that poor kernel set within seven days after

anthesis was due to either lack of silk development or asynchrony. Poor kernel set after seven days was due to both poor silk development and insufficient pollen. Since the pollen shed period is longer silking (about seven days), it was observed that silks that develop prior to anthesis had a high chance of being sufficiently pollinated and hence kernel set and grain yield is generally adequate. Much of the thinking on the degree of susceptibility of maize (especially during silking) to stress has been based on research that showed yield reduction per day of stress on developmental stages of a maize hybrid (Fig. 1.8; Araus *et al.* (2012)). Yield reduction between 0-10 days after pollination is predominantly due to zygotic abortions and sluggish endosperm cell division which affects kernel numbers (Westgate and Boyer, 1986; Schussler and Westgate, 1995). The period afterwards (about 10-21 days after pollination), affect cell enlargement which reduces final kernel mass (Schussler and Westgate, 1995; Westgate, 2000). In Figure 1.8, the outer two lines represent the upper and lower limits of yield reduction determined from various experiments; the middle line is the average. The greatest yield reduction for the set of hybrids was recorded between 80 to 85 days after planting, that is, during the silking period (Fig. 1.8).



**Fig. 1.8 Relationship between yield loss per day of stress and growth stage in a maize hybrid bred in the 1960s. Outer lines show range of experimental results, middle line shows the average. Although experiments were not run to evaluate effects of stress occurring before 50 days after planting (Araus *et al.*, 2012)**

In a two year study conducted by Pandey *et al.* (2000) in a semi-arid Sahelian environment, maize was grown under five irrigation treatments providing deficit irrigation levels at vegetative and reproductive phases. Results showed that water deficit imposed at the vegetative phase (before flowering) reduced grain yield by 11.1% and 6.6% in the first and

second year, respectively. Water deficit at the vegetative through to early reproductive phases reduced yield by 22.6% and 28.2% for the first and second year, respectively. Imposition of deficit irrigation at both the vegetative and all the reproductive phases reduced yield by 52%. Generally, in all experiments, yield reduction was associated with a reduction in kernel numbers. This study showed that any level of water stress has a negative impact on maize grain yield.

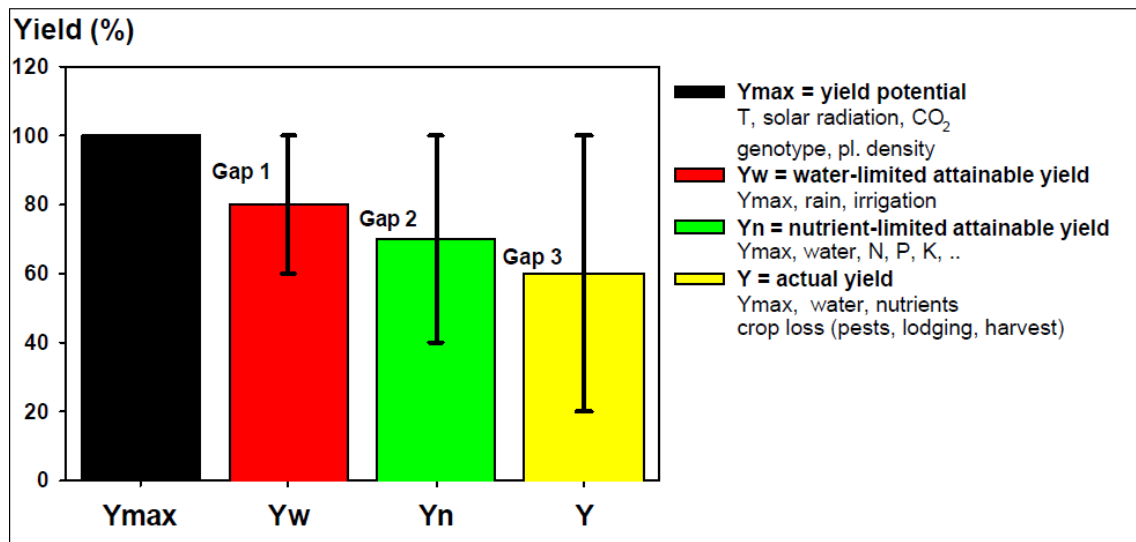
## 1.8 Yield potential versus actual yield

Yield potential ( $Y_{\max}$ ) is defined as the maximum yield that a genotype can achieve under optimum environmental conditions (Evans and Fischer, 1999; Dobermann *et al.*, 2003; Blum, 2005). The optimum environment referred to is where plant growth determining factors such as water and nutrient availability, solar radiation, temperature and  $\text{CO}_2$  concentration are not limiting (Evans and Fischer, 1999; Tollenaar and Lee, 2002; Dobermann *et al.*, 2003; Blum, 2005). The  $Y_{\max}$  (Fig. 1.9) can be quantified by: (i) theoretical calculations from components of yield and radiation use efficiency (RUE); (ii) measurements in well-controlled, small-scale experiments which eliminate biotic and abiotic stresses (such as water, nutrients, and pests); and (iii) estimation by crop simulation models (Dobermann *et al.*, 2003).

There is always a yield gap between  $Y_{\max}$  and actual yield ( $Y$ ) or between yields in optimal and stress conditions (Fig. 1.9) (Dobermann *et al.*, 2003). Introgression of drought tolerance in maize genotypes aims to narrow the gap between  $Y_{\max}$  and  $Y$  under drought stress (Cattivelli *et al.*, 2008). Studies have suggested that yield under well watered conditions ( $\text{GY}_{\text{ww}} = Y_{\max}$ )<sup>1</sup> could be used as a reasonable predictor of grain yield under moderate stress levels ( $\text{GY}_{\text{s}} = Y$ )<sup>2</sup>, only when  $\text{GY}_{\text{s}}/\text{GY}_{\text{ww}} = 70\text{-}90\%$  (Barker *et al.*, 2004). However, when yields under stress are less than 60-70% of  $Y_{\max}$ , it is more effective to select genotypes under stress environments. Under severe stress,  $Y_{\max}$  should be used in combination with specific secondary traits. The generally negative correlation between yield in unstressed and severely stress environments indicates the need to breed for specific adaptation (Barker *et al.*, 2004).

<sup>1</sup> ( $\text{GY}_{\text{ww}} = Y_{\max}$ ) Grain yield under well watered conditions is equal to  $Y_{\max}$

<sup>2</sup> ( $\text{GY}_{\text{s}} = Y$ ) Grain yield under stress conditions is equal to  $Y$



**Fig. 1.9 Yield gaps under different stress conditions (Dobermann *et al.*, 2003).**  
T = Transpiration; pl = Plant; Ymax = Maximum yield; N = Nitrogen; P = Phosphorus;  
K = Pottassium; Y = Yield

## 1.9 Breeding for drought stress environments

Typically breeding programmes focus on: (i) selecting for yield (Donald, 1968); (ii) defect elimination (Donald, 1968); and (iii) evaluation of experimental genotypes across a large number of environments (i.e., locations and seasons) before commercialization (Tollenaar and Lee, 2010). Defect elimination involves the removal of less fit genotypes or correction of physical imperfections in the gene pool during selection processes (Tollenaar and Lee, 2010) or incorporation of, for example, earliness in genotypes that are prone to late season drought. Breeding programmes based on selection for yield *per se* only intended to improve crop yield by hybridization of “promising parents” that have excellent combining ability for yield (Donald, 1968). Such promising parents are selected based on their performance from a large pool of potential candidate genotypes.

A fourth approach to breeding and selection, termed ideotype breeding by Donald (1968) will be discussed. An ideotype is defined as an ideal plant type. It is a specific model plant which is set as a breeding target. Since the term was introduced by Donald in 1968, plant breeders have employed the concept defining and redefining what they considered to be a desirable plant. Following Donald’s introduction of the wheat ideotype, Mock and Pearce (1975) proposed a maize ideotype for optimum production environments which had the following characteristics:

- i. Stiff vertically oriented leaves above the ear with horizontal leaves below the ear
- ii. Maximum and efficient rates of photosynthesis
- iii. Short anthesis to silking interval



- iv. Efficient translocation of photosynthate into grain
- v. Ear shoot prolificacy
- vi. Small tassel size
- vii. Cold-tolerance of germinating seeds and developing seedlings
- viii. Photoperiod insensitivity
- ix. As long grain-filling period as practically possible
- x. Slow leaf senescence

But the ideotype breeding approach has not increased breeding progress as may have been anticipated (Hamblin, 1993) probably because of issues such as lack of robust data management systems, time consumption and precision. Also Tollenaar and Lee (2010) argued that selecting and placing intensive pressure on just one of the 10 traits listed by Mock and Pearce (1975) does not guarantee a substantial increment in grain yield which is a far complex trait than the genetics underlying the individual traits. However, this is when the hypothesis of constant systems capacity (CSC) as described by Yan and Wallace (1995) becomes important. The hypothesis states that a subset of interdependent traits within a species constitutes a biological system that has a constant capacity. The interrelationships among traits are a vital consideration in ideotype breeding. Each trait may be independent or interdependent on some or all of the other traits (Yan and Wallace, 1995). That is why Yan and Wallace (1995) stated that the CSC concept requires, “*a simultaneous and compromising optimisation of the levels of each of the multiple traits of the system, to maximise the function of the system as a whole in contrast to pursuing the extreme and seemingly most desirable level for each single trait*”. The ideotype approach therefore remains a useful concept because it generates assumptions for research and stimulates thinking on which traits may contribute to improved adaptation and yield. Plant breeders have developed some techniques to deal with selection of multiple traits. These include tandem selection, independent culling, index selection, etc. For details on these techniques, readers are referred to a review article by Luby and Shaw (2008).

### **1.9.1 Sources of genes for drought tolerance**

Breeders rely on external germplasm sources of drought tolerance when their own germplasm do not have sufficiently higher frequency of drought tolerance related genes. Rarely can germplasm sources be well adapted and at the same time high yielding in the target environment and therefore, additional breeding attempts are required (Edmeades *et al.*, 1997c). An intelligent choice of source germplasm plays a critical role because it determines the frequency of desirable traits at the onset of a breeding program (Edmeades *et al.*, 1997b; Hallauer *et al.*, 2010). The probability of obtaining drought tolerant hybrids is higher when the source population has a high level of drought tolerance. The most

popular sources used by the International Maize and Wheat Improvement Center (CIMMYT) are landraces, elite germplasm with known drought tolerance, existing high yielding genotypes and adapted tropical lowland genotypes (Edmeades *et al.*, 1997c).

One of the most important aspects in maize breeding is the development of populations from the known germplasm sources. A population is a large group of interbreeding individuals (Acquaah, 2007). CIMMYT has been developing source populations for drought tolerance using two approaches: (i) recurrent selection of elite germplasm; and (ii) screening a wide range of improved and unimproved germplasm for unique sources of drought tolerance. These are then combined into a single gene pool which is further improved for agronomic performance (Edmeades *et al.*, 1997c).

Acquaah (2007) defined recurrent selection as a cyclical improvement technique aimed at gradually concentrating desirable alleles in a population. The procedure exploits mainly additive, partial dominance to over-dominance type of gene action (Acquaah, 2007). According to Blum (1988), unidentified drought adaptive alleles exist at relatively high frequencies in existing breeding populations. Based on this premise, CIMMYT embarked on a recurrent breeding program to develop drought tolerant populations from within the organisation's elite germplasm. The selected elite sources were either relatively drought tolerant (e.g. Tuxpeño Sequía) or broadly adapted germplasm (e.g. Pool 16 Sequía, Pool 18 Sequía, Pool 26 Sequía and La Posta Sequía) (Edmeades *et al.*, 1997c). Full-sib recurrent selection consisted of eight cycles ( $C_0 - C_8$ ; each cycle taking two years) with selection indices comprising relatively high leaf and stem elongation rate, short anthesis-silking interval, delayed foliar senescence, grain yield and canopy temperature (Peña-Valdivia *et al.*, 1997). The evaluation and selection of genotypes in each cycle was accompanied by use of testers in a line by tester mating design. Selection with tester genotypes is important for traits with low heritability. The method exploits other gene actions apart from additivity (Edmeades *et al.*, 1997c). In addition, test crosses enable the establishment of general and specific combining ability, which can be a good indication of broad and specific adaptation, respectively (Hallauer *et al.*, 2010).

Evaluation of improved inbreds showed a significant gain in drought tolerance and grain yield (about  $100 \text{ kg}^{-1} \text{ ha}^{-1} \text{ cycle}^{-1}$ ) when comparisons between  $C_0$  and  $C_8$  were made (Bolaños *et al.*, 1993; Edmeades *et al.*, 1997c; Peña-Valdivia *et al.*, 1997). The improvement from  $C_0$  to  $C_8$  was perhaps due to enhanced agronomical, morphological, biochemical and physiological traits that affect plant efficiency, e.g. water use efficiency, photosynthesis and assimilate partitioning (Bolaños *et al.*, 1993). Today CIMMYT drought tolerant donor lines are available in the public domain (Table 1.2) (Edmeades *et al.*, 1997a).

**Table 1.2 Some of the drought tolerance donors from CIMMYT**

DTMA	Pedigree	HG	Colour	Texture	Grain yield (t ha <sup>-1</sup> ) <sup>a</sup>		
					Drought	Drought + heat	Well-watered
251	La Posta Sequia C7-F18-3-2-1-1 <sup>b</sup>	B	White	Flint	3.16	1.13	7.04
217	DTPWC9-F24-4-3-1 <sup>b</sup>	AB	White	Flint	3.1	1.43	6.97
-	DTPYC9-F46-1-2-1-1-1 <sup>b</sup>	AB	Yellow	Flint	3.07	1.23	6.73
-	DTPYC9-F46-1-2-1-1-2 <sup>b</sup>	AB	Yellow	Flint	3.07	1.58	7.12
103	POB.502 c3 F2-10-3-2-1 <sup>b</sup>	B	White	Flint	3.07	1.17	7.54
	La Posta Sequia C7-F64-2-6-2-2 <sup>b</sup>	B	White	Flint	3.06	1.39	7.72
261	La Posta Sequia C7-F180-3-1-1-1 <sup>b</sup>	B	White	Flint	3.03	1.13	7.94
	Check (CML442/CML444)				2.36	0.96	7.7
	Number of locations				7	3	7
	H				0.64	0.5	0.84
	<b>Trial mean</b>				2.58	1.13	6.88

<sup>a</sup>adjusted means (best linear unbiased prediction); <sup>b</sup>performance when testcrossed with CML539  
DTMA = Drought tolerance maize for Africa; HG = Heterotic groups

### 1.9.2 Evaluation of genotypes in multi-environmental trials

Firstly, breeders create new gene combinations and useful variability by intercrossing parent lines that possess desirable characteristics, or by introgression of useful genes into available germplasm. This is achieved through conventional breeding and/or genetic engineering procedures (Bänziger *et al.*, 2000). The *cspB* gene in the Genuity Droughtguard hybrid (Monsanto Company) is an example of a genetically engineered “drought tolerant” hybrid (Monsanto, 2013). Secondly, resultant genotypes are evaluated in target environments (Bänziger *et al.*, 2000). Evaluation process can be divided into two phases: (i) **screening phase** which involves many genotypes with few or no replications at few sites; and (ii) **testing phase** where successful genotypes or their descendants are evaluated with more replicates and at many sites (Bänziger *et al.*, 2000). Some breeders prefer classification of the evaluation process into initial, intermediate and advanced phases. At each phase, the breeder eliminates poor performers, which ultimately reduces the total number of genotypes and magnitude of variation in the genotype pool. At the testing or advanced phase, selection is more specific and the best hybrids are advanced towards commercialization.

Usually, conventional maize breeders use traits such as resistance to diseases and pests, plant type, desirable grain and yield potential as selection indices during the screening or

initial phase. Screening is generally done under optimum growing conditions (Bänziger *et al.*, 2000). This is because, under favourable conditions, genetic variance, heritability and therefore breeding progress for grain yield are greatest (Bänziger *et al.*, 2006). Genotypes are only evaluated under drought stress at an advanced testing phase in many locations. At this stage, genetic variation is low (Bänziger *et al.*, 2000) and the objective is the advancement of the best genotypes to the advanced testing phase.

Maize breeders are reluctant to carry out genotype screening in drought stress prone environments due to a number of reasons:

- i. heritability and genetic variances for grain yield usually decrease under abiotic stress as yield levels fall (Blum *et al.*, 1988; Bolaños and Edmeades, 1996; Bänziger *et al.*, 2004).
- ii. stress experiments often produce rankings that differ significantly from one experiment to another due to high genetic by environment (GxE) interactions caused by abiotic stress which makes selection and breeding progress difficult (Bänziger *et al.*, 2006).
- iii. breeders assume that selection under high-yielding conditions (favourable levels of fertility and water) will also increase grain yield under abiotic stress conditions, but Blum (2005) warns that most breeders still believe the myth that there is superior genotypes over all environments, for example, the assumption that performance under high N translates to performance under low N as reported by Mungoma and Mwambula (1996) is not always correct.
- iv. farmers in high-yielding and high-input conditions are usually more attractive targets for the private seed sector than the 'average', resource-poor farmers who normally farm in abiotic stress prone environments.

But research at CIMMYT (Bänziger *et al.*, 2000) and other studies (Atlin and Frey, 1990; Ceccarelli *et al.*, 1992; Banziger *et al.*, 1997), proved to some degree that the above apprehensions can be overcome and rapid breeding progress can be achieved by extensive screening under target stress environments. In favour of target screening breeding programmes, the following has to be considered (Bänziger *et al.*, 2000):

- i. The notion that selection gains in an unstressed target environment are higher than under stress is of little or no help in improving yield in the target environment.
- ii. No breeder would expect to improve disease resistance in maize by selecting in a virtually disease-free environment, yet breeders routinely expect to increase drought tolerance and low N stress tolerance by selecting mainly in favourable environments.

- iii. When genotypes are selected under favourable conditions, the much needed genetic variation for stress tolerance may be lost. Yet on the other hand, selection under drought could lead to full expression of stress tolerance traits.
- iv. Global climate change further increases the degree of abiotic stress (Abraha and Savage, 2006) and there is need to come up with climate ready genotypes for all environments.
- v. It is possible to develop drought tolerant genotypes. Considerable selection gains under managed stress have been revealed for the past three decades by studies conducted by CIMMYT and others (Bolaños and Edmeades, 1993a,1993b; Bolaños *et al.*, 1993; Chapman *et al.*, 1997; Chapman and Edmeades, 1999).

Nevertheless, some issues still remain major obstacles e.g.

- i. The lack of control of stress conditions;
- ii. Highly variable yield (too high or too low to be useful for selection);
- iii. Very high error variances;
- iv. High GxE interactions;
- v. Negative correlation between drought tolerance and  $Y_{max}$  (Jensen and Cavalieri, 1983).

It will not be worthwhile for breeders to pursue drought stress breeding programmes in an environment that has no justifiable short or long term benefits either economically or genetically. On the other hand, successful breeding is merely the art of intelligent compromise; “you lose one thing and gain the other” (Bolaños and Edmeades, 1993b).

### **1.9.3 Yield adaptation**

Genotypes that consistently rank above the general mean across environments (quantitative GxE interactions) are widely adapted and those that change markedly in rank order from one environment to another are inconsistently adapted. The aim of breeding for wide adaptation is to obtain genotypes that perform well in nearly all environments while specific adaptation focuses on genotypes that have higher site specific yields in a defined subset of target environments (Monneveux *et al.*, 2008). The argument of breeding for either wide or specific adaptation is solved by considering the yield benefit to the farmer and cost of hybrid development versus revenue to the breeder.

Usually, genotype adaptation and yield stability are regarded as similar. But other authors have resorted to using the term adaptation of a genotype in reference to performance in space while the term stability is used in reference to performance over time and space (Monneveux *et al.*, 2008). A highly stable genotype is therefore one that performs well in

nearly all environments and over many years. An immense amount of literature covers the subject of GxE interaction, but it is still difficult to effectively identify the best genotype when GxE interactions are high. High GxE interactions for quantitative traits such as grain yield reduce genotypic and phenotypic correlations and consequently lower response to selection (Bänziger *et al.*, 2000).

The FAO (2013) categorised hybrids into three, viz. those that have: (i) uniform superiority over all environments; (ii) relatively better performance over all environments; and (iii) relatively better performance in favourable environments. Due to the enormous variation in the attributes of the environment, it can be accepted that the environment is undefined or it is rather a random occurrence of unlimited variation. If so, the breeder's attempt to develop widely adapted genotypes will be practically challenging (FAO, 2012).

#### **1.9.4 Phenotypic stability**

According to Becker and Léon (1988): *"The basic cause of differences between genotypes in their yield stability is the wide occurrence of genotype-environmental interactions (GxE interactions), i.e. the ranking of genotypes depends on the particular environmental conditions in which they are grown...phenotypic stability refers to fluctuations in the phenotypic expression of yield while the genotypic compositions of the varieties or populations remain stable"*. The phenotypic expression referred here is not just yield but any other observed and/or measured phenotype. Phenotypic stability is defined in many ways depending on how the scientist wants to look at the problem. It can be divided into two concepts: (i) static and (ii) dynamic stability (Gray, 1982; Lin *et al.*, 1986). Static stability refers to unchanged performance of genotypes regardless of any variation in environmental conditions (Tollenaar and Lee, 2002). A stable genotype in this case has a minimum variance across different environments. Dynamic stability permits a predictable response to environments. A stable genotype is one that has minimal interactions with the environment. Static and dynamic stability were also termed biological and agronomic stability, respectively by Becker (1981). According to Becker (1981), breeders use the term stability to characterize a genotype that always shows constant yield under any environmental condition (static stability). However, that has been regarded as undesirable by most agronomists because it refers to genotypes that do not respond to improved growing conditions (Becker, 1981). The objective of a breeding program is to develop a genotype that gives expected yield proportional to the productivity level of the respective environments. Therefore, agronomic stability is desirable for it describes performance of genotypes consistent with an environment's level of productivity, which is a vital property in crop production. That is it describes a genotype with no GxE interactions (Gray, 1982; Becker and Léon, 1988).

## 1.10 Statistical methods of analysing trial data

Several statistical methods are available to analyse GxE interactions. Only the most important methods used in this study are discussed. Other methods will be elaborated in the methodology section of each of the subsequent research.

### 1.10.1 Analysis of variance

The analysis of variance (ANOVA) is a statistical analysis technique that is very commonly used in plant breeding; therefore only basic principles as applied to multi-location trials and GxE interactions will be discussed with a more extensive review written by Blum (2010). It is important to state that the ANOVA is performed based on three main models, viz. random, fixed and mixed effects. When conclusions are limited only to the sample used, a fixed model is applied. And lastly when experiments have both random and fixed factors, a mixed model is applied.

A basic ANOVA model (Annicchiarico, 1997) is therefore:

$$Y_{ijk} = \mu + G_i + E_j + GE_{ij} + R_m + e_{ijk} \quad \text{Eqn 1.3}$$

Where  $\sum G_i = \sum E_j = \sum GE_{ij} = 0$

$Y_{ijk}$  is the measured trait of the  $i^{\text{th}}$  hybrid in the  $j^{\text{th}}$  environment;  $\mu$  is the grand mean;  $G_i$  is the  $i^{\text{th}}$  hybrid effect;  $E_j$  is the  $j^{\text{th}}$  environment effect;  $GE_{ij}$  is the  $ij^{\text{th}}$  interaction effect;  $R_m$  is the  $m^{\text{th}}$  replication within environments;  $e_{ijk}$  is the random error.

When analysing multi-environmental trials (METs), a stable genotype is one that has minimum or no GxE interaction with respect to all testing locations. If  $Y_{ijk}$  is the  $i^{\text{th}}$  genotype yield for environments  $j^{\text{th}}$  and  $m^{\text{th}}$  replication, the  $GE_{ij}$  interaction effects of the  $i^{\text{th}}$  genotype across the  $j^{\text{th}}$  environments and  $m^{\text{th}}$  replication should be non-significant. Conversely, a genotype that is selected for specific adaptation is one that has high GxE interaction with respect to that specific environment.

Annicchiarico (1997) presented an ANOVA model for the evaluation of genotypes in a multilocal trial as:

$$Y_{ijk} = \mu + G_i + L_j + S_k + GL_{ij} + LS_{jk} + GS_{ik} + GLS_{ijk} + R_m + e_{ijkm} \quad \text{Eqn 1.4}$$

Where  $Y_{ijk}$  is yield of the  $i^{\text{th}}$  genotype in the  $j^{\text{th}}$  environment,  $k^{\text{th}}$  season and  $m^{\text{th}}$  replication,  $G_i$  is the  $i^{\text{th}}$  genotype effect,  $L_j$  is the  $j^{\text{th}}$  location effect,  $S_k$  is the  $k^{\text{th}}$  seasonal effect,  $GL_{ij}$  is the  $ij^{\text{th}}$  interaction effect,  $LS_{jk}$  is the  $jk^{\text{th}}$  interaction effect,  $GS_{ik}$  is the  $ik^{\text{th}}$  interaction effect,  $GLS_{ijk}$  is the  $ijk^{\text{th}}$  interaction effect,  $R_m$  is the  $m^{\text{th}}$  replication effect and  $e_{ijkm}$  is the  $ijkm^{\text{th}}$  random error

effect. In the ANOVA model, L and S collectively comprise the environment  $E_{jk}$ , while  $GL_{ij}$ ,  $LS_{jk}$ ,  $GS_{ik}$  and  $GLS_{ijk}$  collectively comprise the GxE interaction component.

The ANOVA enables the effects, variances of effects and differences of variances among effects to be estimated. The variance components are important in estimation of broad sense heritability ( $H^2$ ) values using the methods suggested by Burton and Devane (1953):

$$\text{Genotypic variance } (\sigma_g^2) = \frac{MS_g - MS_e}{r} \quad \text{Eqn 1.5}$$

$$\text{Environmental variance } (\sigma_e^2) = MS_e \quad \text{Eqn 1.6}$$

$$\text{Phenotypic variance } (\sigma_{ph}^2) = \sigma_g^2 + \sigma_e^2 \quad \text{Eqn 1.7}$$

Where  $MS_g$  and  $MS_e$  are the mean sum of squares for the genotypes and error in the analysis of variance, respectively, and  $r$  is the number of replications.

Using Eqn 1.5, 1.6 and 1.7, broad sense heritability can be calculated as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_{ph}^2} \times 100 \quad \text{Eqn 1.8}$$

Heritability estimates provide information about the extent to which a particular character can be transmitted to successive generations (Haq *et al.*, 2008). It has been reported that heritability for grain yield decreases under drought environments (Bänziger *et al.*, 2000; Bruce *et al.*, 2002). This is mainly due to a high GxE variance component that forms part of the total phenotypic variance. The GxE interaction is incorporated into the denominator of the heritability equation and consequently, it reduces the  $H^2$  value (DeLacy *et al.*, 1996). DeLacy *et al.* (1996) described a number of ways in which the GxE interaction component can be minimized e.g. increasing replications of fewer genotypes in fewer environments and the power to correctly detect GxE interaction effects and the mathematical ability to (by transformation) induce or remove the interaction effects. This illustrates a basic tension between research targets: whether to cover many locations with many genotypes and fewer replications or minimize environmental variation to precisely detect small differences between genotypes. But ultimately, breeding for drought environments cannot escape the existence of GxE interactions.



### 1.10.2 Joint regression analysis

The power of regression analysis lies in its ability to allow a breeder to examine and explore spatial relationships and derive possible reasons behind spatial patterns. In addition and most importantly is its use in interpreting the GxE interaction (non-additive) component in the ANOVA model. To estimate stability parameters, the  $GE_{ij}$  component can be partitioned into two parts, that is, the regression effect and unexplained  $GE_{ij}$  (Yau, 1995; Monsanto, 2013):

$$GE_{ij} = b_i E_j + \delta_{ij} \quad \text{Eqn 1.9}$$

Where  $b_i$  is the linear regression coefficient,  $E_j$  is the  $j^{\text{th}}$  environment and  $\delta_{ij}$  is the deviation or unexplained GxE interaction. The linear regression model can also be described as follows (Yau, 1995):

$$Y_{ij} = [G_i] + [b_i E_j] + \epsilon_{ij} \quad \text{Eqn 1.10}$$

Where  $\epsilon_{ij}$  comprises both the unexplained GxE interaction and the experimental error. The three important indices in regression analysis are the individual genotype means ( $Y_{ij}$ ) for each location, the environmental index (EI; the mean for all genotypes in a location) or simply the population mean (PM) and regression coefficient  $b_i$ . From Eqn 1.10 above, a graph of the form  $y=mx+c$  can be plotted where  $y = Y_{ij}$ ,  $m=b_i$ ,  $E_j = \text{EI (location mean)}$ ,  $c=G_i$  and  $\epsilon_{ij}$ , the experimental error (or deviation from  $E_j$ ) could be represented by error bars on the line graph, if necessary. The PM serves as a standard of comparison. This is the procedure popularised by Finlay and Wilkinson (1963). The PM has regression coefficient of 1.0 when PM values are plotted against themselves. Genotypes with  $b_i$  equal to 1.0 have average stability over all environments. It is important to note that a stable genotype is not just the one that constantly give the same amount of yield in all locations (i.e. no GxE interaction), but one that consistently performs better relative to the level of productivity of the respective environments.

### 1.10.3 Additive main effects and multiplicative interactions and GGE biplot analysis

Even though ANOVA can quantify the main effects and interactions, it falls short on explaining the nature of the GxE interaction component. The regression approach only explains a small portion of the interaction sum of squares component and that is only when the pattern fits a simple linear regression model. However, the Additive Main effects and Multiplicative Interactions (AMMI), model advocated by Gauch (1988) was found to be suitable for handling both the main effects and GxE interactions in METs more effectively and efficiently. The success of this technique is in the amalgamation of ANOVA and principal component analysis (PCA) (Eqn 1.13). The AMMI first applies the additive ANOVA model to

two way data and then applies the PCA to the multiplicative residual which is the interaction component. Because of its inclusiveness of ANOVA and PCA, the AMMI model is superior to both PCA and to the regression model as far as GxE interaction is concerned (Yau, 1995; Annicchiarico, 1997). The AMMI model is defined as follows:

$$Y_{ij} = \mu + G_i + E_j + \sum_{k=1}^n \lambda_k \alpha_{ik} \gamma_{jk} + e_{ij} \quad \text{Eqn 1.11}$$

Where  $Y_{ij}$  is the yield of the  $i^{\text{th}}$  genotype in the  $j^{\text{th}}$  environment,  $\mu$  is the grand mean,  $G_i$  and  $E_j$  are the genotype and environment deviations from the grand mean, respectively,  $\lambda_k$  is the Eigen value of the PCA analysis axis  $k$ ,  $\alpha_{ik}$  and  $\gamma_{jk}$  are the  $i^{\text{th}}$  genotype and the  $k^{\text{th}}$  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. AMMI analysis can be conducted in the software packages such as GenStat and Statistical Analysis Software (SAS). Graphical representation of data fitted in the AMMI model as biplots provides a clear relationship of genotypes to environments.

According to Yan *et al.* (2007) the biplot display of G and GxE effects as relevant to genotype evaluation is better done using the Genotype and Genotype by Environment (GGE) biplot analysis than the AMMI analysis. The model is based on the Sites Regression (SREG) Linear-bilinear model which can be written as:

$$\bar{Y}_{ij} = \mu_j + \sum_{k=1}^n \lambda_k \alpha_{ik} \gamma_{jk} + e_{ij} \quad \text{Eqn 1.12}$$

Where  $\bar{Y}_{ij}$  is the cell mean for genotype  $i$  in the  $j^{\text{th}}$  environment ( $i = 1, 2 \dots e$ );  $\mu_j$  is the mean value of genotypes in environment  $j$  ( $j = 1, 2 \dots e$ ). The other terms are as defined in Eqn 1.13. The GGE biplot is recognized for its ability to clearly depict the “who won where”, which is an important attribute in multi-location trials. However this can also be established from AMMI biplots (Yan *et al.*, 2007).

## 1.11 Future breeding methodologies

### 1.11.1 Management of stress

The choice of a selection strategy is critical when breeding for drought tolerance. According to Bänziger *et al.* (2000), the key to breeding for both drought and low N stress tolerance is careful management of stress. In the case of drought this is done by conducting experiments partly or entirely in the dry season and managing stress through irrigation, whereas for N, this is done by conducting experiments in fields that are depleted of N. The objective is not

to simulate farmers' field conditions, but a clearly defined stress that is relevant in the farmers' fields. This is called the direct method of selection and is often used as a breeding strategy at CIMMYT. Two water regimes are used, that is, well watered (WW) and water stressed (WS). The water stress regime is divided into intermediate (IS) and severe stress (SS) (Vasal *et al.*, 1996; Magorokosho *et al.*, 2003; Moser *et al.*, 2006).

When selecting or screening for drought tolerance, different water regimes serve different purposes of exposing genetic variation for specific traits (Bruce *et al.*, 2002). For example, WW regimes allow for assessment of  $Y_{max}$ . Before anthesis, water stress affects stover biomass yield, plant height, number of leaves and leaf area, and the number of ovules and ears (Moser, 2004). Intermediate stress (water withdrawn during late flowering and grain filling) is used to expose variation for lower leaf senescence and grain yield while severe stress (no water applied from three weeks before silking and onwards) is used to expose variation for tassel blasting, barrenness and ASI (Bruce *et al.*, 2002; Bänziger, *et al.*, 2004).

The second method involves screening of genotypes under randomly occurring stress conditions with an expectation that the crop will be exposed to random levels of stress. But, Bänziger *et al.* (2000) warns that this may slow down breeding progress due to interaction and confounding effects of different levels of stresses. It is possible that each time a different stress mechanism can be selected for. Consequently, it becomes difficult to relate the plant's performance to a strictly defined stress mechanism. On the other hand, selection under managed stress allows for precise timing, intensity and uniformity of stress to be instituted (Bänziger *et al.*, 2000; Moser *et al.*, 2006).

### **1.11.2 Secondary traits and phenotypic correlations with yield**

Nearly all breeding programmes use grain yield as the main selection criterion and as a primary trait indicative of genotype performance (Bruce *et al.*, 2002; Magorokosho *et al.*, 2003). In addition to grain yield, secondary traits are widely used. As mentioned earlier (section 1.6), plants possess secondary traits that are both independent and interdependent at several developmental and morphological levels (Yan and Wallace, 1995). Independent traits do not have genetic and physiological controls that alter nor have negative correlations with any other traits. They are controlled by one or few genes, therefore; they usually have high heritabilities and are relatively easy to breed for if germplasm with a desired trait is identified. Conversely, interdependent traits have genetic and physiological controls that alter the expression of other traits. They are either negatively or positively correlated with other traits (Yan and Wallace, 1995). Most traits relevant to plant growth and development are interdependent. For this reason, the concept of CSC of interdependent traits is worth considering.

Linear association between genetic values of two traits X and Y is termed genetic correlation (Bernardo, 2002). Observed phenotypic correlation between, for instance, traits X and Y is a result of genetic and/or environmental correlations (Isik, 2009). Genetic correlations of X and Y result from genetic effects that contribute to both traits; whereas environmental correlation results from environmental conditions causing a positive ( or negative) correlation between two distinct traits that may have independent genetic effects (Yan and Wallace, 1995). Phenotypic correlations in part caused by the environment are not reliable as variations from one environment to the other are always high (Bänziger *et al.*, 2000). Genetic correlations are more important because they indicate the degree to which direct selection for one trait indirectly influences the response to selection for the second trait (Luby and Shaw, 2008). Under drought stress, phenotypic correlations between secondary traits are predominantly affected by environmental variations which may conceal genetic effects (Luby and Shaw, 2008).

A suitable secondary trait is one that is: (i) genetically associated with grain yield under drought; (ii) highly heritable; (iii) cheaper, stable and feasible to measure; (iv) not associated with yield penalty under ideal growing conditions; (v) observed at or before flowering, so that undesirable parents are not crossed (this does not apply at the testing phase); and (vi) a reliable estimator of yield potential before harvest (Edmeades *et al.*, 1997a; Bänziger *et al.*, 2000; Bruce *et al.*, 2002; Monneveux *et al.*, 2008). There are various proposed traits, but few meet these criteria. Based on experience at CIMMYT and Pioneer Seed Company, the following secondary traits have been rated the most important under drought stress: reduced bareness; ears per plant (EPP); ASI; stay green; and to a lesser extent, leaf rolling or epinasty (Bänziger *et al.*, 2000; Bruce *et al.*, 2002; Moser, 2004). Generally, these traits range from medium to highly heritable and to being very positively correlated with grain yield (Bänziger *et al.*, 2000; Magorokosho *et al.*, 2003). Comprehensive descriptions of these key traits including how to measure them under abiotic stress are reviewed by Bänziger *et al.* (2000).

Since heritability for grain yield declines under severe drought stress (Bänziger *et al.*, 2000; Moser, 2004), many other authors reported that secondary traits assume real significance in selection efficiency especially when yields fall below 50-60% of  $Y_{\max}$  (Bänziger and Laffite, 1997; Bänziger *et al.*, 2000). In their study, Bänziger and Laffite (1997) reported a 20% increase in selection gains when selection procedures incorporated secondary traits. According to Bänziger *et al.* (2000) secondary traits are mostly valuable under abiotic stress conditions for a number of reasons: (i) they demonstrate widely the degree to which a crop was stressed by drought, e.g. leaf rolling; (ii) if they are observed before flowering, they can

be used for selecting desirable crossing parents; and (iii) they improve precision with which drought or low N tolerant genotypes are identified.

### **1.11.3 New phenotyping techniques**

Selection strategies based on grain yield, reduced ASI and other secondary traits produced gains of up to 144 kg ha<sup>-1</sup> year<sup>-1</sup> under drought stress (Chapman and Edmeades, 1999). There is a need to fully explore gene-to-phenotype relationships and increase the precision in phenotyping techniques. Some of these existing techniques include measuring chlorophyll content, leaf stomatal conductance, leaf senescence, plant water status using Scholander pressure chamber, etc.

#### **1.11.3.1 Chlorophyll content**

Chlorophyll concentration in a plant tissue relates to its photosynthetic capacity and could be a good indicator of general plant health and stress (Barton, 2001). Plant physiologists have demonstrated that chlorophyll pigments are broken down when the plant is subjected to stress inducers such as drought, causing a change in spectral reflectance and speeds up early leaf senescence (Barton, 2001). According to Tollenaar and Wu (1999), yield improvements in temperate regions were attributed to efficient resource capture and use. This was more evident under stress. Resource capture (mainly CO<sub>2</sub> and solar radiation) largely depends on leaf health (Cairns *et al.*, 2012). Genotypes with longer stay-green have increased duration of incident solar radiation capture. It can be hypothesized that genotypes that are drought tolerant will maintain a relatively high concentration of chlorophyll than others at the same level of stress. A portable chlorophyll meter is used for chlorophyll content measurement and the methodology is based on measuring light that is transmitted through a leaf. A study by Cairns *et al.* (2012) observed that, with time, chlorophyll content (measured in SPAD units) decreased in all treatments (i.e. well watered and water stress), but remained relatively higher in well watered genotypes than drought stressed. In the same study, a significant and positive correlation between chlorophyll content and grain yield was observed.

#### **1.11.3.2 Stomatal conductance**

The primary substrate for photosynthesis is CO<sub>2</sub> which is coupled with a release of water through the plant's stoma. As discussed earlier, biomass is directly proportional to transpiration. Transpiration and CO<sub>2</sub> intake largely depend on the plant's stomatal conductance. In Cairns *et al.* (2012) study, there was a correlation between stomatal conductance and grain yield in inbred lines and hybrids. Stomatal conductance is measured using a leaf porometer such as AP4 (Delta-T Devices) or SC1 (Decagon Devices, Inc.)

models. Unlike chlorophyll content, it is possible that stomatal conductance could correlate both positively and negatively with yield (Cairns *et al.*, 2012). The same has been found to occur with leaf rolling (Edmeades, 2013).

#### **1.11.3.3 Normalised difference vegetation index**

Leaf senescence and general plant health are typically measured using visual scores, but the non-destructive spectroradiometric techniques could provide a faster and more reliable method. The technique is based on changes in the red and near-infrared (NIR) spectra caused by light absorption in the photosynthetic tissues. Unlike the chlorophyll meter, the spectroradiometer (e.g. GreenSeeker®) measures Normalised Difference Vegetation Index (NDVI) of light reflected by the leaves or plant canopy (Cairns *et al.*, 2012). A microprocessor within the GreenSeeker® (AgriOptics, New Zealand) sensor analyses the reflected light and calculates NDVI as:

$$\text{NDVI} = [(\rho\text{NIR} - \rho\text{VIS}) / (\rho\text{NIR} + \rho\text{VIS})] \quad \text{Eqn 1.13}$$

Where  $\rho\text{VIS}$  is reflectance of the visible light and  $\rho\text{NIR}$  is reflectance of the near infra-red light. The NDVI values range from 0 to 0.99 and values around 0.6 and above indicate a healthy leaf.

#### **1.11.4 Molecular breeding and genetic engineering**

The growth and application of biotechnology to plant breeding which involves genetic engineering and molecular breeding has accelerated crop breeding. Plant biotechnology which began around 1980s led to production of transgenic plants using *Agrobacterium spp* (Funke *et al.*, 2006; Gómez-Barbero *et al.*, 2008) Molecular markers are being developed to create genetic maps, and exploit genetic linkages between markers and important crop traits. In many crops, molecular breeding is currently a standard practice. However, even though there has been considerable success in many crops (e.g. *Bacillus thuringiensis* (Bt) transformed maize for pest control (Gómez-Barbero *et al.*, 2008; Hellmich and Hellmich, 2012) and Roundup Ready® crops for weed control (Funke *et al.*, 2006)) complex traits such as drought tolerance have proven to be a challenge.

Drought tolerance is a quantitative trait with complex phenotype and genetic controls. Though an understanding of the nature of drought and its effects on crops is important, the knowledge of the genetic basis of drought tolerance in crop plants is a prerequisite for developing superior genotypes through both conventional and molecular breeding (McWilliam, 1989; Fleury *et al.*, 2010). Because of the complexity of the genetic control of drought tolerance, marker assisted selection (MAS) has not contributed significantly to crop

improvement for dry environments. Breeding strategies to this day have largely relied on conventional methods (Fleury *et al.*, 2010). This is largely because the identification of quantitative traits loci (QTLs) associated with the drought tolerance trait has proven to be very difficult (Yang *et al.*, 2007). The high genotype by environment interactions resulting from drought environments complicates the identification of stable QTLs (Yang *et al.*, 2007). Under field conditions gene expression tend to vary from one environment to the other and therefore association between genes at the phenotype becomes inconsistent. It is important to mention that there has been some success in mapping of genetic markers for individual traits that are known to be associated with drought tolerance e.g. ASI and leaf elongation (Ribaut *et al.*, 1996).

In spite of the difficulties, breeding programmes will always have to incorporate biotechnology, genetics and trait integration (Syngenta, 2012). Research to uncover stable QTLs for drought tolerance is an ongoing process. Three multinational breeding companies, Monsanto, Syngenta and DuPont Pioneer, have recently independently announced drought tolerant hybrids which were either developed through marker assisted selection (MAS) or genetic engineering technology. Monsanto's Genuity Droughtguard® is the first genetically engineered maize drought hybrid which was produced by adding a *cspB* gene that enables bacteria to continue growing in cold environments (Monsanto, 2013). DuPont Pioneer, through classical breeding approaches and MAS, developed and released more than 25 Optimum® AQUAmax™ hybrids (Becker *et al.*, 2011). Syngenta released the Agrisure Artesian™ hybrids using proprietary Gene Blueprinting™ technology to identify genes that protect crops from moisture stress (Syngenta, 2012). These hybrids have not yet been tested in Africa, and so, their performance under African conditions is unknown. Furthermore, it is unclear whether one gene inserted into the Droughtguard® would effectively solve the complexity of drought stress. But even if protection against drought is proved, will Africa adopt a genetically modified (GM) Droughtguard crop? If AQUAmax and Syngenta's yield protection is acceptably higher, they have a better chance to be adopted in Africa where reluctance to genetically modified crops is still high.

#### **1.10.5 Doubled haploid technology**

One of the most important ways to increase genetic gains and accelerate the development and deployment of improved genotypes is to reduce the time needed for inbred line development. This was made possible with the introduction of doubled haploid technology in maize breeding (Prasanna *et al.*, 2012). A doubled haploid (DH) genotype is formed when haploid cells ( $n$ ) are induced artificially to undergo chromosome doubling, *in vivo* (Prasanna *et al.*, 2012). The use of monoploids (or haploids) in breeding was first pioneered by Chase

(1947). The procedure today involves (i) a donor plant and (ii) an inducer (which can be a line or single cross or population) (Geiger and Gordillo, 2009). Haploid inducers are specialised genetic stocks which when crossed to a diploid (normal) maize plant, results in an ear with both diploid ( $2n$ ) and a fraction of haploid ( $n$ ) kernels. The haploids are identified by the anthocyanin colouration in the kernels. The haploid kernels have a regular triploid ( $3n$ ) endosperm and hence, they are capable of undergoing normal germination which produces viable seedlings similar to diploid kernels (Prasanna *et al.*, 2012). The seedlings are treated with colchicine or other chromosome doubling agents. After a successful doubling (to create D0 generation), the D0 lines are selfed leading to homozygous and homogenous progenies (called DH lines) (Geiger and Gordillo, 2009). The DH technology shortens breeding cycles by producing a completely homozygous line in 2-3 generations and the technology simplifies the inheritance of traits (Prasanna *et al.*, 2012).

## **1.12 Conclusion**

Even though a considerable amount of research has been done, new breeding strategies are still needed. Previously, selection for drought tolerance was carried out under favourable environments and it was assumed that genotypes will perform similarly under drought conditions. Breeding based on this assumption seem to have achieved little progress. However, MDS sites such as those developed by CIMMYT have proven to be a more successful approach. With a wider understanding of drought stress and subsequent plant responses, it will be possible to identify phenotypes linked to performance of maize under drought stress. This, along with the identification of stable genetic markers associated with drought tolerance traits will speed up hybrid development processes. In addition, the use of the doubled haploid technology will significantly increase breeding efficiency.



## 1.13 References

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## Chapter 2

### Characterisation of F<sub>1</sub> maize (*Zea mays* L.) hybrids under random drought stress

#### Abstract

The growth and development of maize (*Zea mays* L.) grain is severely hindered by drought stress. There is thus an increasing need to breed maize hybrids with improved drought tolerance for sub-Saharan Africa. Genetic variation for drought tolerance was reported as available in existing germplasm. The objective of this study was to characterise the drought tolerance of single cross F<sub>1</sub> maize hybrids developed from selected drought tolerant donor inbreds (both CIMMYT and PANNAR lines) and PANNAR's elite inbred lines. A 12x12 factorial North Carolina design II mating design was used to produce the single cross F<sub>1</sub> hybrids in the 2012 winter season. In the 2012/13 summer season, 70 successful F<sub>1</sub> hybrid crosses out of the expected 144 were evaluated in four random drought stress (RDS) environments: Cedara and Greytown (South Africa) and Devonias and Agriculture Research Trust farm (ART; Zimbabwe). The 70 experimental F<sub>1</sub> hybrids plus 10 commercial single cross F<sub>1</sub> hybrids as checks were evaluated in an 8x10 row by column design. Data was analysed using restricted maximum likelihood in GenStat (16<sup>th</sup> edition). Grain yield was significantly different ( $P < 0.05$ ) across all locations. Heritability for grain yield ranged from 0.56 (Greytown) to 0.69 (Cedara). The Wald statistic for general combining ability (GCA) and specific combining ability (SCA) were significant ( $P < 0.05$ ). Inbreds DT7, DT2 and UL8 had high GCA effects. Hybrid EH79 (DT7xUL8) had the highest SCA effect. Based on the GCA:SCA ratio there was a significant contribution of both additive and non-additive gene action to grain yield. Based on performance of the hybrid crosses, inbred lines with high GCA effects were recommended for use in future hybrid development. The best F<sub>1</sub> hybrids were recommended for consideration as parents for three-way and double cross hybrids or for release as F<sub>1</sub> commercial hybrids.

**Key words:** Drought tolerance, maize, hybrids, environments, secondary traits, GxE interaction

**Terms used:** The term “hybrid” was used to refer to the 70 experimental hybrids and 10 commercial hybrid checks. The term “inbred lines” was used interchangeably with the term “inbreds”. The convention “genotype x environment” interaction was used instead of “hybrid x environment” interaction

## 2.1 Introduction

The growth, development and yield of maize (*Zea mays* L.) are severely hindered by drought stress (Bänziger *et al.*, 2000). There is thus an increasing need to breed maize hybrids with improved drought tolerance for sub-Saharan Africa where drought is common. However, the complexity of the drought phenomenon and the limited knowledge of the genetic basis of plant responses to drought stress have slowed down breeding progress (Edmeades, 2013). Even though it has been reported that there is genetic variation for drought tolerance in existing elite lines, it seems breeding strategies and targeted technologies have not kept up with the pace needed to meet the rising demand for food (Bänziger *et al.*, 2000). It has been reported that the occurrence of drought in many areas is highly unpredictable (Eberhart and Russell, 1965; Bänziger *et al.*, 2000; Edmeades, 2013) giving rise to genotype x location x season interactions that are difficult to interpret. Nevertheless, there is a pressing need to produce maize hybrids that are stable under randomly occurring drought (Edmeades *et al.*, 2000; Andjelkovic *et al.*, 2008; Edmeades, 2013).

Grain yield gain is a function of yield determining traits (Bänziger *et al.*, 2000). In particular, a shorter anthesis-silking interval (ASI), increased ears per plant (EPP) and higher leaf senescence (LSEN) have been suggested as ideal secondary traits that can be used to improve maize tolerance to stress (Edmeades *et al.*, 1997). These and other stress adaptive traits are reviewed by Duvick (2005). According to Duvick (2005), yield measured under drought showed that newer hybrids were more tolerant to drought than hybrids released around the 1930s owing to improvements in stress adaptive traits. From early to newer hybrids, trends show a gradual increase in upright leaves (Meghji *et al.*, 1984), a decrease in tassel size, shorter plants and an increased number of leaves per plant, an increase in leaf-rolling under drought stress, improved staygreen (or delayed leaf senescence), a shorter ASI, an increase in EPP, an increased mass per kernel (Westgate, 2000), and increased grain starch and plant standability (Bänziger and Lafitte, 1997).

The success of breeding for drought tolerance in maize depends on the ability to select and test hybrids in various environments prone to drought (Bänziger *et al.*, 2000). Drought amplifies genotype x environment (GxE) interaction effects, resulting in differential responses of hybrids from one environment to another. Simultaneously, however, such testing allows the identification of stable hybrids that interact less with the environments in which they are grown and are thus more stable. Various techniques have been developed to analyse GxE interaction patterns to determine the stability of hybrids (Finlay and Wilkinson, 1963; Eberhart and Russell, 1965; Lin and Binns, 1988; Zobel *et al.*, 1988; Annicchiarico, 1997). The most important aspect of multi-environmental testing (MET) is that it enables the

characterisation of yield performance, stability and adaptability of hybrids, and the determination of genetic association of secondary traits with grain yield (GY).

If GY, stability of performance and favourable secondary traits are genetically controlled, then the choice of good parental lines should be carefully done (Hallauer *et al.*, 2010). The development of drought tolerant hybrids involves the intelligent choice of parental lines with heritable genetic variation for drought tolerance. This is followed by methodical crossing of the parental lines to create new genetic combinations in the resultant progeny. In addition, the selection of environments within a target population of environments is crucial (Edmeades *et al.*, 2000; Hallauer *et al.*, 2010).

When the progeny of parental lines crossed in a suitable mating design are compared over a series of environments, selections can include the use of general and specific combining ability across environments and *per se* performance of the hybrid progeny. General combining ability (GCA) quantifies the average performance of the hybrids of an inbred in a series of crosses with other inbreds expressed as a deviation from the overall mean of all the crosses. It reflects the ability of an individual line to transmit its genetic superiority to its hybrids when crossed with other lines (Betrán *et al.*, 2003). Specific combining ability (SCA) is the deviation of a cross from its expected value where the expected value is the sum of the general combining abilities of its two parental lines (Hallauer *et al.*, 2010). Breeders are interested in the best parent (based on GCA) for use in breeding programmes and the best hybrid (based on SCA) for the market (Hallauer *et al.*, 2010).

The overall objective of this study was to characterise, under random drought stress, the performance of experimental F<sub>1</sub> maize hybrids generated from crosses between drought tolerant inbred lines (sourced from PANNAR and CIMMYT lines) and PANNAR elite inbred lines.

The specific objectives were to:

- i. evaluate the yield performance of F<sub>1</sub> single cross maize hybrids across four environments and determine the best performing genotypes;
- ii. estimate GCA and SCA for grain yield (GY) of parental lines and hybrid progeny and identify best inbred lines for hybrid development;
- iii. determine the relationships between measured and derived traits with GY;  
and
- iv. assess the GxE interactions, stability and adaptability of the F<sub>1</sub> hybrids and identify the most stable hybrids.

## 2.2 Materials and methods

### 2.2.1 Germplasm and mating design

The experimental F<sub>1</sub> maize hybrids evaluated for drought tolerance in this study were generated in 12x12 factorial (North Carolina II) mating design scheme during the winter season of 2012. The crosses were conducted at PANNAR farm in Letsitele (23°52'41.0"S 30°23'30.1"E, 528 masl<sup>1</sup>) in the Limpopo Province in South Africa. The parents comprised of 10 inbreds with known drought tolerance obtained from CIMMYT and PANNAR (Pty Ltd, Greytown) (coded as DT for drought tolerance) and 10 inbreds with known drought susceptibility obtained from PANNAR (coded as UT for unknown tolerance). The DT inbred lines were used as female parents while the UT lines were male parents. Two control inbred lines, one with established drought tolerance and one with known susceptibility were included as both female and male in the breeding design to constitute a 12x12 factorial mating design. From the expected 144 hybrids, only 70 experimental F<sub>1</sub> single cross hybrids were successfully generated (see Appendix 2.1). The CIMMYT inbred lines and the 10 commercial F<sub>1</sub> hybrids as checks (used as controls in the field evaluation of the experimental hybrids) are presented in Table 2.1. For proprietary reasons, the pedigrees of the PANNAR lines are not presented. The relative maturity of commercial hybrids was obtained from PANNAR unpublished data. In the succeeding sections, four maturity groups were considered based on days after planting (DAP), that is, very early (VE; 60-65 DAP), early (E; 65-70 DAP), medium (M; 70-75 DAP) and late (L; 75+ and above DAP). The number of hybrids in each maturity group varied. Commercial hybrids CH1, CH3, CH4 and CH7 were used as benchmarks for each of the maturity groups.

**Table 2.1 Commercial hybrid checks and CIMMYT lines used in the experiment**

Inbred line (CIMMYT Pedigree)*	Code	Commercial hybrids	Code	Maturity
La Posta Seq C7-F103-2-1-1-1-B-B-B	DT1	PAN 3M-01	CH1	VE
La Posta Seq C7-F86-3-1-1-1-B-B-B/	DT2	PAN 4M-21	CH2	M
La Posta Sequia C7-F64-2-6-2-2	DT3	PAN 4M-19	CH3	E
CML 442	DT9	PAN 53	CH4	M
CML 444	DT10	PAN 63	CH5	M
CML 395	P_CNTRL	PAN 67	CH6	M
		PAN 7M-81	CH7	L
		PAN 8M-91	CH8	L
		PAN 8M-93	CH9	L
		PAN 413	CH10	M

\*PANNAR inbred lines are not shown for proprietary reasons. Commercial hybrids were from different maturity groups. CH = Commercial hybrids; DT = Drought tolerance; CML = CIMMYT maize lines; VE = Very early; E = Early; M = Medium; L = Late

<sup>1</sup> Meters above sea level

## 2.2.2 Test environments and weather data

The 70 experimental F<sub>1</sub> hybrids and 10 commercial F<sub>1</sub> hybrids as checks were evaluated at four environments (in the 2012/13 summer season) that could potentially be affected by random drought during the various growth phases of the maize plants evaluated. Two of the environments, Greytown and Cedara, are in South Africa and the other two, ART and Devonian, are in Zimbabwe. Environmental descriptions are presented in Table 2.2. The seasonal rainfall at each environment was measured from 1 December 2012 to 31 May 2013, that is, from planting to harvesting. The average weekly rainfall from December 2012 to May 2013 was also recorded (Table 2.2). Based on grain yield (GY) at each environment, Cedara and Devonian were considered high potential (HP) whereas Greytown and ART farm were considered the low potential (LP) environments.

**Table 2.2 Average weekly rainfalls (mm) per test environment measured from planting to harvesting (December 2012 to May 2013), location altitude and GPS coordinates**

	Cedara	Greytown	ART	Devonia
Altitude (masl)	1068	1092	1480	1527
GPS coordinates	29°32'48.1"S 30°15'57.9"E	29°04'53.2"S 30°39'11.7"E	17°15'36.0"S 31°30'00.0"E	17°43'5.82"S 31°24'2.85"E
Week 1	1.31	3.46	9.00	10.00
Week 2	2.98	4.74	4.57	3.45
Week 3	2.76	5.23	5.86	2.31
Week 4	5.96	1.17	0.71	3.40
Week 5	10.16	11.34	17.86	8.50
Week 6	5.70	0.50	10.14	13.25
Week 7	5.66	1.69	6.00	3.00
Week 8	1.60	1.51	1.79	1.23
Week 9	1.01	7.86	0.00	2.12
Week 10	5.98	2.66	1.57	1.54
Week 11	3.30	2.97	4.71	10.20
Week 12	4.10	3.12	0.00	0.00
Week 13	2.90	1.93	0.86	4.21
Week 14	6.27	1.16	2.36	3.10
Week 15	6.42	7.03	4.00	6.10
Week 16	0.15	3.91	2.14	3.81
Week 17	1.38	1.03	2.86	2.85
Week 18	2.18	1.63	2.29	2.98
Week 19	3.23	1.37	0.00	1.10
Week 20	2.21	1.58	0.00	0.32
Week 21	10.20	2.28	1.36	0.00
Week 22	0.00	0.89	0.00	0.00
Week 23	3.23	4.40	2.00	3.20
Week 24	0.00	3.90	1.20	3.50
Total (mm)	88.69	76.86	81.28	90.17

ART = Agriculture Research Trust; masl = meters above sea level; GPS = Global positioning System



### 2.2.3 Trial management

General management practices were followed at all environments. No supplemental irrigation was applied. Weeds were controlled using herbicides and hand weeding was done as required.

### 2.2.4 Field trial design and data collection

The 70 experimental F<sub>1</sub> hybrids and 10 commercial hybrid checks were evaluated at each of the four environments in an 8x10 row by column design with three replications. The four field trials were planted by hand in the first week of December 2012. The trials at each environment had different randomizations. Due to low quantities of seed, single row plots each 4.40 m long were used. Inter-row and intra-row spacing were 0.76 m and 0.22 m, respectively, providing a plant population of 59 900 plants ha<sup>-1</sup>. Measured and derived traits are presented in Table 2.3.

**Table 2.3 Traits measured or derived from the 2012/2013 RDS environments**

Traits measured	Description
Grain yield (GY)	Mass of shelled grain adjusted to t ha <sup>-1</sup> at 12.5% grain moisture content. Trials were harvested by hand when grain moisture of the late check hybrid was below 18%.
Days to anthesis anthesis date (AD)	Number of days from planting to 50% of plants shedding pollen
Silking date (SD)	Number of days from planting to 50% of plants with silks approximately 2 cm long
Anthesis silking interval (ASI)	SD minus DTA
Ears per plant (EPP)	Number of cobs with at least one kernel, divided by the total number of plants per plot
Leaf senescence (LSEN)	Scored on a scale from 1 to 9 i.e. 1 = green leaves to 9 = dead leaves
Grey Leaf Spot (GLS)	Ratings using scale from 1 to 9 (1 = clean leaves to 9 = fully covered by diseases)
<i>Phaesphaeria</i> (PHAE)	Ratings using scale from 1 to 9 (1 = clean leaves to 9 = fully covered by diseases)
Plant height (cm) (PH)	Distance between the base of a plant to the top of the tassel
Ear height (cm) (EH)	Distance between the base of the plant to the point of insertion of the bottom ear
Ear diameter (ED)	Score of ear diameter from 1-5 (1 thick ear to 5 = very thin ear)
Ear position (EP)	Ratio of EH to PH
Lodging Percentage (LP)	Percentage of lodged plants per plot
Shelling percentage (SP)	Shelled grain mass divided by gross mass x 100
Husk dry (HD)	Score on a scale from 1 to 9, that is, from green husks to brown husks of the ear

## 2.2.5 Data analysis

### 2.2.5.1 REML analysis of data

All data were analysed in GenStat 16th edition. Data for yield and secondary traits were subjected to REML analysis for each environment using the following model:

$$Y_{ij} = \mu + G_i + r_k + e_{ij} \quad \text{Eqn 2.1}$$

Homogeneity of variances test was conducted using Bartlett (1937) method before combined analysis of data. The hypothesised test for the equality of variances across the four environments against the alternative that variances are unequal for at least two environments are as given below:

$$\text{Null hypothesis } H_0: \sigma_1^2 = \sigma_2^2 = \dots = \sigma_4^2$$

$$\text{Alternate hypothesis } H_a: \sigma_i^2 \neq \sigma_j^2 \text{ for at least one pair (i,j)} \quad \text{Eqn 2.2}$$

The across environment analysis was performed using the following model:

$$Y_{ijk} = \mu + G_i + E_j + GE_{ij} + r_k + e_{ijk} \quad \text{Eqn 2.3}$$

Where  $Y_{ijk}$  is the measured trait of the  $i^{\text{th}}$  hybrid in the  $j^{\text{th}}$  environment;  $G_i$  is the  $i^{\text{th}}$  hybrid effect;  $E_j$  is the  $j^{\text{th}}$  environment effect;  $GE_{ij}$  is the  $ij^{\text{th}}$  interaction effect;  $r_k$  is the  $k^{\text{th}}$  replication within environments;  $e_{ijk}$  is the random error.

### 2.2.5.2 AMMI analysis of GxE interactions

The GxE interaction was partitioned using the Additive Main effects and Multiplicative Interactions (AMMI) procedure in GenStat:

$$Y_{ij} = \mu + G_i + E_j + \sum_{k=1}^n \lambda_k \alpha_{ik} \gamma_{jk} + e_{ij} \quad \text{Eqn 2.4}$$

Where  $Y_{ij}$  is the yield of the  $i^{\text{th}}$  hybrid in the  $j^{\text{th}}$  environment;  $\mu$  is the grand mean;  $G_i$  and  $E_j$  are the hybrid and environment deviations from the grand mean, respectively;  $\lambda_k$  is the Eigen value of the  $k^{\text{th}}$  principal component analysis axis;  $\alpha_{ik}$  and  $\gamma_{jk}$  are, respectively, the hybrid and the environmental principal component scores for axis  $k$ ;  $n$  is the number of principal components and  $e_{ij}$  is random error (Gauch, 1988).

### 2.2.5.3 The stability and adaptation analysis

The stability and adaptation of hybrids were quantified and characterised using regression analysis (Finlay and Wilkinson, 1963), cultivar superiority measure (Lin and Binns, 1988), and AMMI biplots (Gauch and Zobel, 1996). To compare the overall standard deviations for

the traits at each environment to each other, they were expressed as coefficients of variation (CV%).

The cultivar superiority index ( $P_i$ ) (Lin and Binns, 1988), regression coefficient ( $\beta_i$ ) (Eberhart and Russell, 1965) and IPCA1 scores were used to characterise hybrid yield stability. The cultivar superiority measure ( $P_i$ ) was calculated as:

$$P_i = \sum_{j=1}^n (X_{ij} - M_j)^2 / 2n \quad \text{Eqn 2.5}$$

Where,  $X_{ij}$  is yield of the  $i^{\text{th}}$  cultivar in the  $j^{\text{th}}$  location,  $M_j$  is the maximum performance achieved in the  $j^{\text{th}}$  environment and  $n$  is the number of environments. The superiority measure ( $P_i$ ) is defined as the mean squares of the distance between the  $i^{\text{th}}$  experimental hybrid and the maximum response of all hybrids. The smaller the  $P_i$  value, the more stable the hybrid is.

The regression coefficient  $b_i$  for hybrid mean performance at each environment versus environment mean was determined as:

$$Y_{ij} = a_i + b_i m_j + e_{ij} \quad \text{Eqn 2.6}$$

Where  $Y_{ij}$  is the mean performance of the  $i^{\text{th}}$  hybrid in the  $j^{\text{th}}$  environment ( $i = 1, 2, \dots, 80$ ;  $j = 1, 2, \dots, 4$ ),  $a_i$  is the y intercept value,  $b_i$  is the regression coefficient that measures the response of the  $i^{\text{th}}$  variety to varying environments;  $m_j$  is the environmental means and  $e_{ij}$  is the random error. Based on Eberhart and Russell (1965) study, a regression coefficient  $<0.7$  indicate that a hybrid is specifically adapted to low-yielding environments whereas, when  $b_i > 1.3$ , a hybrid is specifically adapted to higher yielding environments This implies that hybrids that have regression coefficients between 0.7 and 1.3 were stable.

#### 2.2.5.4 Least significant differences

Significant differences between hybrids in performance for the measured traits were determined using least significant differences at the 5% significance level ( $LSD_{0.05}$ ).

#### 2.2.5.5 Combining ability

A combining ability analysis across the four environments was conducted for the 7 female x 10 male factorial mating design. The  $F_i$ ,  $M_j$  and  $E_k$  and the interacted effects were considered fixed and the error term was considered random. The following combining ability model was used in REML analysis:

$$Y_{ijk} = \mu + F_i + M_j + E_k + FM_{ij} + FE_{ik} + ME_{jk} + FME_{ijk} + e_{ijk} \quad \text{Eqn 2.7}$$

Where  $Y_{ijk}$  is the yield of single cross hybrid from the  $i^{\text{th}}$  female line crossed with the  $j^{\text{th}}$  male line in the  $k^{\text{th}}$  environment;  $\mu$  is the grand mean;  $F_i$  and  $M_j$  are the average (main) effects of the  $i^{\text{th}}$  female and  $j^{\text{th}}$  male line across environments, respectively, which are equivalent to the GCA effects of the female ( $GCA_f$ ) and male ( $GCA_m$ ) lines, respectively;  $FM_{ij}$  is the female x male interaction effects,  $E_k$  is the  $k^{\text{th}}$  fixed effects of the environment  $E$ ,  $k = 1$  to 4;  $FE_{ik}$  and  $ME_{jk}$  are the interaction effects of the female and male lines with the environments;  $FME_{ijk}$  is the female line x male line (SCA) x environment interaction effects; and  $e_{ijk}$  is the random error term.

The estimates of GCA of parents and SCA of hybrids were obtained as follows (Griffing, 1956):

$$GCA = \frac{1}{n(n-1)} (nX_{i.} - 2X_{..}) \quad \text{Eqn 2.8}$$

$$SCA = X_{ij} - \frac{1}{n-2} (X_{i.} + X_{.j}) + \frac{2}{(n-1)(n-2)} X_{..} \quad \text{Eqn 2.9}$$

Where  $X_{i.}$  and  $X_{.j}$  are the means of the  $i^{\text{th}}$  and  $j^{\text{th}}$  parents, respectively;  $X_{..}$  is the grand mean;  $n$  is the number of parent lines

To compare the contribution of GCA and SCA to the expression of a trait, the combining ability ratio was calculated according to Baker (1978) as follows:

$$GCA:SCA = \frac{2\sigma^2_{GCA}}{2\sigma^2_{GCA} + \sigma^2_{SCA}} \quad \text{Eqn 2.10}$$

To determine the relationship between GCA and test environments, the  $GCA_f$  and  $GCA_m$  effects were correlated with mean yield of the environments. Likewise, the SCA effects were correlated with mean performance of the hybrids at each of the four environments.

### 2.2.5.6 Heritability estimates

Narrow sense heritability ( $h^2$ ) values were estimated using variance components for  $GCA_m$  and  $GCA_f$  and  $\sigma^2_{SCA}$  obtained based on the formulae by Xiang and Li (2001):

$$h^2 = \frac{\sigma^2_A}{\sigma^2_P} = \frac{4\sigma^2_{GCA(f+m)}}{(2\sigma^2_{GCA(f+m)} + \sigma^2_{SCA} + \sigma^2_{GCA_m \times E} + \sigma^2_{GCA_f \times E} + \sigma^2_{SCA \times E} + \sigma^2_e)} \times 100 \quad \text{Eqn 2.11}$$

Where  $\sigma^2_A$  = additive genetic variance which is four times the GCA variance and  $\sigma^2_p$  is total phenotypic variance.

### 2.2.5.6 Relative yield reduction as a percentage

Yield reduction expressed as a percentage was determined as the mean yield in the LP relative to yield of the HP environments as follows:

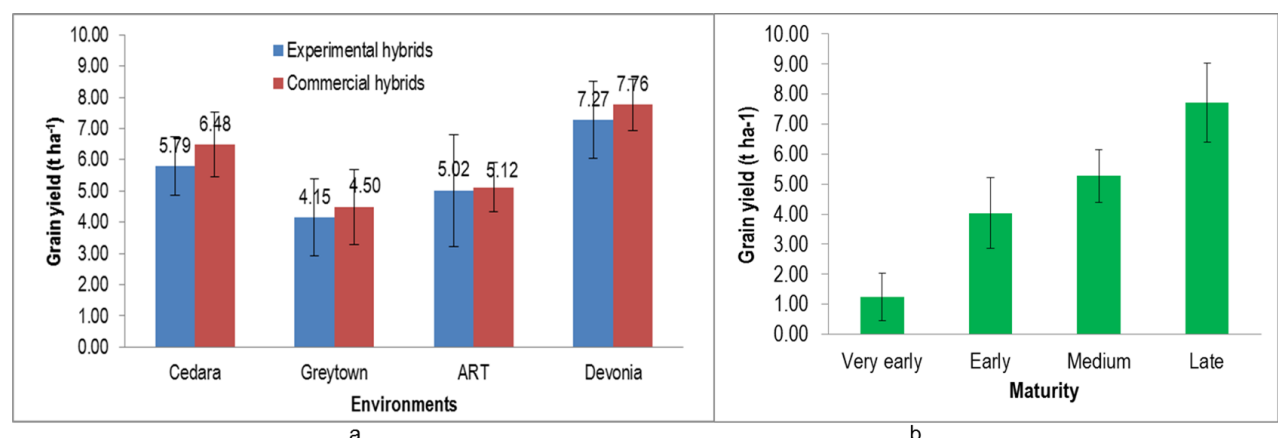
$$\text{Yield reduction\%} = (Y_{LP}/Y_{HP}) * 100 \quad \text{Eqn 2.12}$$

Where  $Y_{LP}$  = yield of the low potential environments, Greytown and ART farm; and  $Y_{HP}$  = yield of the high potential environment, Cedara and Devonina.

## 2.3 Results

### 2.3.1 REML analysis within each environment

Mean yield for each of the four environments was recorded (Fig. 2.1a). Greytown and ART farm experienced mild levels of drought stress particularly between flowering and grain filling. Greytown had the lowest mean yield and also received the least amount of rainfall (Table 2.1). Devonina and Cedara had relatively more favourable conditions during the growing season with better distribution of rainfall. To determine the maturity group with the highest yield, four maturity groups were considered. Mean yield of late maturing hybrids across environments were higher yielding than early maturing (Fig. 2.1b). At each environment, the overall mean of the experimental hybrids was lower than overall mean for the standard checks. Based on the error bars, experimental hybrids were not significantly different from commercial checks (Fig. 2.1a).



**Fig. 2.1 (a.) Location mean yield of 70 experimental single cross hybrids and 10 commercial checks; (b.) Mean yield of very early, early, medium and late maturity.**

The REML analysis for each environment indicated that there were significant ( $P < 0.05$ ) differences among the 80 hybrids for grain yield (GY), days to anthesis (DTA) and anthesis

silking interval (ASI) (Table 2.4). For ears per plant (EPP), the differences between the hybrids were significant ( $P < 0.05$ ) at all environments except Greytown. The mean yield of all hybrids at Greytown was  $4.33 \text{ t ha}^{-1}$  with maximum and minimum yield of  $6.60$  and  $1.20 \text{ t ha}^{-1}$ , respectively. At Devonian, mean yield was  $7.52 \text{ t ha}^{-1}$  with a maximum and minimum yield of  $10.11$  and  $2.43 \text{ t ha}^{-1}$ , respectively. At ART farm, the mean yield was  $5.07 \text{ t ha}^{-1}$  with maximum and minimum yield of  $8.87$  and  $1.90 \text{ t ha}^{-1}$ , respectively. At Cedar, the mean yield was  $6.13 \text{ t ha}^{-1}$  with maximum and minimum yield of  $8.24$  and  $1.42 \text{ t ha}^{-1}$ , respectively. The CVs ranged from  $13.52$  (Devonian) to  $18.61\%$  (Greytown). The ratio of the lowest yielding relative to the highest yielding environment expressed as a percentage was  $57.60\%$ .

**Table 2.4 Summary statistics for four traits measured at each of the four random drought environments**

	Greytown				Devonian			
	GY	DTA	ASI	EPP	GY	DTA	ASI	EPP
Mean	4.33	74.96	0.35	0.98	7.52	68.90	0.59	1.99
LSD <sub>0.05</sub>	1.10	2.03	1.72	0.29	1.18	2.01	1.78	0.18
SE	0.06	1.80	1.24	0.03	0.07	1.72	1.26	0.01
CV%	18.61	1.79	313.81	16.36	13.52	1.91	190.54	9.17
X <sup>2</sup> value	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00
Min	1.23	66.39	-2.69	0.65	2.43	59.50	-2.98	0.94
Max	6.62	82.61	4.88	1.25	10.11	76.86	3.76	1.67
	ART				Cedar			
	GY	DTA	ASI	EPP	GY	DTA	ASI	EPP
Mean	5.07	73.03	0.91	1.15	6.14	79.65	0.81	0.97
LSD <sub>0.05</sub>	1.34	1.75	1.67	0.27	1.78	2.62	2.00	0.28
SE	0.12	1.19	1.06	0.02	0.13	2.60	1.50	0.02
CV%	15.04	1.59	113.25	12.90	16.83	2.32	146.30	14.69
X <sup>2</sup> value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Min	1.90	63.70	-2.66	0.93	1.42	72.00	-2.42	0.79
Max	8.85	83.55	8.67	1.81	8.24	89.33	4.32	1.57

GY = Grain yield; DTA = Days to anthesis; ASI = Anthesis silking interval; EPP = Ears per plant; SE = Standard error; P-value = probability value of significance level; Min = minimum; Max = maximum; CV% = coefficient of variation; LSD = Least significant difference; ART = Agriculture Research Trust

### 2.3.1.1 Open tips at the Greytown environment

Some of the hybrids that were exposed to water stress during the flowering and grain filling period at Greytown had “open tips” (OT): that is, the kernels at the terminal end of the cob were unfilled (Fig. 2.2a) whereas others filled the grain up to the ear tip (Fig. 2.2b). The hybrids were significantly ( $P < 0.05$ ) different for OT ratings (scale 1 - 9, best to worst). The rank order for OT rating (from least affected to worst affected) revealed that EH13, EH54, EH68, EH47, EH33, EH5, EH55, EH46 and EH19 were the top 10 hybrids, whereas, EH23, EH21, EH22, EH25, EH24, EH80, EH61, EH57, EH64 and CH8 were the bottom 10. Importantly, the hybrids least affected by OT were not necessarily the top yielding hybrids.



**Fig. 2.2 (a.) Open tips caused by drought stress; (b.) Ears filled to ear tip. Note: Open tips were measured only at the Greytown environment**

### **2.3.1.2 Ranking of top 20 hybrids at each environment**

Hybrid EH79 was ranked as the top experimental hybrid at Cedara and Devonias, whereas EH28 and EH56 were top ranked at Greytown and ART farm, respectively (Table 2.5). Experimental hybrids out-yielded commercial checks at all environments. There was inconsistent ranking of hybrids from one environment to the other, as confirmed by Spearman's weak correlation coefficients in Table 2.6. Even though they were both favourable environments, rank orders at Devonias and Cedara were not significantly correlated ( $r=0.48$ ).

### **2.3.1.3 Relative yield in low and high potential environments**

The yields of the 70 hybrids plus 10 commercial checks were compared on the basis of categorising the lowest yielding environment (Greytown) as low potential (LP) and the highest yielding environment (Devonias) as high potential (HP) (Table 2.7). The LP environment recorded lower mean GY ( $4.33 \text{ t ha}^{-1}$ ) relative to the HP environment ( $7.52 \text{ t ha}^{-1}$ ). This was possibly due to drought stress; however, other stress factors may have played a role. Hybrid EH60 produced 87.70% of its HP environment yield at the LP environment. Although EH79 was top ranked in the HP environment, EH60 was the best performing EH in terms of realising a greater percentage of its HP yield under the LP conditions.

**Table 2.5 Rank order for grain yield of the top 20 hybrids for each of the four random drought stress environments**

Rank	Greytown	Devonia	ART	Cedara
1	EH29	EH79	EH56	EH79
2	EH60	EH55	EH18	CH4
3	CH8	EH17	EH64	CH9
4	EH16	EH31	EH55	EH29
5	EH79	EH60	EH23	EH66
6	EH28	EH66	EH31	EH19
7	EH80	EH29	EH38	EH44
8	EH32	EH59	EH16	EH23
9	EH27	CH4	CH9	EH60
10	EH72	EH38	EH76	EH12
11	EH44	EH26	EH79	EH18
12	EH38	EH18	CH4	EH52
13	EH66	EH15	EH57	CH2
14	EH21	CH9	EH52	CH8
15	EH31	EH64	EH63	EH71
16	EH53	EH21	EH17	EH64
17	EH40	EH45	EH58	CH5
18	EH18	EH27	CH7	EH27
19	EH20	EH12	EH13	EH46
20	EH52	EH20	EH48	EH70

CH = commercial hybrid check; EH = experimental hybrid; ART = Agriculture Research Trust

**Table 2.6 Spearman's rank correlation coefficients for 70 hybrids plus 10 checks at four random drought stress environments**

	ART	Cedara	Devonia	Greytown
ART	1.00			
Cedara	0.32	1.00		
Devonia	0.27	0.48	1.00	
Greytown	0.16	0.41	0.51*	1.00

\*Significant at 5% significance level; ART = Agriculture Research Trust



**Table 2.7 Mean yield of the top 25 experimental hybrids and commercial hybrid checks at the LP (Greytown and ART) and the HP environments (Cedara and Devonla). The LP yield as a percentage of the HP yield is ranked from highest to lowest. The ranks of each hybrid in the LP and HP environments are in parenthesis**

Hybrid	LP	HP	LP/HP x100	LP/HP Rank	Hybrid	LP	HP	LP/HP x100	LP/HP Rank
EH60	6.50(1)	7.42(2)	87.70	1	EH18	5.05(16)	6.68(10)	75.72	41
EH80	5.61(6)	6.5(80)	86.40	2	EH36	3.92(53)	5.19(62)	75.55	42
EH29	6.03(3)	7.03(4)	85.86	3	EH19	4.25(43)	5.64(43)	75.32	43
EH66	5.76(4)	6.78(8)	84.93	4	EH11	4.49(38)	5.98(31)	75.13	44
EH79	6.45(2)	7.65(1)	84.28	5	EH51	3.73(62)	4.97(68)	74.99	45
CH8	5.17(13)	6.13(25)	84.23	6	EH64	4.74(29)	6.33(15)	74.95	46
EH72	5.47(8)	6.56(12)	83.44	7	CH2	4.09(48)	5.46(50)	74.83	47
EH28	5.62(5)	6.79(7)	82.73	8	EH73	4.42(40)	5.92(37)	74.68	48
EH21	5.33(11)	6.52(13)	81.86	9	EH62	3.91(54)	5.23(59)	74.68	49
EH25	5.06(15)	6.19(23)	81.82	10	EH68	3.82(58)	5.14(63)	74.24	50
EH15	4.77(27)	5.90(38)	80.77	11	EH49	3.68(65)	4.97(69)	74.12	51
EH27	5.37(10)	6.65(11)	80.76	12	EH30	4.14(46)	5.60(44)	74.04	52
EH44	4.81(25)	6.00(29)	80.11	13	EH69	4.02(50)	5.48(49)	73.33	53
CH5	4.87(23)	6.08(26)	80.06	14	EH48	4.30(42)	5.88(39)	73.07	54
EH40	5.01(18)	6.28(20)	79.91	15	EH54	3.63(67)	4.99(67)	72.83	55
EH20	5.01(17)	6.28(19)	79.72	16	EH13	4.03(49)	5.55(45)	72.65	56
EH34	4.73(30)	5.99(30)	79.01	17	EH55	4.56(34)	6.30(18)	72.40	57
EH61	4.18(45)	5.30(57)	78.87	18	EH77	3.99(52)	5.52(47)	72.34	58
EH32	4.90(20)	6.22(22)	78.72	19	EH52	3.89(55)	5.38(54)	72.32	59
EH17	5.43(9)	6.91(5)	78.70	20	EH47	3.82(57)	5.28(58)	72.31	60
CH6	4.66(32)	5.93(36)	78.69	21	EH24	3.53(70)	4.95(70)	71.43	61
EH53	4.75(28)	6.04(28)	78.64	22	CH1	3.65(66)	5.11(65)	71.33	62
CH9	5.00(19)	6.37(14)	78.49	23	EH74	3.23(74)	4.56(74)	70.72	63
EH12	4.80(26)	6.15(24)	77.97	24	EH43	3.62(68)	5.12(64)	70.66	64
EH45	4.62(33)	5.94(34)	77.80	25	EH35	3.78(59)	5.36(56)	70.53	65
EH37	4.70(31)	6.05(27)	77.73	26	EH23	3.84(56)	5.50(48)	69.73	66
EH31	5.53(7)	7.11(3)	77.72	27	EH76	3.74(61)	5.39(52)	69.43	67
EH75	4.02(51)	5.20(61)	77.36	28	EH63	3.76(60)	5.42(51)	69.33	68
CH7	4.88(22)	6.32(16)	77.35	29	EH14	3.61(69)	5.22(60)	69.14	69
CH4	4.88(21)	6.31(17)	77.31	30	EH57	3.71(63)	5.39(53)	68.95	70
EH42	4.50(37)	5.83(40)	77.16	31	EH67	3.48(71)	5.08(66)	68.49	71
EH70	4.43(39)	5.75(41)	77.08	32	EH39	3.31(72)	4.84(71)	68.35	72
EH26	4.81(24)	6.26(21)	76.75	33	EH22	3.30(73)	4.84(72)	68.24	73
CH3	4.34(41)	5.66(42)	76.72	34	EH50	2.73(76)	4.11(76)	66.37	74
EH46	4.11(47)	5.36(55)	76.68	35	EH78	3.10(75)	4.77(73)	64.99	75
EH16	5.16(14)	6.75(9)	76.50	36	EH56	3.70(64)	5.95(33)	62.19	76
EH38	5.26(12)	6.90(6)	76.26	37	CH10	2.52(77)	4.08(77)	61.72	77
EH71	4.52(36)	5.94(35)	76.07	38	EH58	2.52(78)	4.37(75)	57.59	78
EH33	4.2(44)	5.53(46)	75.87	39	EH65	1.30(80)	2.61(79)	49.90	79
EH59	4.53(35)	5.97(32)	75.80	40	EH41	1.61(79)	3.24(78)	49.71	80

### 2.3.2 REML analysis across environments

Results from Bartlett (1937) test revealed equality of variances ( $\sigma_1^2 = \sigma_2^2 = \sigma_3^2 = \sigma_4^2$ ) (Appendix 2.4;  $P > 0.05$ ) between environments. The Wald statistics for hybrids, environments and the interaction between hybrids and environments were highly significant ( $P < 0.05$ ; Table 2.8). The GxE component was then analysed using AMMI, regression coefficient ( $b_i$ ) and cultivar superiority index ( $P_i$ ).

**Table 2.8 REML Wald statistics for fixed effects of grain yield (t ha<sup>-1</sup>) hybrids, environments and GxE interaction**

Fixed term	d.f.	Wald statistic	Wald/d.f	Chi pr.
Row	7	5.05	0.72	0.385
Column	9	28.37	3.15	0.001
Row.Column	63	147.72	2.34	0.001
Hybrid	79	430.00	5.44	0.001
Environment	3	250.69	83.56	0.001
GxE	237	397.44	1.68	0.001
Error	380	257.41	0.68	
Total	778	1516.70	1.95	

GxE = Genotype x environment interaction; d.f. = degree of freedom

#### 2.3.2.1 Additive main effect and multiplicative interaction analysis

The additive main effects and multiplicative interaction (AMMI) ANOVA was conducted for the GxE means for the trait GY across the four environments (Table 2.9). Hybrids (referred to as genotypes) were significantly different. The treatments sum of squares (SS) (that is, hybrids, environments and GxE) accounted for 82.57% of the Total SS (Table 2.9). Hybrids alone contributed 41.81% to the treatments SS whereas environments and GxE accounted for 20.57 and 37.61%, respectively. Thus the contribution of hybrids to the treatment variation was more than double that of the environments. The IPCA1, 2 and residuals for GY were significant ( $P < 0.05$ ), accounting for 46.15, 31.24 and 22.63% of the GxE sum of squares, respectively. The IPCA 1, 2 and residual SS explained 100% of the GxE SS.

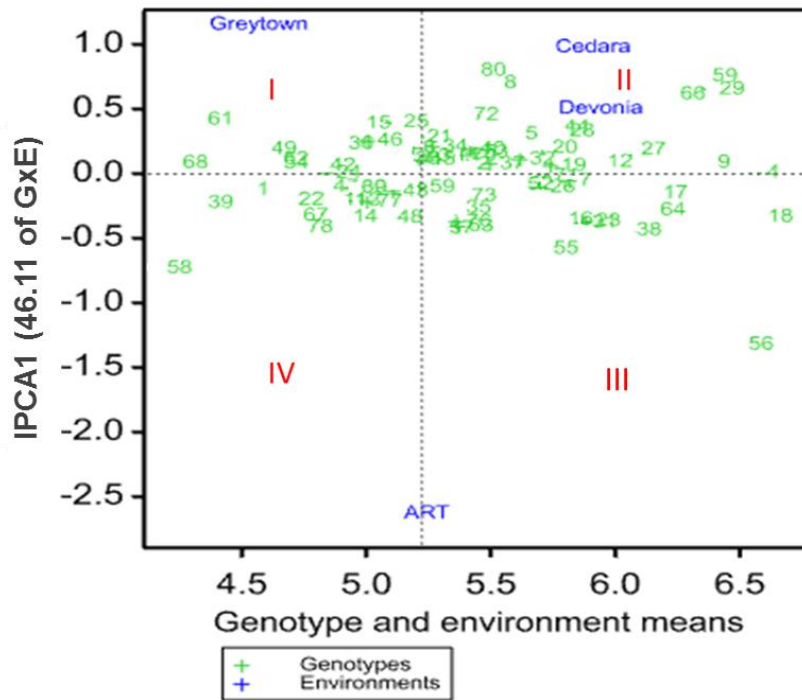
**Table 2.9 AMMI ANOVA for grain yield (t ha<sup>-1</sup>) evaluated across four environments under random drought stress.**

Source	Df	SS	MS	F	F_prob	%SS
Total	779	1877.90	2.41	*	*	
Blocks	9	31.10	3.46	5.99	0.00	1.66
Treatments	319	1550.60	4.86	7.41	0.00	82.60
Genotypes	79	648.30	8.21	12.50	0.00	41.81
Environments	3	319.00	106.33	27.39	0.00	20.26
GxE	237	583.30	2.46	3.75	0.00	37.62
IPCA1	81	269.20	3.32	5.06	0.00	46.15
IPCA2	79	182.20	2.31	3.51	0.00	31.24
Residuals	77	132.00	1.71	2.61	0.00	22.63
Error	451	296.20	0.66	*	*	

Blocks refer to blocks within environments ignoring rows and columns

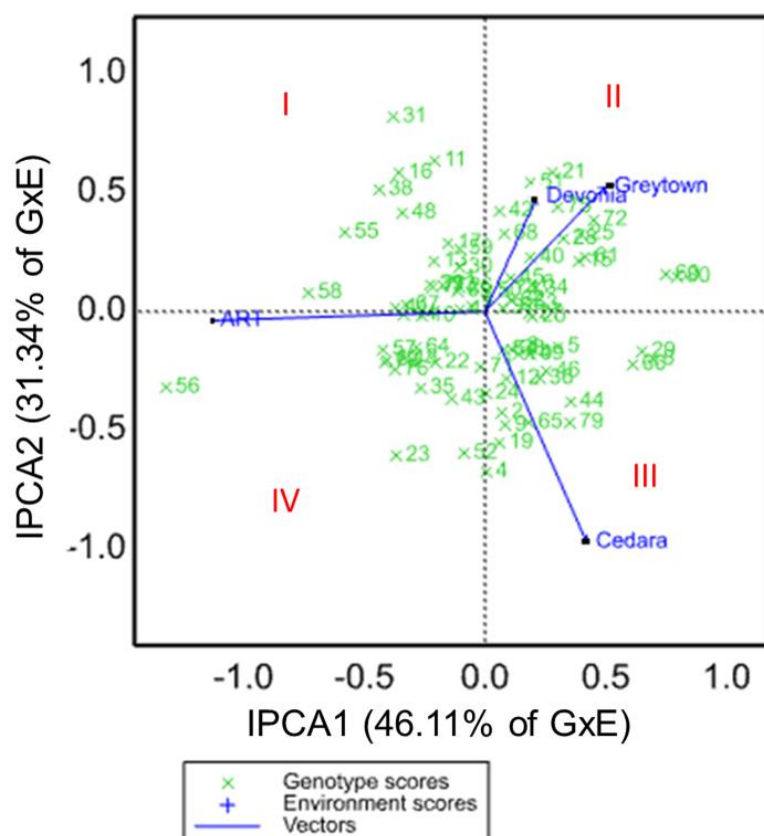
### 2.3.2.2 AMMI biplots

For the IPCA1 versus mean performance biplot (Fig. 2.3), displacement along the y-axis indicated differences in the extent of the interaction between hybrids and environments and displacement along the x-axis indicated differences in hybrid and environment mean performance for GY. Hybrids with IPCA1 scores close to zero had low interaction with the environments and expressed general adaptation whereas hybrids with large scores had high interaction or specific adaptation to the environments, the interaction being positive with environments of the same IPCA1 signage and negative with environments of opposite signage. Hybrid EH56 had the highest IPCA1 score and therefore can be regarded as unstable. Cedara and Devonía environments appeared in the top yielding quadrant II. Greytown was the lowest yielding environment (quadrant I). The ART farm environment had the highest (negative) IPCA1 score indicating it had the highest interaction with hybrids. Greytown had the second highest IPCA1 scores of the four environments but of opposite signage to ART farm. Hybrid EH56 had the highest IPCA1 score of the same signage as ART farm and therefore had a high positive interaction with this environment as confirmed by it being the highest yielder in this environment whereas hybrids EH80, CH8, EH60, EH29, EH59 and EH66 had specific and positive interaction with Cedara and Devonía. The top 10 stable hybrids based on having the lowest IPCA1 scores were EH24, EH4, EH50, EH71, CH7, EH19, EH42, CH2, EH37 and EH54, in that order (Table 2.10). Overall, the environments can be classified into three heterogeneous groups based on their placement in the four quadrants of the biplot. The first group comprised Cedara and Devonía (relatively high potential, stable environments with Cedara tending towards unstable), second group comprised Greytown (low potential, unstable environment) and ART farm in the third group (average potential, highly unstable).



**Fig. 2.3 AMMI biplot of the mean performance ( $t\ ha^{-1}$ ) versus IPCA1 scores of 80 hybrids evaluated in four environments. The numbers from 1-10 were commercial hybrid checks and 11-80 were experimental hybrids**

For the IPCA1 versus IPCA2 biplot (Fig. 2.4), Devonian and Greytown are in the same quadrant with IPCA1 and 2 scores of the same signage and, with the angle between the vectors from the origin to their coordinates less than 90 degrees, their interaction with the hybrids was positively correlated. The ART farm environment had the highest negative IPCA1 score and therefore the environmental factor causing the IPCA1 pattern resulted in hybrids interacting strongly with this environment. The angle between the vectors from the origin to the coordinates of ART farm and Cedara was more than 90 degrees indicating that their interaction with hybrids was negatively correlated. Hybrids which clustered around the origin were generally adapted, whereas hybrids such as EH31 with high IPCA2 score indicated specific adaptation. The extent of the interaction between a genotype and an environment is given by the length of the perpendicular drop down from the genotype coordinate to the environmental vector from the origin.



**Fig. 2.4 AMMI biplot of IPCA1 versus IPCA2 scores for 80 genotypes tested in four environments. The numbers from 1-10 were commercial hybrid checks and 11-80 were experimental hybrids**

### 2.3.2.3 Stability parameters

Hybrids EH79 and EH18 had the lowest  $P_i$  value and therefore they could be regarded as generally adapted (Table 2.10). These two hybrids performed above the commercial hybrid checks in terms of yield. However, a regression coefficient ( $b_i$ ) of 2.15 for EH79 indicated that the hybrid was specifically adapted to high potential conditions, whereas under low potential conditions, yield was greatly reduced (Table 2.10). A regression coefficient less than 0.7 indicated that a hybrid was specifically adapted to low-yielding environments and hybrids with  $b_i > 1.3$  were specifically adapted to higher yielding environments. Hybrids with regression coefficients between 0.7 and 1.3 were EH27, EH62, EH39, EH60, EH25, EH73, EH37, EH76, EH14, EH69, EH54, EH22, EH68, EH34, EH65, EH77, EH29, EH19, EH18, EH71, EH49, EH17, and EH24. Hybrid EH24 also had the lowest IPCA1 score (Table 2.10), confirming its stability. Some hybrids, such as EH26, had negative regression coefficients indicating better performance under low yielding than high yielding environments.

**Table 2.10 Cultivar superiority index ( $P_i$ ) across the four environments, IPCA1 score and regression coefficient ( $b_i$ ), with hybrids ranked from the most stable to the least based on  $P_i$ . The  $b_i$  coefficient corresponds to hybrids ranked from the top yielding to the lowest in the top 30**

Rank	$P_i$	IPCA1	Hybrids	$b_i$
1	EH79	EH24	EH79	2.15
2	EH18	CH4	EH18	1.30
3	CH4	EH50	CH4	2.61
4	CH9	EH71	CH9	1.76
5	EH64	CH7	EH60	1.41
6	EH56	EH19	EH56	1.62
7	EH23	EH42	EH29	1.97
8	EH12	CH2	EH64	1.69
9	EH17	EH37	EH66	1.33
10	EH27	EH54	EH17	0.70
11	EH60	CH9	EH27	0.82
12	EH29	EH68	EH38	1.49
13	CH7	EH74	EH12	0.85
14	EH66	EH12	EH31	1.78
15	EH71	EH52	EH23	1.28
16	EH19	EH59	EH28	1.21
17	EH38	EH47	CH7	0.41
18	EH20	EH26	EH19	0.97
19	EH26	EH30	EH16	1.41
20	EH52	EH62	EH71	0.33
21	EH44	EH33	EH44	0.63
22	EH76	EH32	EH20	1.04
23	EH37	EH69	EH55	0.86
24	EH16	EH45	EH26	-0.74
25	CH5	EH1	EH52	1.13
26	EH31	EH43	EH32	0.37
27	EH32	CH3	CH5	0.06
28	EH28	EH70	EH37	-0.45
29	EH55	EH17	EH53	2.21
30	EH73	EH53	CH8	0.82

$P_i$  = Cultivar superiority index;  $b_i$  = regression coefficient; IPCA1 = Interaction principal component axis (ranking based on absolute values, ignoring signage); EH = Experimental hybrid; CH = commercial hybrid

### 2.3.3 Combining ability analysis for grain yield

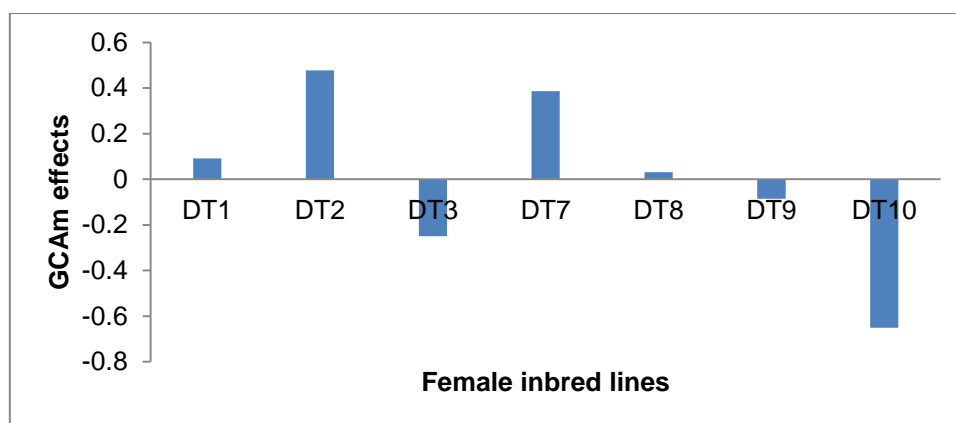
The combining analysis of the seven female and 10 male inbred parents was conducted based on the mean performances of their 70  $F_1$  single crosses across the four environments. The Wald statistics due to  $GCA_m$ ,  $GCA_f$ , SCA,  $GCA_m \times E$  and  $SCA \times E$  for GY were highly

significant ( $P < 0.05$ ) (Table 2.11). However, the interaction of  $GCA_f \times E$  was non-significant ( $P > 0.05$ ). The female parents with the highest positive  $GCA_f$  effects for GY under random drought stress environments were DT7 (0.39), DT2 (0.48) whereas DT3 (-0.25), DT10 (-0.65) had the lowest negative  $GCA_f$  effects (Fig. 2.5). Male parents with the highest positive  $GCA_m$  effects were UT4 (0.17), UT1 (0.25), and UT8 (0.49), whereas UT10 (-0.22), N\_CNTRL (-0.24), and UT6 (-0.50) had the lowest negative  $GCA_m$  effects (Fig. 2.6). During pollinations it was observed that DT2 was a good female (seed) parent but a poor male (pollen) parent. A simple correlation analysis indicated that both  $GCA_m$  and  $GCA_f$  effects were not highly correlated with environment means indicating that additive genetic effects did not contribute to mean yield of each hybrid at each environment (Table 2.12). Correlation coefficients ranged from 0.20 (Greytown) to 0.55 (Cedara). The ratio of  $GCA_f:SCA$  and  $GCA_m:SCA$  (based on the Wald statistic values; Table 2.11) was  $< 1$  indicating the preponderance of SCA over GCA. This implied that grain yield was influenced mainly by non-additive gene action. Histograms of hybrids with significant positive SCA effects (Fig. 2.7) and significant negative SCA effects (Fig. 2.8) are plotted. Hybrids EH79 had the highest positive SCA effects whereas EH65 followed by EH41 had the lowest negative SCA effects (Fig. 2.8). Hybrids that are not shown in Fig 2.7 and 2.8 had SCA effects close to zero. Simple correlation analysis showed that SCA effects were highly positively correlated with yield performance of each corresponding experimental hybrid at each environment (Table 2.13). This indicates that non-additive genetic effects were positively associated with mean yield of each hybrid at each environment.

**Table 2.11 REML analysis of general and specific combining ability for grain yield ( $t\ ha^{-1}$ ) across four environments**

Fixed term	Wald statistic	d.f.	F statistic	chi pr
Replications	108.21	3	36.07	0.0030
Environments	521.00	3	173.67	0.0011
Crosses	416.39	70	5.95	0.0001
GCA for females (f)	29.62	6	4.94	0.0001
GCA for males (m)	18.12	9	2.11	0.0001
SCA (f x m)	56.60	54	1.05	0.0001
Crosses*Environments	615.30	207	2.93	0.0001
GCA <sub>f</sub> *Environments	23.50	18	1.32	0.5010
GCA <sub>m</sub> *Environments	33.10	27	1.23	0.0021
SCA*Environments	714.60	162	4.41	0.0001
Error	588.21	276	2.13	
GCA <sub>f</sub> :SCA	0.52			
GCA <sub>m</sub> :SCA	0.32			

GCA = General Combining ability; SCA = Specific combining ability; d.f. = degrees of freedom



**Fig. 2.5** Mean (t ha<sup>-1</sup>) general combining ability across four environments of seven female lines of the 7x10 factorial mating design for grain yield (t ha<sup>-1</sup>)



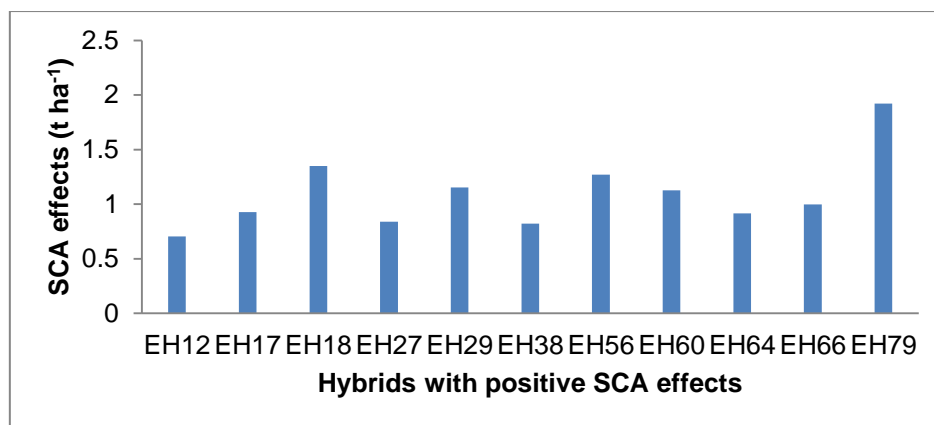
**Fig. 2.6** Mean (t ha<sup>-1</sup>) general combining ability across four environments of seven male lines of the 7x10 factorial mating design for grain yield (t ha<sup>-1</sup>)

**Table 2.12** Standard correlation coefficients between GCA effects of female and male parents and the mean performance of the experimental hybrids for grain yield (t ha<sup>-1</sup>) in each of four environments

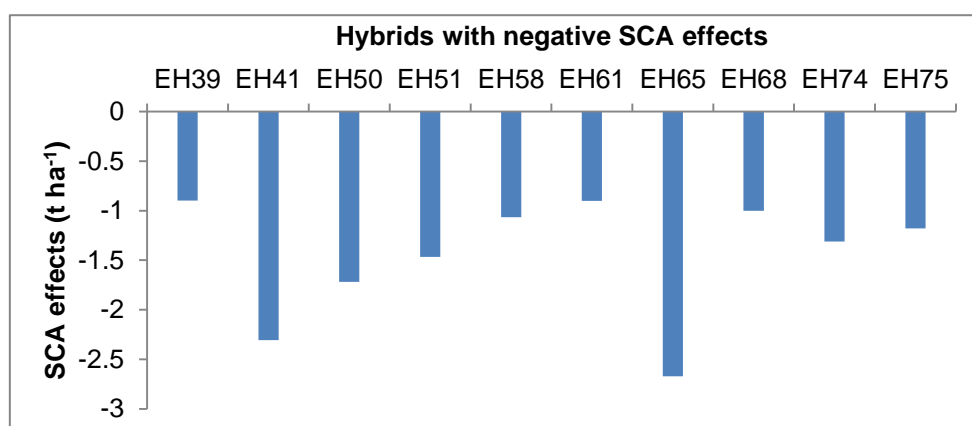
Environment	GCA <sub>f</sub>	GCA <sub>m</sub>
Cedara	0.55	0.28
Greytown	0.38	0.20
ART	0.29	0.54
Devonia	0.48	0.40

GCA = General combining ability; ART = Agriculture Research Trust





**Fig. 2.7** Single cross hybrids with significantly positive SCA effects for grain yield (t ha<sup>-1</sup>) under random drought stress environments



**Fig. 2.8** Single cross hybrids with significantly negative SCA effects for grain yield (t ha<sup>-1</sup>) under random drought stress environments

**Table 2.13** Standard correlation coefficients between SCA effects and the mean performance of each corresponding experimental hybrid for grain yield (t ha<sup>-1</sup>) in each of four environments

Environment	Correlation coefficients
Cedara	0.84
Devonia	0.87
ART	0.81
Greytown	0.78

ART = Agriculture Research Trust

### 2.3.4 Maturity groupings of hybrids

To avoid discarding early maturing hybrids (in favour of late hybrids when selection is based on yield alone), all hybrids including checks were grouped into four maturity groups and LSD<sub>0.05</sub> were conducted relative to the top yielding commercial check within a maturity group (Table 2.14). Experimental hybrids that yielded above the commercial checks within each maturity group were desirable. Maturity groups were based on days to anthesis averaged across all four environments. Four maturity groups were established, i.e. 60-65 (very early; VE), 65-70 (early; E), 70-75 (medium; M) and 75+ days to anthesis (late; L). Of the commercial PANNAR hybrid checks, CH1 was very early maturing (60 DTA), followed by

CH3 (66 DTA), CH4 (73 DTA) and CH7 (78 DTA). These commercial hybrids were used as benchmarks for each of the four groups. The selection of commercial hybrids as benchmarks for maturity groups was based on maturity data provided by PANNAR and results obtained from this study. The other commercial hybrid checks were included in Table 2.14 for comparison with EHs. In the VE maturity group, EH19 (5.74 t ha<sup>-1</sup>) and EH71 (5.71 t ha<sup>-1</sup>) had significantly higher yields relative to CH1, whereas, only EH41 (2.29 t ha<sup>-1</sup>) had significantly lower yield. Therefore selection based on yield favoured EH19 and EH71 in this group. Both EH19 and EH71 were in the top 10 most stable hybrids based on AMMI IPCA1 scores (Table 2.14). In the E group only two hybrids, CH10 (3.74 t ha<sup>-1</sup>) and EH61 (4.32 t ha<sup>-1</sup>), had significantly lower yields relative to the commercial check CH3. The medium group had more experimental hybrids, EH63 (5.37 t ha<sup>-1</sup>), EH75 (4.05 t ha<sup>-1</sup>), EH70 (5.38 t ha<sup>-1</sup>), EH50 (3.50 t ha<sup>-1</sup>), EH15 (4.96 t ha<sup>-1</sup>), (4.96 t ha<sup>-1</sup>), EH11 (4.87 t ha<sup>-1</sup>), EH51 (3.76 t ha<sup>-1</sup>), EH14 (4.90 t ha<sup>-1</sup>), EH72 (5.39 t ha<sup>-1</sup>), EH58 (4.16 t ha<sup>-1</sup>) with significantly (P<0.05) lower yields than the commercial check, CH4 (6.62 t ha<sup>-1</sup>). The late (L) maturity group had only two hybrids, EH74 (3.91 t ha<sup>-1</sup>) and EH65 (2.55 t ha<sup>-1</sup>) that were significantly lower than the commercial check CH7 (5.90 t ha<sup>-1</sup>). The other hybrids produced yields that were not significantly different from the benchmark commercial hybrids.

**Table 2.14 Experimental hybrids placed in four maturity groups based on average days to anthesis across all locations. CH1, CH3, CH4 and CH7 were used as the benchmark commercial hybrids to group the experimental hybrids**

VE	E		M		L
CH1(4.48 t ha <sup>-1</sup> )	CH3 (5.19 t ha <sup>-1</sup> )		CH4 (6.62 t ha <sup>-1</sup> )		CH7 (5.90 t ha <sup>-1</sup> )
EH69	EH53	EH45	EH28	EH79	EH40
EH68	EH43	EH77	EH63*	EH55	EH18
EH54	EH23	EH24	EH75*	EH29	EH34
EH71*	EH67	CH10*	EH70*	EH44	EH20
EH76	EH25	EH49	EH50*	EH17	EH73
EH41*	EH33	EH12	EH64	EH38	EH60
EH19*	EH22	EH78	EH31	CH5	EH16
EH48	EH57	CH2	EH15*		EH32
	EH26	EH59	EH11*		EH27
	EH52	EH62	EH51*		EH21
	EH42	EH30	EH56		EH80
	EH35	CH6	EH14*		CH8
	EH61*	EH39	EH72*		EH36
	EH13	EH37	CH9		EH74*
			EH58*		EH66
					EH65*

CH = Commercial hybrid; EH = Experimental hybrids; VE = Very early; E = Early; M = Medium; L = Late; \*LSD significant at 5%

### 2.3.5 Heritability estimates for grain yield and secondary traits at each environment

Narrow sense heritability ( $h^2$ ) estimates for yield and secondary traits were calculated using variance components obtained from REML. Heritability estimates varied from one environment to another (Table 2.15). Grain yield was highly heritable at Cedara (0.69) followed by ART farm (0.68), whereas at Greytown (0.56) and Devonian (0.60), it had low  $h^2$ . Anthesis silking interval had lower  $h^2$  than DTA for each of the four environments. Of the 13 traits measured, plant height, rows ear<sup>-1</sup>, 1000 kernel mass, grain moisture and DTA were the highest heritable traits at each environment. Husk dry was the least heritable trait. Heritability estimates for number of EPP varied from 0.32 (Greytown) to 0.64 (Cedara). Generally,  $h^2$  estimates were higher at environments that received higher rainfall and that were high yielding i.e. Devonian and Cedara. In LP (Greytown) conditions, heritability for GY trait was lower than in the HP (Cedara) conditions.

**Table 2.15 Narrow sense heritability ( $h^2$ ) and standard error estimates for yield and secondary traits of 80 hybrids including 10 checks evaluated at each of four environments.**

Traits	Cedara	Greytown	ART <sup>a</sup>	Devonian <sup>a</sup>
Grain yield (t ha <sup>-1</sup> )	0.69±0.14	0.56±0.21	0.68±0.18	0.60±0.08
Days to anthesis	0.92±0.29	0.78±0.19	0.74±0.13	0.94±0.19
Anthesis-silking interval	0.76±0.17	0.62±0.13	0.70±0.12	0.85±0.14
Husk dry	0.10±0.57	0.20±0.23	-	-
Ear height (cm)	0.98±9.14	0.75±17.0	-	-
Ears per plant	0.64±0.03	0.45±0.03	0.32±0.02	0.55±0.01
Grain moisture %	0.91±0.01	0.90±0.19	0.75±0.02	0.80±0.13
Grey leaf spot	0.64±0.13	0.87±0.18	0.64±0.35	0.79±0.20
Plant height (cm)	0.82±10.23	0.95±12.54	-	-
Ear diameter	0.65±0.04	0.45±0.09	-	-
Rows ear <sup>-1</sup>	0.96±0.01	0.92±0.23	-	-
1000 kernel-mass	0.91±0.32	0.76±0.42	-	-
Open tips	0.65±0.87	0.68±0.14	-	-

<sup>a</sup>Heritability estimates were not determined for some traits in these environments as these traits were not measured. ART = Agriculture Research Trust

### 2.3.6 Spearman's rank correlations between secondary traits and grain yield across four environments

Spearman's rank correlations between traits were computed for each of the LP and HP environments and significance level expressed at  $P < 0.05$  (Table 2.16 and 2.17). Under the LP conditions (Table 2.16), the rank order of GY was significantly and negatively correlated with ASI ( $r = -0.49$ ) and OT ( $r = -0.44$ ), but positively and significantly correlated to DTA

( $r=0.52$ ), EPP ( $r=0.48$ ), shelling percentage (SP;  $r=0.63$ ) and grain moisture (GM;  $r=0.49$ ). The correlation between DTA and leaf senescence (LSEN;  $r=-0.36$ ) was negative and significant. There was a positive and significant correlation between husk dry (HD) cover and OT (0.54). Under the HP conditions (Table 2.17), the rank order correlation between GY and ASI was positive but not significant as opposed to what was observed under LP conditions. The significant and positive correlation between GY and DTA ( $r=0.72$ ) was stronger than under LP conditions. Days to anthesis and EPP ( $r=-0.05$ ) were very weakly correlated when compared to LP conditions. But under both conditions, the correlation was non-significant. Under HP conditions the rank order of GY was significantly correlated with GLS ( $r=-0.69$ ), LSEN ( $r=-0.28$ ), SP ( $r=0.59$ ), OT ( $r=-0.33$ ), and 1000 kernel mass ( $r=0.19$ ). The SP and OT ( $r=0.42$ ) were themselves significantly and negatively correlated. The significant correlation between GY and Grey Leaf Spot (GLS) was negative ( $r=-0.69$ ); and EPP and GY was positive ( $r=0.31$ ). The GLS and LSEN were positively correlated ( $r=0.58$ ), and the correlation significant. There was a significant and positive correlation between 1000 kernel mass and grain moisture (GM;  $r=0.30$ ). The correlation between SP and GY was positive and significant, but lower than the correlation coefficient under LP conditions. The highest positive and significant correlation was between GM and DTA ( $r=0.78$ ) under HP conditions (Table 2.17). Days to anthesis were negatively ( $r=-0.42$ ) and significantly correlated with GLS. Leaf senescence was positively correlated with GLS ( $r=0.43$ ). Grain moisture and ASI were positively ( $r=0.49$ ) and significantly correlated. The negative correlation between LSEN and HD cover ( $r=-0.37$ ) was significant. Husk dry cover was positively and significantly correlated with GM ( $r=0.44$ ) and negatively and significantly correlated with 1000 kernel mass ( $r=-0.22$ ). The negative correlation between GY and OT was significant.

**Table 2.16 Spearman's rank correlation coefficients between grain yield and secondary traits (in which they were measured) in the LP environment**

	GY	ASI	DTA	EPP	GLS	LSEN	HD	OT	SP	GM
ASI	-0.49*	1.00								
DTA	0.52*	-0.12	1.00							
EPP	0.48*	-0.24	0.30	1.00						
GLS	-0.25	0.32	0.12	-0.15	1.00					
LSEN	-0.36*	0.24*	-0.36*	0.10	0.69*	1.00				
HD	-0.09	0.10	0.07	0.12	0.14	0.08	1.00			
OT	-0.44*	0.02	-0.20	0.02	0.05	0.07	0.54*	1.00		
SP	0.63*	-0.08	0.44	-0.06	-0.13	-0.11	0.06	-0.42	1.00	
GM	0.49*	0.19	0.32*	-0.02	0.07	0.13	0.01	0.16	-0.04	1.00
1000 K-mass	0.30	-0.30	0.02	0.15	-0.19	-0.14	-0.01	0.00	0.18	0.53*

GY = Grain yield; ASI = Anthesis silking interval; DTA = Days to anthesis; EPP = Ears per plant; GLS = Grey leaf spot; LSEN = Leaf senescence; HD = Husk dry cover; OT = Open tips; SP = Shelling percentage; GM = grain moisture; 1000 K-mass = mass of 1000 kernels

**Table 2.17 Spearman's rank correlation coefficients between grain yield and secondary traits (in which they were measured) in the HP environment**

	GY	ASI	DTA	EPP	GLS	LSEN	HD	OT	SP	GM
ASI	0.11	1.00								
DTA	0.72*	-0.21	1.00							
EPP	0.31*	-0.08	-0.05	1.00						
GLS	-0.69*	0.10	-0.42*	0.02	1.00					
LSEN	-0.28*	0.05	0.36*	0.20	0.58*	1.00				
HD	-0.16	0.12	-0.05	0.37	0.27	-0.37	1.00			
OT	0.09	-0.15	-0.19	0.01	-0.01	-0.06	-0.22	1.00		
SP	0.59*	-0.01	-0.01	0.20*	0.10	-0.04	0.39	-0.16	1.00	
GM	0.57*	0.73*	0.78*	-0.24	-0.08	0.08	0.44	-0.03	-0.21*	1.00
1000 K-mass	-0.19*	-0.16	-0.09	0.19	0.04	-0.14	-0.22*	0.09	0.19	-0.30*

GY = Grain yield, DTA = Days to anthesis, ASI = Anthesis-silking interval, EPP = Ears per plant, GLS = Grey leaf spot, LSEN = Leaf senescence, HD = Husk dry cover; OT = Open tips; SP = Shelling percentage, GM = Grain moisture, 1000 K-mass = mass of 1000 kernels; \*significantly correlated at 5% significance level

## 2.4 Discussion

The main objective of this study was to evaluate the yield and related secondary trait performance of 70 F<sub>1</sub> single cross maize hybrids generated in a 7x10 factorial mating design and exposed to random drought at four environments during the 2012/13 season. This data was used for combining ability and stability analysis across environments. Individual and across environments analysis for GY and selected secondary traits indicated significant differences between hybrids in all environments. The interaction between hybrids and environments indicated that hybrid performances were environment dependent. The parent lines used in this study were very diverse in genetic background. This likely led to the production of diverse hybrid progeny and therefore differential performance of the hybrids across environments was to be expected. According Liu *et al.* (2003), the exploitation of inbred lines in a breeding program requires a detailed knowledge of the genetic and historical relationships among the lines. However, in the case where sufficient information on the inbred lines is not available for proprietary reasons, as in this study, the initial breeding objective was to characterise the parental lines and hybrid progeny using combining ability analysis. The results of this study have important implications for breeding, presenting an opportunity to identify promising lines and F<sub>1</sub> hybrids with improved performance under RDS. The differential performance of the hybrids in the different environments presented an opportunity to select for either specific or general adaptation to RDS.

## Effects of the environments on grain yield

The test environments were dissimilar in terms of latitude, altitude and associated climatic conditions (Table 2.1). This would have influenced the performance of the individual experimental hybrids. Cedara and Devonias were the highest yielding environments because seasonal rainfall was relatively favourable. According to Bello *et al.* (2012), precipitation patterns have considerable impact on the expression of a plant's yield potential, particularly during the flowering/grain filling period of maize. Both the seasonal amounts and the distribution are reportedly important. In this study, weekly rainfall distribution and subsequent total amounts appeared to have affected GY. For example, Devonias received the highest and most well distributed seasonal rainfall, followed by Cedara and the GY attained mirrored that order. The timing of stress is very important in maize (Bänziger *et al.*, 2000). At Greytown, water stress coincided with the onset of flowering of the late maturing hybrids between week 6 and 8 after planting while at the ART farm, it coincided mostly with the grain filling phase between weeks 8 to 13. On average, late maturing hybrids had higher GY than the early maturing hybrids. Importantly, for trials with different maturity clusters (even separated by a few days), this study demonstrated that even mild water stress can differentially affect the different maturity clusters and the attained yields.

The impact of water stress on yield was analysed by determining the percentage GY attained between LP (Greytown and ART farm) relative to HP (Cedara and Devonias) environments. This analysis could also be used as a measure of stability. A hybrid that maintains a consistent yield across both LP and HP environments could be regarded as stable. Experimental hybrid EH60 appeared to be the least affected by stress in the LP environment because it attained about 87% of its HP yield, whereas EH41 has the lowest LP/HP ratio (49%). According to Bänziger and Lafitte (1997), when realised yield falls below 50-60% of potential, stress is regarded as severe and the use of secondary traits for selection assume real significance. The difference in GY between hybrids is reportedly a result of either differences in yield potential, stress tolerance or both (Tollenaar and Lee, 2004). In this study, both the inherent yield potential of a hybrid and its ability to tolerate stress were important. According to Tollenaar and Lee (2004), improved stress tolerance is partly attributable to heterosis for yield (also reported by Blum, 1997) exhibited by hybrid crosses. In maize (Betrán *et al.*, 2003) and pearl millet (*Pennisetum glaucum*) (Yadav *et al.*, 2000), high estimates of heterosis for yield were obtained under stress conditions. If heterosis occurs for stress tolerance, then parents that express good combining in association with maximising heterosis for stress tolerance would be vitally important.

Rainfall distribution and amount are among the important factors that define the rainy season and in turn determine planting date. Maize hybrids in most production regions are generally classified into E, M, and L maturity groups, based on days to anthesis. In this study, the sub-group of VE was included. Very early, E, M and L hybrids are suitable for very short, short, moderately long and long rainy seasons, respectively. The clustering of hybrids by maturity and GY was an important step in comparing hybrid yield performance within the same maturity group. In the VE group, EH19 and EH71 performed significantly higher than the commercial checks CH1, whereas EH41 performed significantly lower than CH1. The hybrids EH19 and EH71 are recommended for environments with relatively short rainy seasons. Further testing is recommended to confirm maturity clusters and repeatability of performance.

### **Interpreting genotype by environment interaction patterns**

To interpret the GxE interaction patterns, the AMMI model was used. In the AMMI analysis, the GxE interaction was highly significant ( $P < 0.05$ ), explaining 37.62% of the treatment SS variation. The main effects for hybrid alone accounted for 41.80%, compared to 20.57% attributed to the environmental effect. This was indicative of the sizeable amount of exploitable phenotypic variation present in the hybrids evaluated across the four environments. The GxE interaction was of the crossover type as revealed by inconsistent ranking of hybrids across environments and very weak Spearman's rank correlations.

The significant GxE interaction component meant that hybrids differed in terms of their stability and adaptation across environments. Hybrids by environment interactions are common under abiotic stresses, make breeding progress difficult (Bänziger *et al.*, 2006). A characterisation of the stability of performance of hybrids across environments was done using AMMI biplots. The biplots indicated that the hybrids ranged from highly unstable to stable. Hybrids that had high IPCA1 scores interacted with specific environments and they may be selected for specific adaptation. If a breeder is pursuing specific adaptation, hybrids EH80, EH8, EH60, EH29 and EH66 could be selected for Devon and Cedara (Fig. 2.7). But if the objective is general adaptation to all four environments, hybrids with the lowest IPCA1 scores will be preferred (e.g. EH24, EH4, EH50, EH71, EH7, EH19; Table 2.11).

Breeders may use the cultivar superiority index,  $P_i$ , in selecting for stability, however, since the  $P_i$ -value is calculated across all locations for each hybrid it provides a measure of general adaptation. Using the  $P_i$ , EH79 and EH18 can be recommended as generally adapted. The classifications for stability based on the Finlay and Wilkinson (1963) regression coefficient were, when  $b_i < 0.7$ , a hybrid is specifically adapted to low-yielding locations; and

$b_i > 1.3$ , a hybrid is specifically adapted to higher yielding locations (Eberhart and Russell, 1965) and therefore for  $b_i$  between 0.7 and 1.3, a hybrid was generally adapted to all locations. Eberhart and Russell (1965) reported that hybrids with regression coefficients less than one ( $b_i < 1$ ) usually had average yield below the grand mean. Breeders always require hybrids with above average yield across all environments. Finlay and Wilkinson (1963) and Eberhart and Russell (1965) described a stable hybrid as one with  $b_i = 1.0$ . On this basis, hybrids that were the most stable (i.e.  $0.7 \leq b_i \leq 1.3$ ) were EH27, EH62, EH39, EH60, EH25, EH73, EH37 (these had  $0.7 \leq b_i \leq 1.0$ ) EH76, EH14, EH69, EH54, EH22, EH68, EH34, EH65, EH77, EH29, EH19, EH18, EH71, EH49, EH17 and EH24 (these had  $1.0 < b_i < 1.3$ ).

### **Combining ability for grain yield**

Maize inbred lines can themselves have excellent performance for several traits but unless they are also excellent parents in hybrid combinations they may not be of direct use for the generation of commercially successful hybrids (Hallauer *et al.*, 2010). This is why analysis of GCA and SCA was important in selecting suitable lines for hybrid generation. Variance due to SCA was greater than GCA indicating that GY is mainly influenced by non-additive gene action. In turn, this means that the non-additive gene action for GY could be exploited through heterosis expressed via the hybridization of inbred parental lines (Betrán *et al.*, 2003). Lines such as DT2 and DT7 had high GCA for GY. High GCA indicates that an inbred line will contribute a high level of additive gene action to its hybrid combinations for the trait of interest. There were hybrid combinations that had high GCA for both parents in combination with high SCA such as DT7xUT8, DT2xUT2 and DT2xUT1. On the other hand, there were hybrids with one parent with high and the other with low GCA in combination with high SCA e.g. DT2xUT10, DT7xUT10, DT1xP\_CNTRL and DT7xUT5. Also, there were some hybrids with both parents having negative GCA but high SCA, for example, DT10xN\_CNTRL and DT3xUT10. Hybrid EH65 (with DT6 and UT10 as parent lines) had the lowest SCA. These results suggest that both additive and non-additive (dominance and epistatic) gene actions collectively contributed to grain yield performance depending on the combination of parents. Hallauer *et al.* (2010) stated that hybrid vigour is a manifestation of high SCA. Hybrid EH79 which manifested the highest SCA could have its parent lines classified into two distinct heterotic groups (HG), whereas parents of EH41 and EH65 could be classified into one HG because they were related. A heterotic group represents inbred lines that are genetically closely related and less related to those in other heterotic groups. Inbred lines from different heterotic groups are commonly crossed to optimise heterosis (Hallauer *et al.*, 2010). Clearly, the GCA and SCA values are important parameters in order to identify inbred lines for potential use as parents in hybrid combinations.



## Narrow sense heritability estimates

In this study, some traits were highly heritable whereas others were not. Traits with low  $h^2$  indicate the extent of the effect of environmental factors on their phenotypic variation. In this study,  $h^2$  for secondary traits such as rows ear<sup>-1</sup>, 1000 kernel mass, DTA, ASI, PH, and ear diameter were greater than  $h^2$  for GY in each of the environments. Heritability estimates for HD cover in each of the four environments were the lowest (<0.20), suggesting a greater influence of environmental effects on this trait. Rows ear<sup>-1</sup> had the highest  $h^2$  in each environment. This is in agreement with studies by Hallauer *et al.* (2010). This was followed by GM, PH, EH and 1000 kernel mass with  $h^2$  ranging from 0.75 to 0.95. High  $h^2$  indicates that these traits were largely under additive gene control and that environmental factors did not greatly affect their phenotypic variation. It is important to note that this study was carried out under mild drought stress conditions and therefore the results cannot be extrapolated to high stress conditions.

## Relationships between secondary traits and yield

Nearly all breeding programmes use GY as the main selection criterion and as a primary trait indicative of hybrid performance in maize. In addition to GY, secondary traits are also widely used. Plants possess traits that are both positively and negatively associated with each other (Yan and Wallace, 1995). According to Barker *et al.* (2005), the ease of measurement of secondary traits alone does not justify their use. The author suggested that, “*useful traits are those that are correlated with yield under stress, cheap and fast to measure, highly heritable, stable in expression and not associated with yield loss under unstressed conditions*”. In most studies, the main secondary traits that fit this criterion under drought are EPP, ASI, and LSEN. In this study, under LP conditions ASI, DTA, LSEN, EPP, OT, SP and GM were also found to be important as the traits were significantly correlated with GY. Under HP conditions, DTA, SP, GLS and GM were more important. To increase GY, breeders can select for hybrids with shorter ASI, extended stay-green, prolificacy and ears that fill to the tip. In this study, open tips may have been caused by kernel abortion and poor kernel set as reported by other authors (Frey, 1981; Blum, 1996; Edmeades *et al.*, 2000). Bassetti and Wesgate (1994) and Edmeades (2013) described a progressive loss of kernels starting from the tip to the base as one of the major phenotypic expressions of the effects of drought stress. As was observed, ears with OT are likely to have low shelling percentage. If drought stress caused OT, then hybrids that are tolerant to drought should express relatively normal tip-fill under drought conditions.

Under both LP and HP the correlation between DTA and GY was positive and significant ( $P < 0.05$ ). The results obtained here suggest the greater importance of DTA over ASI in trial evaluations. Bolaños and Edmeades (1996) observed that late maturing maize hybrids with shorter ASI produced higher yield under drought stress than hybrids that were early maturing but with longer ASI. The positive correlation between DTA and GM at harvest under both LP ( $r = 0.32$ ) and HP ( $r = 0.78$ ) indicates that the traits are both measures of maturity in maize. The wide range of adaptation of maize hybrids to latitude and elevation suggests that maturity has largely been exploited in maize breeding programmes. This is certainly because the trait is highly heritable (Bänziger *et al.*, 2000) and therefore relatively easy to modify.

The significant correlation between ASI and GY ( $r = 0.11$ ; Table 2.15) under LP agrees with studies by CIMMYT and many others such as Edmeades *et al.* (1999) who reported that selection based on GY and reduced ASI resulted in significant gains of up to  $144 \text{ kg ha}^{-1} \text{ year}^{-1}$  under drought stress. Under HP, however, the correlation was weak and non-significant. Perhaps, it was because all emerging silks were sufficiently pollinated by neighbouring hybrids in the same experiment under HP conditions. Since drought stress delays days to silking (Bänziger *et al.*, 2000), the impact of large ASI would only be noticeable if some hybrids produce silks outside of the general period bracketing pollen shed for the hybrids tested. The interaction between drought stress and ASI could only be properly evaluated in a hybrid grown in an isolation plot where it is the only source of pollen. However, when a trial is composed of a range of hybrids, flowering at different times from day of planting, the pollination period will be extended especially under HP. Monneveux *et al.* (2008) highlighted the need to explore other secondary traits apart from ASI. This is in agreement with a report by Edmeades (2013) which stated that there is evidence that ASI has been improved in modern hybrids to a level where its correlation with GY has stabilised.

The positive relationship between LSEN and GLS ( $r = 0.69$ ) under LP indicated that disease pressure could have intensified LSEN. This suggests that under drought stress conditions, the visual separation of LSEN due to drought stress or GLS may not be easy. A study on turf grass (*Stenotaphrum secundatum*) showed that abiotic stresses such as drought can have effects that mimic GLS (Vann, 2014). This is because GLS lesions are brown and can coalesce with the browning caused by drought stress. The interaction between these stress factors caused a rapid decline in chlorophyll content as shown by a gradual change in leaf greenness from pre-to post-flowering period. The rate of LSEN under drought was reported to be accelerated during the grain filling period (Borras *et al.*, 2003). Yet, maintaining an active green leaf area plays a role in reducing the effects of drought during grain filling by keeping a higher photosynthetic rate (Edmeades, 2013).

## 2.5 Conclusion

Due to the genetic diversity of the parent lines from CIMMYT and PANNAR, the  $F_1$  single cross hybrids were significantly different based on yield and secondary traits performance. The top yielding hybrids at each environment were EH79 (Devonia and Cedara), EH29 (Greytown) and EH56 (ART). Hybrid EH79 was top yielding across all environments. These hybrids can be recommended for future testing across more environments.

The determination of the GCA and SCA effects of the inbreds revealed inbred lines that could be used as potential parents for commercial hybrid development. Lines DT2, DT7, UT8, UT1 and UT4 had the highest GCA effects. The first and second highest yielding hybrids (EH79 and EH18) had inbred lines with high GCA. The high correlation between SCA and yield for each hybrid and the higher variance component of SCA relative to that of GCA indicated the relative importance of SCA over GCA for GY. Inbred lines DT2, DT7, UT8 and UT6 could be used as sources of drought tolerance in crosses or backcrosses. The crosses with high SCA can either be evaluated for release as commercial hybrids or as parents of 3-way and double cross hybrids. Techniques such as marker assisted selection could be implemented to identify markers associated with non-additive gene action. However, the complexity of the drought tolerance traits could make the exercise fruitless.

The relatively high heritability at some environments for yield meant that significant progress can be achieved by selecting  $F_1$  hybrids based on yield alone. However, traits such as DTA, EPP, GM and 1000 kernel mass were more positively and significantly correlated with grain yield than other traits and had higher heritability estimates. These traits can be used to facilitate selection of better hybrids under drought conditions.

Hybrids that had longer growing periods have more time for grain filling compared to early hybrids. These had higher mean yields than the shorter season hybrids. Selection should therefore be made within maturity groups so that early hybrids may be identified for target environments with short growing seasons.

Using regression coefficients,  $b_i$  EH76, EH14, EH69, EH54, EH22, EH68, EH34, EH65, EH77, EH29, EH19, EH18, EH71, EH49, EH17, and EH2 were more stable and performed above the grand mean. Based on  $P_i$ , EH79 and EH18 were the most stable hybrids. The AMMI biplots were used to study the interactions between hybrids and the environments in which they were tested. Hybrids EH24, EH4, EH50, EH71, CH7, EH19, EH42, CH2, EH37 and EH54 were more stable as they had the lowest IPCA1 scores. AMMI biplots provided a better visualisation of hybrid-environment interaction than did  $P_i$  and  $b_i$ .

Future work should focus on assessing repeatability of performance of inbred lines and hybrids under random and managed drought conditions over a number of season and geographical locations. The information will be useful in the classification of the hybrids for either general or specific adaptation.

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## Appendices 2

### Appendix 2.1 The 7x10 mating design with DT lines as females, 10 UT lines as males and the two control lines P\_CNTRL and N\_CNTRL

		Male lines									
		UT1	UT2	UT3	UT4	UT5	UT6	UT7	UT8	P_CNTRL	N_CNTRL
Female lines	DT1	DT1xUT1	DT1xUT2	DT1xUT3	DT1xUT4	DT1xUT5	DT1xUT6	DT1xUT7	DT1xUT8	DT1xP_CNTRL	DT1xN_CNTRL
	DT2	DT2xUT1	DT2xUT2	DT2xUT3	DT2xUT4	DT2xUT5	DT2xUT6	DT2xUT7	DT2xUT8	DT2xP_CNTRL	DT2xN_CNTRL
	DT3	DT3xUT1	DT3xUT2	DT3xUT3	DT3xUT4	DT3xUT5	DT3xUT6	DT3xUT7	DT3xUT8	DT3xP_CNTRL	DT3xN_CNTRL
	DT4	DT4xUT1	DT4xUT2	DT4xUT3	DT4xUT4	DT4xUT5	DT4xUT6	DT4xUT7	DT4xUT8	DT4xP_CNTRL	DT4xN_CNTRL
	DT5	DT5xUT1	DT5xUT2	DT5xUT3	DT5xUT4	DT5xUT5	DT5xUT6	DT5xUT7	DT5xUT8	DT5xP_CNTRL	DT5xN_CNTRL
	DT6	DT6xUT1	DT6xUT2	DT6xUT3	DT6xUT4	DT6xUT5	DT6xUT6	DT6xUT7	DT6xUT8	DT6xP_CNTRL	DT6xN_CNTRL
	DT7	DT7xUT1	DT7xUT2	DT7xUT3	DT7xUT4	DT7xUT5	DT7xUT6	DT7xUT7	DT7xUT8	DT7xP_CNTRL	DT7xN_CNTRL

DT = Drought tolerant; UT = Unknown tolerance

### Appendix 2.2 Field layout of 80 hybrids, with each cell representing hybrids in one replication of a row x column design

Border										
Border	1	9	17	25	33	41	49	57	65	73
	2	10	18	26	34	42	50	58	66	74
	3	11	19	27	35	43	51	59	67	75
	4	12	20	28	36	44	52	60	68	76
	5	13	21	29	37	45	53	61	69	77
	6	14	22	30	38	46	54	62	70	78
	7	15	23	31	39	47	55	63	71	79
	8	16	24	32	40	48	56	64	72	80
Border										



**Appendix 2.3 Seventy single crosses (EH11-EH80) generated from the 7x10 mating design. The list excludes the 10 commercial hybrid checks**

Hybrid	Pedigree	Hybrid	Pedigree
EH11	DT1xUT1	EH46	DT2xP_CNTRL
EH12	DT1xUT2	EH47	DT3xUT3
EH13	DT1xUT3	EH48	DT8xUT3
EH14	DT1xUT5	EH49	DT10xUT2
EH15	DT1xUT6	EH50	DT3xN_CNTRL
EH16	DT2xUT3	EH51	DT10xUT5
EH17	DT1xP_CNTRL	EH52	DT9xUT5
EH18	DT2xUT2	EH53	DT2xUT5
EH19	DT2xUT1	EH54	DT3xUT6
EH20	DT2xUT6	EH55	DT8xUT2
EH21	DT3xUT8	EH56	DT3xUT5
EH22	DT3xP_CNTRL	EH57	DT10xUT4
EH23	DT8xUT1	EH58	DT9xUT6
EH24	DT8xUT6	EH59	DT2xUT10
EH25	DT7xUT1	EH60	DT2xUT8
EH26	DT7xUT3	EH61	DT1xUTN_CNTRL
EH27	DT7xUT6	EH62	DT1xUT10
EH28	DT7xN_CNTRL	EH63	DT3xUT10
EH29	DT10xUT8	EH64	DT1xUT4
EH30	DT10xUT3	EH65	DT10xUT6
EH31	DT10xN_CNTRL	EH66	DT7xUT10
EH32	DT2xN_CNTRL	EH67	DT8xN_CNTRL
EH33	DT9xUT1	EH68	DT7xUT4
EH34	DT9xUT2	EH69	DT9xN_CNTRL
EH35	DT9xUT4	EH70	DT2xUT4
EH36	DT9xUT5	EH71	DT7xP_CNTRL
EH37	DT8xUT8	EH72	DT8xUT5
EH38	DT9xUT7	EH73	DT8xUT4
EH39	DT9xUT10	EH74	DT10xUT1
EH40	DT9xP_CNTRL	EH75	DT3xUT2
EH41	DT10xUT5	EH76	DT7xUT5
EH42	DT10xP_CNTRL	EH77	DT3xUT1
EH43	DT1xUT8	EH78	DT8xN_CNTRL
EH44	DT3xUT4	EH79	DT7xUT8
EH45	DT7xUT2	EH80	DT8xUT4

EH =Experimental hybrid; DT = Drought tolerant; UT = Unknown tolerance

**Appendix 2.4 Bartlett's test for homogeneity of variances**

Source of variation	Df	Chi probability
Environment	3	0.08

## Chapter 3

### Evaluation of the drought tolerance of F<sub>1</sub> maize (*Zea mays* L.) hybrids under managed drought stress

#### Abstract

Drought stress significantly lowers the production of maize (*Zea mays* L.) grain in areas where there is no supplementary irrigation. The main objective of this study was to evaluate the drought tolerance of F<sub>1</sub> maize hybrids under managed drought stress (MDS). Three trials were conducted in three environments during the rain free winter season: water stressed (CHIS-WS) and well watered (CHIS-WW) at Chisumbanje and water stressed at Save Valley (SAVE-WS) experimental stations in Zimbabwe. The F<sub>1</sub> hybrids were developed by crossing inbred lines with known drought tolerance from CIMMYT and PANNAR with PANNAR elite inbred lines. A 12x12 factorial (North Carolina design II) mating design was used. One hundred F<sub>1</sub> experimental hybrids (EHs) were successfully produced from the expected 144. The EH including 10 commercial hybrid (CH) checks were evaluated in a 10x11 row-by-column design using single row plots of 4.4 m in length. Plant population density was 59 900 plants ha<sup>-1</sup>. The CHIS-WW trial was well watered from planting until maturity. The SAVE-WS and CHIS-WS trials were water stressed from about two weeks before flowering. At each environment there were significant differences ( $P < 0.05$ ) among the hybrids for grain yield (GY) and other traits. The CHIS-WS and SAVE-WS trials produced 33.7 and 58.1%, respectively, of the mean grain yield of the CHIS-WW environment. Change in hybrid rank order indicated the presence of crossover interactions between the hybrids and environments. Combined ANOVA revealed a significant ( $P < 0.05$ ) genotype x environment (GxE) interaction. The AMMI2 model of the additive main effects and multiplicative interactions (AMMI) analysis accounted for 100% of the GxE interaction sum of squares. The AMMI1 biplot indicated that EH24 combined both stability and high yield. Experimental hybrids EH24, EH61, EH46 and EH68 were classified as potentially drought tolerant hybrids and should be further evaluated for stability of drought tolerance over a greater range of drought stressed environments.

**Key words:** Drought tolerance, maize, hybrids, drought stress, water stress, GxE interactions, combining ability

**Terms used:** The term “hybrid” was used to refer to the 100 experimental hybrids and 10 commercial hybrid checks. In the discussion, the convention “genotype x environment” interaction was used instead of “hybrid x environment”. The term “inbred line” was used interchangeably with “inbreds”. The term “trial” refers to the 110 hybrids tested under well watered and water stressed conditions at Chisumbanje and water stressed conditions at Save Valley . An environment refers to each of the three trial conditions the hybrids were subjected to.

### 3.1 Introduction

Maize (*Zea mays* L.) is the staple food for more than 300 million people, many of whom suffer from hunger, malnutrition (Edmeades, 2013) and food insecurity. Where maize is the staple crop in dryland areas, food insecurity can often be attributable to drought stress that can reduce maize grain productivity by up to 50% (Boyer, 1982; Wang *et al.*, 2003; Moser, 2004; Bänziger *et al.*, 2006; Edmeades, 2008; Kumar *et al.*, 2008; Edmeades, 2013). The effect of drought is likely to be worsened in future by global climate change (Abraha and Savage, 2006; Lu *et al.*, 2011; Edmeades, 2013). This change, together with the food needs of the increasing human population is expected to exacerbate global food insecurity (Lu *et al.*, 2011). According to Edmeades (2013), the requirement for drought tolerant maize hybrids is thus particularly relevant in sub-Saharan Africa to meet an urgent need to boost yields in a region that relies predominantly on rainfall for maize production.

Drought is a multi-dimensional stress that induces a variety of crop responses (Blum, 1996). The crop responses to drought that are observed at the whole plant level are a result of intricate biochemical reactions at various stages of the plant's growth and development (Bänziger *et al.*, 2000). Summarising the effects of drought, Bänziger *et al.* (2000) stated: *"...drought can affect maize production by decreasing plant stand during the seedling stage, by decreasing leaf area development and photosynthesis rate during the pre-flowering period, by decreasing ear development during the two weeks bracketing flowering, and by decreasing photosynthesis and inducing early leaf senescence during grain-filling"*. Although all maize growth stages are susceptible to drought (Bänziger *et al.*, 2000), it has been reported that the crop is most susceptible during the flowering and grain filling period (Claassen and Shaw, 1970; Grant *et al.*, 1989; Schussler and Westgate, 1995; Chapman and Edmeades, 1999; Bänziger *et al.*, 2000; Magorokosho *et al.*, 2003; Barker *et al.*, 2005; Mhike *et al.*, 2012).

Owing to the complexity of the drought phenomenon and subsequent plant responses, scientific methods can only reasonably explore the effects of drought by investigating specific parts of the whole plant (Blum, 1996). According to Blum (1996), this resultant lack of holistic information about the effects of drought on maize leads to simplified and speculative conclusions and unfortunately this remains the case to this day. For example, the role of leaf rolling is not properly understood, with some authors claiming its positive role in yield determination while others suggest otherwise (Edmeades, 2013). Nonetheless studies on the various facets of drought stress have produced invaluable conclusions such as the role of abscisic acid (ABA) in controlling the plant's response to drought (Yang *et al.*, 2013). The main approach to developing drought tolerant genotypes is breeding

and testing of the hybrids under random drought stress (RDS) and/or managed drought stress (MDS) environments.

The occurrence of drought in all natural maize production environments is random, that is, largely unquantifiable and unpredictable throughout the growing seasons (Edmeades, 2008). Therefore, the difficulties of testing maize in RDS have significant implications for breeding methodologies. Under RDS, the timing of the drought cannot be properly quantified and controlled. As a result of a combination of stresses, breeders tend to unintentionally select for different stress mechanisms expressed in the experimental hybrids (Bänziger *et al.*, 2000). This challenge has led to the establishment of defined MDS environments by the International Maize and Wheat Improvement Centre (CIMMYT). The MDS environments are rain-free, where the timing, duration and intensity of drought can be carefully managed through controlled application of irrigation (Edmeades, 2008; Edmeades, 2013). Drought stress is generally induced on experimental hybrids either at flowering and/or at the grain filling stage. It has been reported that the use of MDS environments at CIMMYT has achieved significant yield gains (Edmeades, 2013).

Regardless of the testing environment, the identification of heritable genetic variation for tolerance to drought remains the first requirement for the successful development of drought tolerant hybrids (Edmeades, 2013). Blum (1988) hypothesized that there is indeed drought adaptive alleles in breeding populations. This was recently supported by Carena (2013) who concluded that unexploited genetic variability can be used for the development of drought tolerant maize hybrids.

The main objective of this study, therefore, was to develop  $F_1$  hybrids from drought tolerant donors and available elite commercial lines, and to evaluate the drought tolerance of  $F_1$  progeny under MDS.

The specific objectives were to:

- (i) determine the yield performance of  $F_1$  hybrids under well watered (WW) and water stressed (WS) conditions;
- (ii) calculate percentage yield reduction of the  $F_1$  hybrids under water stress (WS);
- (iii) determine GxE interactions, yield stability and adaptability of the hybrids selected; and
- (iv) estimate general and specific combining ability under WW and WS conditions.

## 3.2 Materials and methods

### 3.2.1 Germplasm

The experimental F<sub>1</sub> single-cross maize hybrids evaluated for drought tolerance in this study were developed during the summer season of 2012/13 using a 12x12 factorial (North Carolina II) mating design scheme (see Chapter 2). Only 100 F<sub>1</sub> single cross hybrids were successfully produced from the expected 144. The drought tolerance of the 100 experimental F<sub>1</sub> single-cross experimental hybrids (coded as EH) plus 10 commercial hybrid checks (coded as CH) were evaluated in three environments under MDS. Due to the large number of failed crosses, the combining ability analysis was conducted on 72 of the 100 experimental hybrids that constituted a complete 8x9 factorial mating design (Appendix 3.2).

### 3.2.2 Environment information

The F<sub>1</sub> hybrids were evaluated in two locations, one at Chisumbanje (20°46'12.3"S 32°13'43.5"E; 401 masl<sup>1</sup>) experimental station and the other at the Save Valley (20°32'32.1"S 32°05'01.5"E; 430 masl) experimental station in Zimbabwe, during the rain free winter season of 2013. Chisumbanje has montmorillonite 2:1<sup>2</sup> soils and received 86 mm of rainfall during the experiment period (from June to December 2013) in that season. The Save Valley has alluvial clay soil and received seasonal rainfall of 90 mm during the experiment period (from June to December 2013). The two locations fall under climatic region five of Zimbabwe's ecological zones<sup>3</sup> (Appendix 3.6). Region five receives annual and very erratic rainfall of <450 mm (FAO, 2006) per annum with average temperatures ranging from 19 to 29 degrees Celsius which is conducive for off-season maize production. The two environments at Chisumbanje were well watered (CHIS-WW) and water stressed (CHIS-WS) while the Save Valley environment was water stressed (SAVE-WS).

### 3.2.3 Management and water stress induction

General management practices were followed. Weeds were controlled by both herbicides and by hand-hoeing when necessary. For CHIS-WW environments, irrigation was scheduled from planting through to maturity as per CIMMYT's standard practice (Bänziger *et al.*, 2000) (incident rainfall recorded in Table 3.1). The management of irrigation for drought induction at CHIS-WS and SAVE-WS was done as described by Bänziger *et al.* (2000). Irrigation was withdrawn about two weeks before the first plant tasselled and 20 mm reapplied once, two weeks after 50% silking. Stress was induced to achieve a range in grain yield (GY) of 1-3 t

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<sup>1</sup> Metres above sea level

<sup>2</sup> Each layer consisting of 2 silica tetrahedral sheets around an aluminium octahedral sheet

<sup>3</sup> Zimbabwe is divided into five agro-ecological regions, known as natural regions, on the basis of soil, climate and vegetation regimes

ha<sup>-1</sup>. Such a stress level is reported to cause, among other results, a delay in silk formation which results in anthesis silking interval being extended by 4 to 8 days (Bänziger *et al.*, 2000). This level of stress can result in an average of 0.3 to 0.7 ears plant<sup>-1</sup>. Moisture probes (PR2 Profile Probe; Delta-T Devices) were installed at intervals across the field, from 0 to 200 cm deep, to quantify the percentage soil moisture (Appendix 3.3).

**Table 3.1 Weekly rainfall data for Chisumbanje and Save Valley research stations recorded from June-December 2013**

	Save valley (mm)	Chisumbanje (mm)
June	3	2
July	0	0
August	0	0
September	2	1
October	10	7
November	25	26
December	50	50
<b>Total</b>	<b>90</b>	<b>86</b>

### 3.2.4 Experimental design and data collection

The 110 F<sub>1</sub> hybrids were evaluated in a 10x11 row by column design with three replications at both locations (Appendix 3.1). The CHIS-WW and CHIS-WS trials were planted on 2 June 2013 whereas the SAVE-WS trial was planted on 3 June 2013. Inter- and intra-row spacing was 0.76 m and 0.22 m, respectively, providing a plant population of 59 900 plants ha<sup>-1</sup>. The plots were double rows, 4.4 m in length, with 40 plants per plot. Sprinkler irrigation was used at the SAVE-WS environment and flood irrigation at the CHIS environments. The CHIS-WW and CHIS-WS trials were planted 20 m apart from each other to limit lateral drainage. Anthesis and silking commenced 65 days after planting (DAP) at CHIS-WS and CHIS-WW environments and 67 DAP at SAVE-WS. Fifty percent anthesis and silking was recorded on 7 September 2013, 10 September 2013 and 15 September 2013 at CHIS-WS, CHIS-WW and Save-WW, respectively. Quantitative and qualitative data were collected (or derived) on selected agronomic traits (Table 3.2).

**Table 3.2 Agronomic traits measured or derived from the winter trials conducted in Chisumbanje and Save Valley in Zimbabwe in 2013**

Traits measured	Description
Grain yield (GY)	Mass of shelled grain converted to t ha <sup>-1</sup> at 12.5% grain moisture content (see Chapter 2)
Days to anthesis (DTA)	Number of days from planting to 50% of plants per plot shedding pollen
Silking date (SD)	Number of days from planting to 50% of plants per plot with silks about 2 cm long
Anthesis silking interval (ASI)	SD minus AD
Ears per plant (EPP)	Number of ears with at least one grain per plant, divided by the total number of plants per plot
Leaf senescence (LSEN)	Scored on a scale from 1 (green leaves) to 9 (dead leaves with no green tissue)
SPAD	Measured chlorophyll content using SPAD <sup>1</sup> 502 chlorophyll meter (in SPAD units)
Plant height (cm) (PH)	Distance between the base of a plant to the top of insertion of the tassel
Ear height (cm) (EH)	Distance between the base of the plant to the point of insertion of the lowest primary ear
Ear position (EP)	Ratio of EH to PH
Lodge percentage (LP)	Percentage of lodged plants per plot
Shelling percentage (SP)	Shelled grain mass divided by gross mass x 100%

### 3.2.5 Data analysis

All data was analysed in GenStat 16<sup>th</sup> edition. Analysis of variance was conducted using data from the individual locations. The REML procedure was used for the combined analysis across environments. Combining ability estimates, heritability estimates, percentage yield difference, Spearman's rank and standard correlation coefficients and AMMI were obtained as described in Chapter 2. Statistical significance was determined at 5% level. Homogeneity of variances was tested using the Bartlett's test (Bartlett, 1937) as described in Chapter 2 (Appendix 3.4). The stability and adaptation of hybrids were quantified and characterised using AMMI biplots of the relevant AMMI models.

## 3.3 Results

### 3.3.1 Yield performance at each environment

The ANOVA of each of the three environments under MDS revealed that the 110 hybrids were significantly ( $P < 0.05$ ) different for grain (GY), ears per plant (EPP), days to anthesis (DTA) and anthesis silking interval (ASI) (Table 3.3, 3.4 and 3.5). The CHIS-WW and SAVE-

<sup>1</sup> Technique quantifies the health of your crops by measuring chlorophyll content



WS environments had the highest mean yields of 4.18 t ha<sup>-1</sup> and 2.43 t ha<sup>-1</sup>, respectively. The coefficient of variation (CV) ranged from 13.14 (CHIS-WW) to 26.60% (CHIS-WS).

**Table 3.3 Mean squares of four traits at the well watered environment in Chisumbanje**

Source of variation	d.f.	GY	EPP	DTA	ASI
Rep	2	0.49*	0.23	29.46*	1.48
Genotype	109	4.50*	0.11*	72.64*	25.72*
Error	218	0.40	0.02	5.10	10.46
Total	329	3.10	0.05	60.68	14.20

Rep = Replications; GY = Grain yield; EPP = Ears per plant; DTA = Days to anthesis; ASI = Anthesis silking interval; \*significant at 5% significant level

**Table 3.4 Mean squares of four traits at the water stress environment in Chisumbanje**

Source of variation	d.f.	GY	EPP	DTA	ASI
Rep	2	0.81*	0.03	15.24	6.07*
Genotype	109	10.08*	0.19*	30.14*	20.34*
Error	218	0.13	0.07	4.63	3.24
Total	329	2.14	0.08	46.80	22.17

Rep = Replications; GY = Grain yield; EPP = Ears per plant; DTA = Days to anthesis; ASI = Anthesis silking interval; \*significant at 5% significant level

**Table 3.5 Mean squares of four traits at the water stress environment in Save Valley**

Source of variation	d.f.	GY	EPP	DTA	ASI
Rep	2	1.40*	2.15	3.21	8.74*
Genotype	109*	8.24*	0.01*	2.42*	14.35*
Error	218	0.10	0.32	1.75	2.46
Total	329	1.98	0.17	3.25	2.23

Rep = Replications; GY = Grain yield; EPP = Ears per plant; DTA = Days to anthesis; ASI = Anthesis silking interval; \*significant at 5% significant level

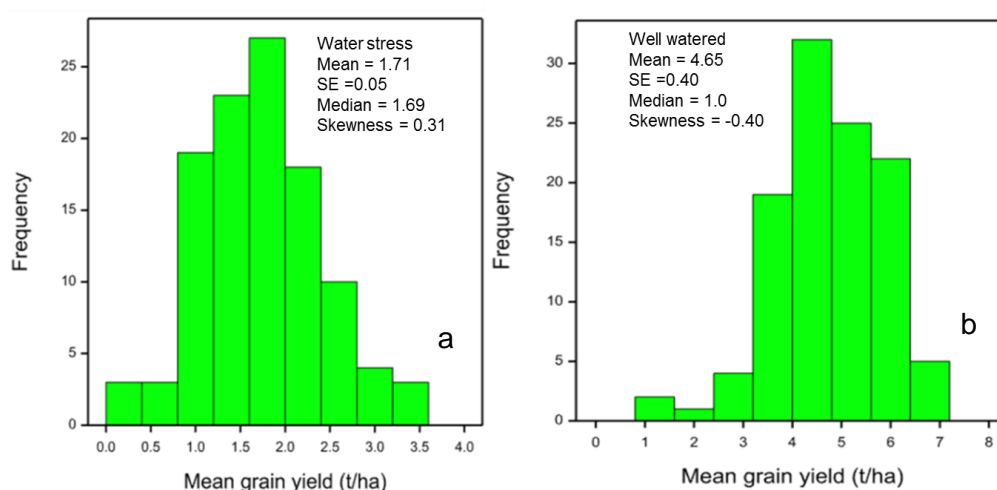
**Table 3.6 Summary statistics for GY (t ha<sup>-1</sup>) at each of the three environments**

	CHIS-WS	CHIS-WW	SAVE-WS
Mean	1.41 (t ha <sup>-1</sup> )	4.18 (t ha <sup>-1</sup> )	2.43 (t ha <sup>-1</sup> )
Min	0.01	1.00	0.22
Max	3.32	6.81	4.28
CV%	26.60	13.16	19.50
SE	0.37	0.54	0.40
P-value	0.00	0.00	0.00

SE = Standard error; Min = Minimum; Max = Maximum, CV% = Coefficient of variation percentage; P-value = F test from ANOVA; CHIS-WW = Chisumbanje well watered; CHIS-WS = Chisumbanje water stress; SAVE-WS = Save water stress

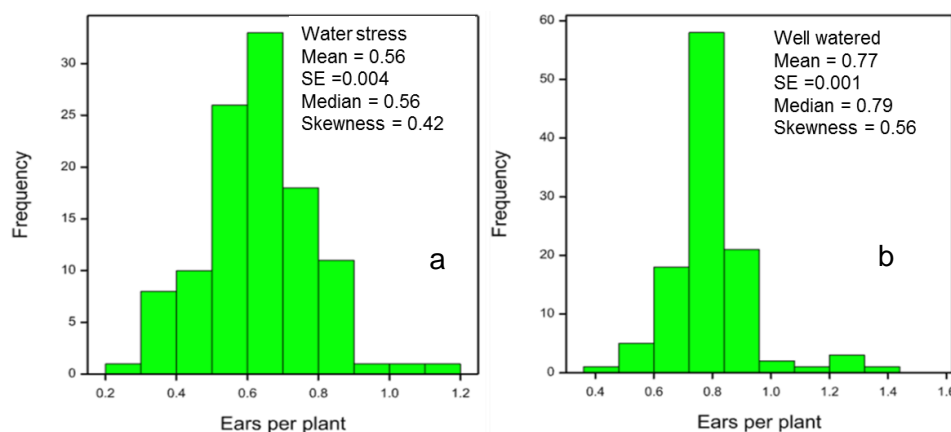
### 3.3.2 Frequency distributions for selected traits under water stress and well watered conditions

The data for the two water stress environments (CHIS-WS and SAVE-WS) were averaged as their error variances were homogenous based on the Bartlett's test (Appendix 3.4). The distribution for GY under WS conditions was positively skewed whereas under WW, it was negatively skewed (Fig. 3.1). The distributions under WS and WW had low ( $1.71 \text{ t ha}^{-1}$ ) and high ( $4.65 \text{ t ha}^{-1}$ ) mean yields, respectively. Forty nine hybrids had yields of between  $1.5\text{-}2.0 \text{ t ha}^{-1}$  under WS. Under WW, 85 hybrids had yields between  $4\text{-}6 \text{ t ha}^{-1}$  with four hybrids yielding above  $6 \text{ t ha}^{-1}$ .



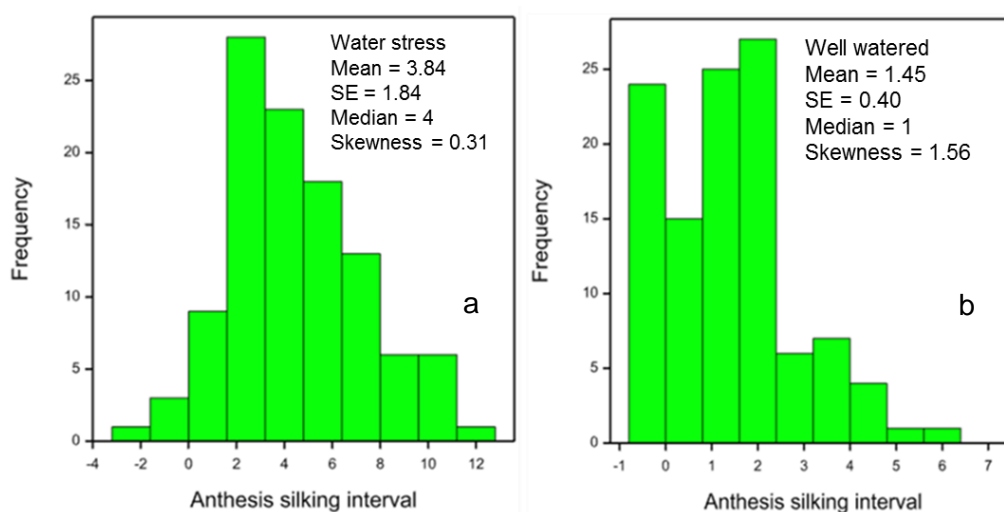
**Fig. 3.1** Frequency distributions of grain yield ( $\text{t ha}^{-1}$ ) of 110 F<sub>1</sub> hybrids evaluated under (a.) water stressed (mean of Chisumbanje-WS and Save Valley WS) and (b.) well watered (Chisumbanje-WW) conditions

Under WS, ears per plant (EPP) were positively skewed under both WS and WW conditions. Thirty eight hybrids produced between  $0.6\text{-}0.7$  EPP under WS whereas WW conditions 58 hybrids had  $0.7\text{-}0.9$  EPP (Fig. 3.5). There were more EPP recorded under WW ( $0.77$ ) than WS ( $0.56$ ) under water stress conditions (Fig. 3.2).



**Fig. 3.2** Frequency distributions of ears per plant of 110 F<sub>1</sub> hybrids evaluated under (a.) water stressed (mean of Chisumbanje-WS and Save Valley WS) and (b.) well watered (Chisumbanje-WW) conditions

Water stress appeared to have had a marked effect on anthesis-silking interval (ASI). Under WS, ASI values ranged from -2 to 12 days whereas it only ranged from -1 to 6 days under WW conditions. Anthesis silking interval was positively skewed under both WW (1.56) and WS (0.31) conditions. Average ASI under WS was 3.84, higher than under WW (1.45) conditions (Fig. 3.3).



**Fig. 3.3** Frequency distributions of anthesis-silking interval (t ha<sup>-1</sup>) of 110 F<sub>1</sub> hybrids evaluated under (a.) water stressed (mean of Chisumbanje-WS and Save Valley WS) and (b.) well watered (Chisumbanje-WW) conditions

### 3.3.3 Rank order of hybrids for grain yield

Hybrids ranked inconsistently across environments (Table 3.7) for GY as confirmed by the negative Spearman's rank correlation coefficients between the rank orders of the three environments (Table 3.8). However, EH24 was the exception in terms of inconsistent ranking, appearing in the top five in all three environments. EH46 appeared in the top three in the CHIS-WS and CHIS-WW environments only. EH24 could be regarded as stable and

generally adapted because its yields were consistently high under both WW and WS conditions. On the other hand, hybrids such as EH46, EH68 and EH61 with high yields in CHIS-WS, CHIS-WW and SAVE-WS, respectively, were specifically adapted to these environments.

**Table 3.7 Rank order of the top 20 hybrids for grain yield ( $\text{t ha}^{-1}$ ) at each of the three managed random drought stress environments**

RANK	CHIS-WS	CHIS-WW	SAVE-WS
1	EH46	EH68	EH61
2	EH16	EH24	EH24
3	EH100	EH46	EH48
4	EH44	EH17	EH26
5	EH24	EH12	EH38
6	EH48	EH105	EH77
7	EH93	EH82	EH72
8	EH85	EH65	EH18
9	EH18	EH61	EH93
10	EH80	EH15	EH19
11	EH20	EH79	EH53
12	EH38	EH11	EH65
13	EH14	EH34	CH5
14	EH19	EH19	EH98
15	EH17	EH39	EH12
16	EH61	EH14	EH74
17	CH2	EH70	EH11
18	EH75	EH100	EH66
19	EH53	EH18	EH80
20	EH106	EH72	EH47

EH = Experimental hybrid; CH = Commercial hybrid checks; CHIS-WS = Chisumbanje water stress; CHIS-WW = Chisumbanje well watered and SAVE-WS = Save water stress

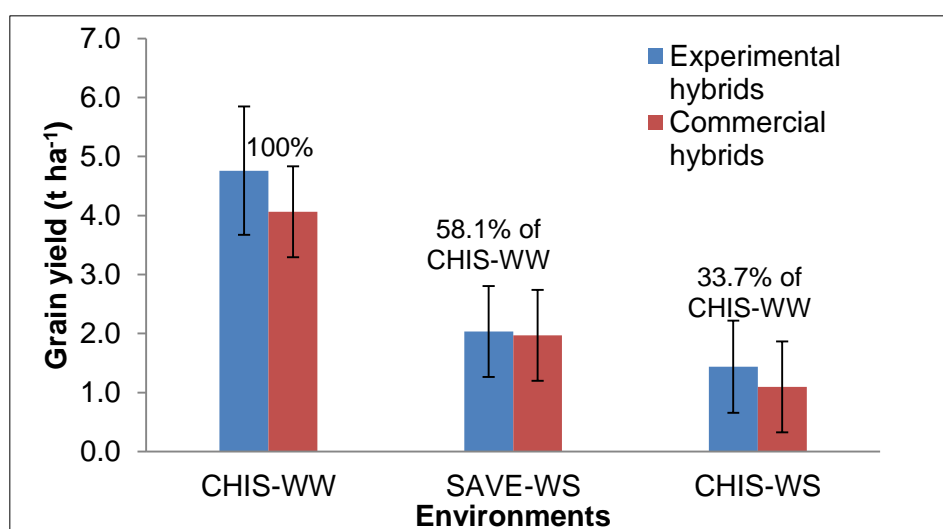
**Table 3.8 Spearman's rank correlation coefficients between the three managed drought stress environments for grain yield ( $\text{t ha}^{-1}$ )**

	CHIS-WS	CHIS-WW	SAVE-WS
CHIS-WS	-	-0.013	-0.010
CHIS-WW		-	-0.020
SAVE-WS			-

CHIS-WS = Chisumbanje water stress; CHIS-WW = Chisumbanje well watered and SAVE-WS = Save water stress

### 3.3.4 Yield under water stress relative to well watered conditions

The performance of each hybrid under WW conditions (i.e. CHIS-WW) was regarded as a measure of a hybrid's yield potential<sup>1</sup> (HYP), whereas the mean performance of all the hybrids in each environment was regarded as each trial's yield potential (TYP). Based on mean performance for GY, CHIS-WS and SAVE-WS, achieved 33.7% (1.41 t ha<sup>-1</sup>) and 58.1% (2.43 t ha<sup>-1</sup>), respectively, of the yield achieved under WW conditions (4.18 t ha<sup>-1</sup>). Based on mean yield, CHIS-WS was the most stressed environment and its CV (26.60%) was higher than that of SAVE-WS (19.50%) which had the second highest mean yield. The CHIS-WW environment had a CV of 13.14% (Fig. 3.4).



**Fig. 3.4 Mean yields of the two water stressed (WS) environments relative to mean yield of the well watered (WW) environment. The relative yield percentages indicates mean of both experimental hybrids and commercial checks**

The mean yield of each of the experimental and commercial hybrids under the WW conditions of CHIS-WW represented the HYP under the optimal conditions established in this set of environments ( $HYP=Y_{WW}$ ), whereas their mean yield across CHIS-WS and SAVE-WS represented their HYP under the water stress conditions established in this set of environments ( $HYP=Y_{WS}$ ). The rank order of the top 20 hybrids was determined for  $Y_{WS}$  expressed as a percentage of  $Y_{WW}$  (Table 3.9). Only EH53 and EH38 had a  $Y_{WS}/Y_{WW}$  greater than 70%. Hybrids with  $Y_{WS}/Y_{WW}$  between 60-70% were EH48, EH92, EH53 and EH38. Of the 110 hybrids tested, EH106 had  $Y_{WS}/Y_{WW}$  below 60%. The rank order of the hybrids based on  $Y_{WS}$  was not consistent with the rank order based on  $Y_{WW}$ . This is indicated by the very weak and insignificant Spearman's rank correlation coefficient ( $r=0.08$ ) between  $Y_{WS}$  and  $Y_{WW}$  ( $P>0.05$ ) (Appendix 3.5). This indicated that the yield potential of a hybrid under WW does not predict the hybrid's ability under WS conditions to realise a high proportion of

<sup>1</sup> All other factors were assumed optimum and therefore yield reduction relative to HYP was only assumed to be due to water stress.

its HYP under WW conditions, that is,  $Y_{ww}$  for a hybrid does not reliably predict its  $Y_{ws}/Y_{ww}$  relative to the other hybrids under evaluation and vice versa. For example, hybrid EH38 produced 3.52 and 3.01 t ha<sup>-1</sup> under WW and WS conditions, respectively and even though it realised the highest percentage of its  $Y_{ww}$  under WS conditions (85.62%), it ranked a lowly 98 under WW conditions. Conversely, EH24 with a  $Y_{ww}$  of 6.72 t ha<sup>-1</sup> ranked second under WW conditions but with a yield of 3.50 t ha<sup>-1</sup> realised only 52.09% of  $Y_{ww}$  under water stress. Hybrids with a high  $Y_{ws}/Y_{ww}$  are considered stable across non-stress and stress conditions because they maintain similar yields under both conditions. On an absolute basis, EH24 would be regarded as superior to EH38 under both WW and WS conditions, but unstable (due to lower  $Y_{ws}/Y_{ww}$ ) when compared to EH38.

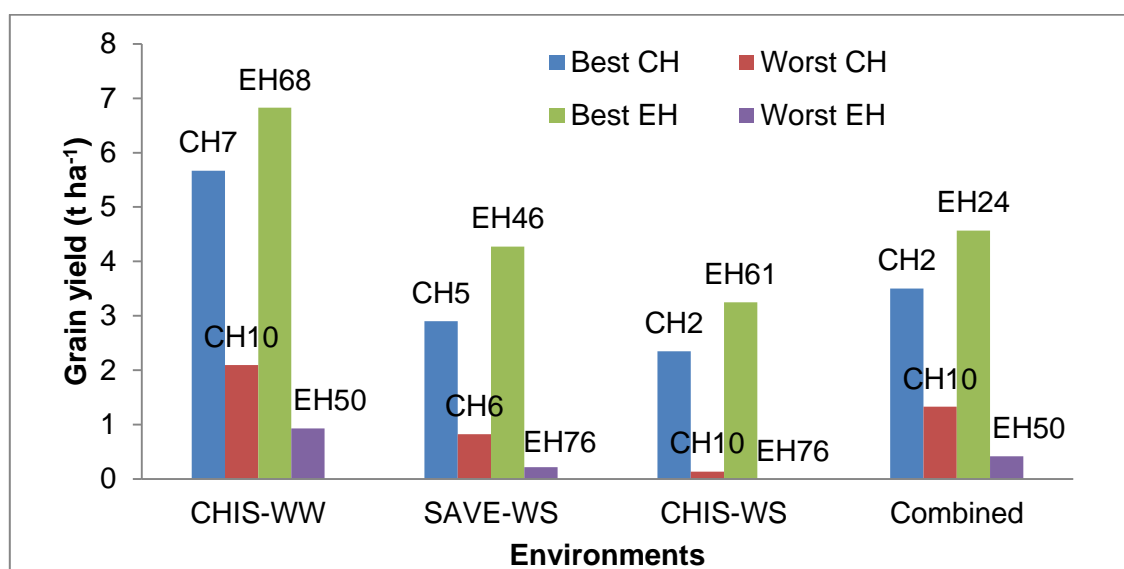
**Table 3.9 Rank orders of top 20 experimental hybrids for percentage grain yield under water stressed conditions relative to well watered conditions. Hybrid ranking under well watered conditions in parenthesis**

Entry	WW	WS	( $Y_{ws}/Y_{ww}$ ) *100	Rank order ( $Y_{ws}/Y_{ww}$ )x100
EH38	3.52(98)	3.01	85.62	1
EH53	3.70(93)	2.59	70.02	2
EH92	3.46(100)	2.20	63.62	3
EH48	5.44(32)	3.30	60.73	4
EH93	5.11(40)	3.02	59.18	5
EH45	3.74(92)	2.08	55.70	6
EH66	3.78(91)	2.10	55.57	7
EH98	4.34(66)	2.41	55.55	8
EH95	4.05(81)	2.22	54.82	9
EH61	6.28(8)	3.32	52.81	10
EH24	6.72(2)	3.50	52.09	11
CH5	3.84(88)	1.93	50.23	12
EH18	5.86(21)	2.94	50.09	13
EH44	4.63(57)	2.28	49.37	14
EH59	3.11(105)	1.53	49.34	15
EH77	5.38(33)	2.63	48.89	16
CH10	1.97(108)	0.96	48.48	17
EH22	3.57(97)	1.71	47.77	18
EH96	4.15(75)	1.98	47.60	19
EH106	4.93(46)	2.32	47.11	20

EH = Experimental hybrids; CH = Commercial hybrids; WS = Water stressed (mean yield across CHIS-WS and SAVE-WS); WW = Well watered

The highest yielding experimental hybrids under CHIS-WW, SAVE-WS and CHIS-WS were EH68 (6.82 t ha<sup>-1</sup>), which was also the highest yield for all the hybrids in the three environments), EH61 (4.28 t ha<sup>-1</sup>) and EH46 (3.25 ha<sup>-1</sup>), respectively. Hybrid EH76 had the lowest yield at both CHIS-WW (0.93 t ha<sup>-1</sup>) and CHIS-WS (0.00 t ha<sup>-1</sup>), which was also the

lowest yield for all the hybrids in the three environments, while EH50 ( $0.22 \text{ t ha}^{-1}$ ) was the lowest yielder at SAVE-WS (Fig. 3.5). The top yielding commercial hybrids at CHIS-WW, SAVE-WS and CHIS-WS were CH7 ( $5.67 \text{ t ha}^{-1}$ ), CH5 ( $2.90 \text{ t ha}^{-1}$ ) and CH2 ( $2.35 \text{ t ha}^{-1}$ ), respectively. Commercial hybrid CH10, had the lowest yield at CHIS-WW ( $2.10 \text{ t ha}^{-1}$ ) and CHIS-WS ( $0.14 \text{ t ha}^{-1}$ ), whereas CH6 ( $0.83 \text{ t ha}^{-1}$ ) had the lowest yield at SAVE-WS (Fig. 3.5). On average across all three environments, EH24 ( $4.57 \text{ t ha}^{-1}$ ) was the top yielding experimental hybrid and EH76 ( $0.42 \text{ t ha}^{-1}$ ) was the lowest whereas CH2 ( $3.49 \text{ t ha}^{-1}$ ) was the top yielding the commercial hybrid and CH10 ( $1.33 \text{ t ha}^{-1}$ ) was the lowest. The mean of the EH was higher ( $4.57 \text{ t ha}^{-1}$ ) compared to the mean of CH ( $3.50 \text{ t ha}^{-1}$ ) across all environments. The best EH at each of the three environments performed above the best CH at each respective environment. Conversely, the worst EH at each of the three environments achieved yields below the worst CH (Table 3.10). Using  $\text{LSD}_{0.05}$ , there was no significant difference between the best performing EH and the best performing CH, but the difference between the worst EH and worst CH was significant ( $P < 0.05$ ).



**Fig. 3.5** Best experimental and commercial hybrids versus worst experimental and commercial hybrid checks at each environment and mean of the best hybrid across all three environments versus mean of the worst hybrid across three environments. CH = commercial hybrid; EH = experimental hybrid; CHIS-WW = Chisumbanje well watered; CHIS-WS = Chisumbanje water stress; SAVE-WS = Save water stress

**Table 3.10** Comparison of best experimental hybrids relative to commercial hybrids expressed as a percentage at each environment, mean of the two water stress environments and across the three environments

	CHIS-WS	CHIS-WW	SAVE-WS	WS	Combined
Best EH/Best CH	138.27	120.35	147.25	154.61	136.76
Worst EH/Best CH	0.0	0.44	0.27	0.0	0.0

EH = Experimental hybrids; CH = Commercial hybrids; CHIS-WS = Chisumbanje water stress; CHIS-WW = Chisumbanje well watered; SAVE-WS = Save water stress; WS = Water stress

### 3.3.4.1 Phenotypic correlations among traits for CHIS-WS and SAVE-WS

Data for the two water stressed environments was averaged as the variances were homogenous based on the Bartlett's test (Bartlett, 1937). Grain yield and EPP had the highest, positive ( $r=0.63$ ) and significant ( $P<0.05$ ) standard correlation coefficient among all the traits for the water stressed environments (Table 3.11). Days to anthesis ( $r=-0.27$ ) and ASI ( $r=-0.31$ ) were negatively and significantly ( $P<0.05$ ) correlated with GY. Anthesis silking interval was negatively ( $r=-0.22$ ) and significantly correlated with EPP. Shelling percentage and EPP were negatively ( $r = -0.16$ ) and significantly correlated. The NP were negatively ( $r=-0.46$ ) and significantly correlated to EPP. The correlation between PH and EP was negative ( $r=-0.21$ ) and significant. Leaf senescence (LSEN) was very weakly correlated with all traits except with LP ( $r=0.17$ ). Most of the other correlations between the traits were very weak and close to zero (Table 3.11).

**Table 3.11 Phenotypic correlation coefficients between measured and derived traits averaged across all experimental and commercial hybrids and across the two water stressed environments**

	GY	EPP	DTA	ASI	EP	PH	NP	LP	LSEN	SP
EPP	0.63*	-								
DTA	-0.27*	-0.19*	-							
ASI	-0.31*	-0.22*	0.06	-						
EP	0.01	-0.02	-0.04	-0.05	-					
PH	0.05	0.02	-0.03	-0.02	-0.21	-				
NP	-0.04	-0.46*	-0.01	0.05	0.04*	0.11	-			
LP	-0.07	-0.05	0.06	0.00	0.03	0.07	-0.03	-		
LSEN	-0.04	-0.06	0.08	0.12	0.02	0.00	0.10	0.17*	-	
SP	0.06	-0.16*	0.07	-0.05	-0.01	-0.09	-0.04	-0.13	0.00	-

GY= Grain yield; DTA = Days to anthesis; ASI = ears per plant; LSEN = Leaf senescence; SP = Shelling percentage; LP = Lodge percentage; PH = Plant height (cm)

### 3.3.4.2 Phenotypic correlations coefficients among traits under well watered conditions

Under the WW conditions of CHIS-WW (Table 3.12), the positive correlation ( $r=0.57$ ) between GY and EPP was less than under WS conditions, but remained significant ( $P<0.05$ ). The ASI was negatively ( $r=-0.28$ ) correlated with GY whereas DTA was positively ( $r=0.13$ ) correlated with GY. The correlation between ASI and DTA were positively ( $r=0.27$ ) and significantly correlated. This was higher than the correlation coefficient under WS conditions. The correlation between EPP and ASI went from  $r=-0.22$  under WS to  $r=-0.32$  under WW conditions ( $P<0.05$ ). The EPP were negatively ( $r=-0.18$ ) and significantly correlated with SP. This was close to what was observed under WS conditions. The NP was positively ( $r=0.45$ ) correlated with GY, but the correlation was not significant. Conversely, under WS conditions, the correlation was very weak and negative. The LSEN was negatively



( $r = -0.35$ ) and significantly correlated with GY, whereas under WS, it was very weak and not significant. The PH and EP were positively ( $r = 0.18$ ) and significantly correlated.

**Table 3.12 Phenotypic correlation coefficients between measured and derived traits averaged across all experimental and commercial hybrids under well watered conditions**

	GY	EPP	DTA	ASI	EP	PH	NP	LP	LSEN	SP
EPP	0.57*	-								
DTA	0.13*	-0.15*	-							
ASI	-0.28*	-0.32*	0.27*	-						
EP	0.10	-0.02	0.11	0.05	-					
PH	0.16*	0.02	0.11	0.04	0.18*	-				
NP	0.45*	0.01	-0.15*	-0.02	0.00	0.03	-			
LP	-0.07	-0.19*	0.04	0.12*	-0.01	0.14	0.27*	-		
LSEN	-0.35*	-0.10	-0.05	0.05	-0.06	0.01	-0.03	0.10	-	
SP	-0.04	-0.18*	0.09	0.20*	-0.14	-0.14	-0.23*	-0.11	-0.05	-

GY= Grain yield; DTA = Days to anthesis; ASI = ears per plant; LSEN = Leaf senescence, SP = shelling percentage; LP = Lodge percentage; PH = Plant height (cm)

### 3.3.5 Yield performance across environments

The REML analysis revealed the main effects of hybrids, environments and GxE interaction were significant for GY, DTA and ASI ( $P < 0.05$ ). The GxE interaction was not significant for EPP (Table 3.13).

**Table 3.13 WALD statistics for the REML analysis across the three managed drought stressed environments, CHIS-WW, CHIS-WS and SAVE-WS for four selected traits**

Source of variation	d.f.	GY	EPP	DTA	ASI
Rep	2	0.49	0.23*	29.46*	1.48
Hybrid	109	4.50*	0.11*	72.64*	25.72*
Environment	2	845.69*	6.91*	23177.15*	751.21*
Hybrid*Environment	218	1.00*	0.03	10.16*	12.18*
Error	656	0.40	0.02	5.10	10.46
Total	987	3.10	0.05	60.68	14.20

GY = grain yield, EPP = Ears per plant, DTA = days to anthesis, ASI = anthesis silking interval, Rep = replication

#### 3.3.5.1 AMMI analysis of GxE interaction for grain yield

The REML analysis (Table 3.14) indicated significant ( $P < 0.05$ ) GxE interaction for GY which was partitioned using the AMMI ANOVA. In the AMMI ANOVA of the 110 hybrids across the three environments for GY, hybrids, environments and GxE interaction effects were significant ( $P < 0.05$ ; Table 3.14). Hybrids sum of squares (SS) accounted for 18.78% of the Treatment SS, whereas Environments and GxE SS accounted for 71.92% and 9.30%, respectively. The Treatment SS (i.e. Hybrids + Environments + GxE SS) accounted for

93.1% of the Total SS. The environment effects were the predominant source of variation followed by hybrids and GxE. The AMMI2 model explained 100% of the GxE SS with the first principal component axis (IPCA1) accounting for 67.7% of the GxE SS and the second principal component axis (IPCA2) explained 32.3%.

**Table 3.14 AMMI ANOVA for grain yield (t ha<sup>-1</sup>) of 110 hybrids evaluated in three managed drought stress environments during winter season in Zimbabwe**

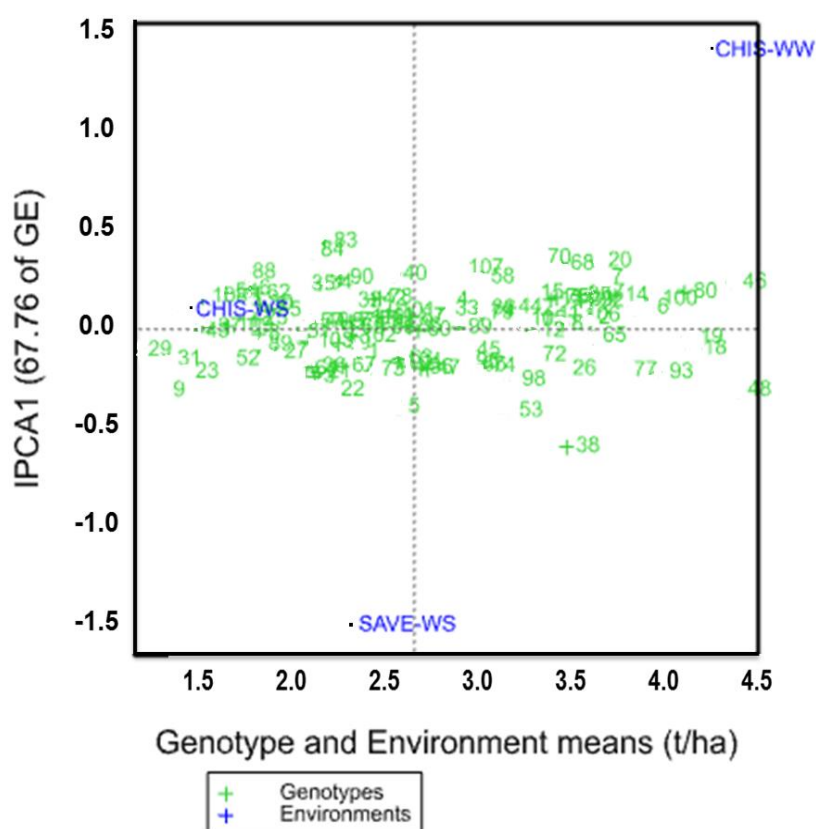
Source	Df	SS	MS	F	F_prob	%SS
Total	842	2998.80	3.56	*	*	
Block	9	9.90	1.10	4.24	0.00	0.33
Treatments	329	2792.50	8.50	21.78	0.00	0.93
Genotypes	109	524.30	4.80	12.34	0.00	18.78
Environments	2	2008.60	1004.30	607.51	0.00	0.72
Interactions	218	259.70	1.20	3.06	0.00	9.30
IPCA1	110	175.90	1.60	4.10	0.00	67.73
IPCA2	108	83.70	0.80	1.99	0.00	32.23
Error	504	196.40	0.40	*	*	

DF = Degrees of freedom; SS = Sum of squares; MS = Mean squares; GxE = Genotype x environment; IPCA = Interaction principal component axis. The block source of variation refers to blocks within environments ignoring rows and columns

### 3.3.5.2 AMMI biplot analysis

The hybrids and environments displayed both negative and positive IPCA1 and IPCA2 scores. In the AMMI1 biplot, the two WS environments, CHIS-WS and SAVE-WS performed below the grand mean with positive and negative IPCA1 scores, respectively while the WW environment, CHIS-WW performed well above the grand mean and had a positive IPCA1 score (Fig. 3.6). The mean performances of the three environments together with their IPCA1 scores of -1.54 for SAVE-WS, +0.25 for CHIS-WS and +1.40 for CHIS-WW resulted in the environments appearing in three different quadrants of the biplot indicating that they discriminated differently between the hybrids. Hybrids that had the same IPCA1 sign as an environment interacted strongly and positively with the environment whereas hybrids with opposite signage to an environment interacted negatively with it. The higher the IPCA1 score the greater the magnitude of the interaction (e.g. EH38 with SAVE-WS). Hybrids with IPCA1 scores close to zero (e.g. EH24, EH99, EH61 and EH18) presented low differential response to changes in the environment and were regarded as generally adapted. Environments CHIS-WW and SAVE-WS had the largest IPCA1 scores of the three environments but with positive and negative signage, respectively. Environment CHIS-WS had an IPCA1 score close to zero and therefore had a lower interaction with the hybrids compared to the other two environments. Hybrids 46 and 48 were the highest yielding with a positive and negative IPCA1 signage (Fig. 3.6). Overall, the hybrids were distributed in a narrow band between

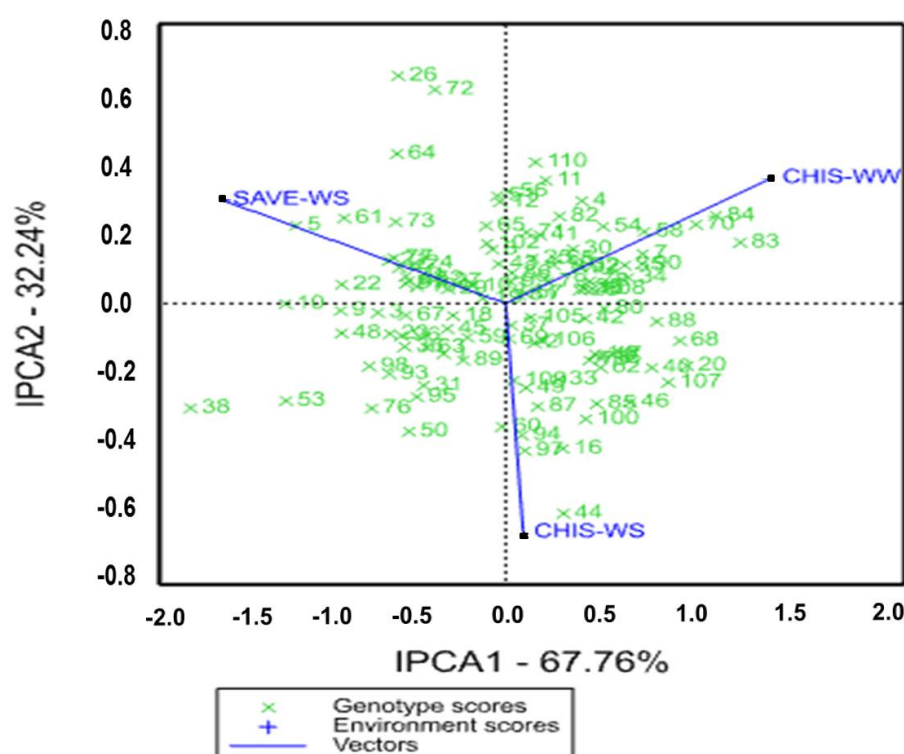
positive and negative IPCA scores but differed considerably between the lowest and highest performing hybrid in mean yield.



**Fig. 3.6 AMMI1 biplot of 110 F<sub>1</sub> single cross maize hybrids evaluated in three environments for grain yield**

In the AMMI2 biplot (Fig. 3.7), the vectors from the origin to the coordinates of the environments are presented. The angle formed between the vectors of any two environments provides an indication of the extent of the correlation between their rank ordering of the hybrids. The angles between the three environments were almost identical and all of them larger than 90 degrees indicating very weak correlations between environments in their rank ordering of the hybrids. CHIS-WS had the shortest vector followed by SAVE-WS whereas CHIS-WW had the longest vector; the length of the vector indicative of the extent of the interaction between the hybrids and the respective environments. Hybrids in the same quadrant as an environment interacted strongly with that environment. The extent of the interaction is indicated by the length of the perpendicular drop down vector from the hybrid coordinate to the environmental vector. Hybrids EH84, EH83 and EH88 positively interacted with CHIS-WW, whereas EH44, EH97 and EH16 positively interacted with CHIS-WS. Environment SAVE-WS interacted strongly with EH61 and EH5. Hybrids such as EH105, EH18, EH35 and EH37 that were close to the origin were non-sensitive to

the environmental factors driving IPCA1 and IPCA2, EH26 was more responsive as they were further from the origin.



**Fig. 3.7 AMMI2 biplot of 110 F<sub>1</sub> single cross maize evaluated in three environments for grain yield (t ha<sup>-1</sup>)**

The top hybrid (based on the GenStat 16 AMMI procedure) for each environment were EH68, EH46 and EH61 in CHIS-WW, CHIS-WS and CHIS-WS, respectively. Only EH24 and EH46 were listed among the top four in two of the three environments (Table 3.15).

**Table 3.15 Ranking of the first four AMMI selections of 110 hybrids (commercial and experimental) evaluated in water stressed and well watered environments**

			Top four hybrids			
Environment IPCA1						
Environments	Mean (t ha <sup>-1</sup> )	Score	1	2	3	4
CHIS-WW	4.18	1.54	EH68	EH24	EH46	EH17
CHIS-WS	1.41	0.25	EH46	EH16	EH100	EH44
SAVE-WS	2.43	-1.40	EH61	EH24	EH48	EH26

EH = Experimental hybrids; CH = Commercial hybrids; CHIS-WS = Chisumbanje water stress; CHIS-WW = Chisumbanje well watered; WS = Water stress; SAVE-WS = Save Valley water stress; IPCA = Interaction principal component axis

### 3.3.6 Combining ability analysis of nine traits of 72 F<sub>1</sub> hybrid crosses

Combining ability analysis was conducted on the 72 crosses from the 8 (female DT lines) x 9 (male UT lines) factorial mating design to estimate the general combining ability effects for the female (GCA<sub>f</sub>) and male lines (GCA<sub>m</sub>) and the specific combining ability (SCA) effects. Only the GCA and SCA effects for GY are presented and discussed. The combining ability analyses were done separately on the well watered environments, CHIS-WW and across the two water stress environments, CHIS-WS and Save-WS. This was done separately to establish the GCA and SCA effects under WW conditions versus WS conditions separately. To determine the relative contribution of GCA effects representing additive genetic effects and SCA effects representing non-additive genetic effects to the inheritance of the selected traits, the combining ability ratio was calculated according to Baker (1978):

$$\text{GCA:SCA} = \frac{2\sigma^2_{\text{GCA}}}{2\sigma^2_{\text{GCA}} + \sigma^2_{\text{SCA}}}.$$

All variance components were determined using REML.

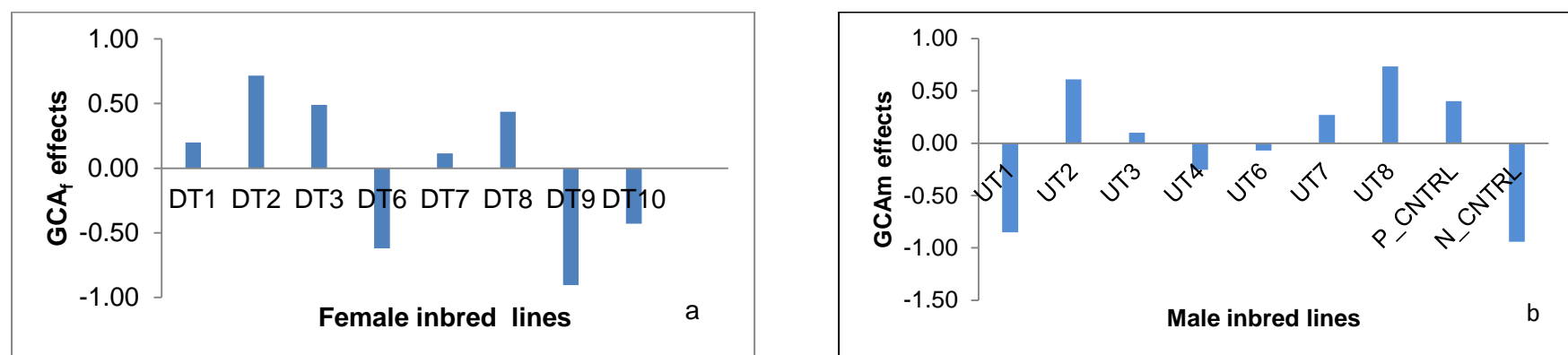
#### 3.3.6.1 Well watered conditions

The Wald statistics for GCA<sub>f</sub> and GCA<sub>m</sub> and the SCA effects for GY and secondary traits were significant ( $P < 0.05$ ) under WW (Table 3.16). The magnitude of GCA<sub>f</sub> was higher in six of the nine traits than GCA<sub>m</sub>. All traits had a GCA:SCA ratio less than 1, ranging from 0.43 to 0.81, indicating the preponderance of non-additive over additive gene action in their inheritance. Assuming the absence of epistasis, GCA corresponds to the variance of the additive effects and SCA to the variance of dominance effects. The CV% for the various traits ranged from 3.10% to 14.61% indicative of a low experimental error in the evaluation of the traits. Female lines with positive and therefore desirable GCA<sub>f</sub> effects for GY (from lowest to highest) were DT7 (0.11), DT1 (0.19), DT8 (0.44), DT3 (0.49), DT2 (0.72), with DT2 having the highest GCA<sub>f</sub> effect. The female lines DT10 (-0.43), DT6 (-0.62), DT9 (-0.90), had negative GCA<sub>f</sub> effects, with DT9 having the highest negative GCA (Fig. 3.8a). Male lines with positive GCA effects for GY were UT3 (0.10), UT7 (0.27), P\_CNTRL (0.40), UT2 (0.61), UT8 (0.73). Male lines UT6 (-0.07), UT4 (-0.25), UT1 (-0.85), N\_CNTRL (-0.94) had negative GCA<sub>m</sub> effects (Fig. 3.8b). Hybrids EH24 (1.79; DT8xP\_CNTRL), EH46 (1.78; DT10xP\_CNTRL) and EH68 (1.88; DT3xUL3)) had the highest positive SCA effects (Fig. 3.9a) whereas EH59 (-1.82; DT7xP\_CNTRL), EH23 (-2.10; DT8xN\_CNTRL), and EH76 (-3.93; DT8xUL6) had the highest negative SCA effects (Fig. 3.9b).

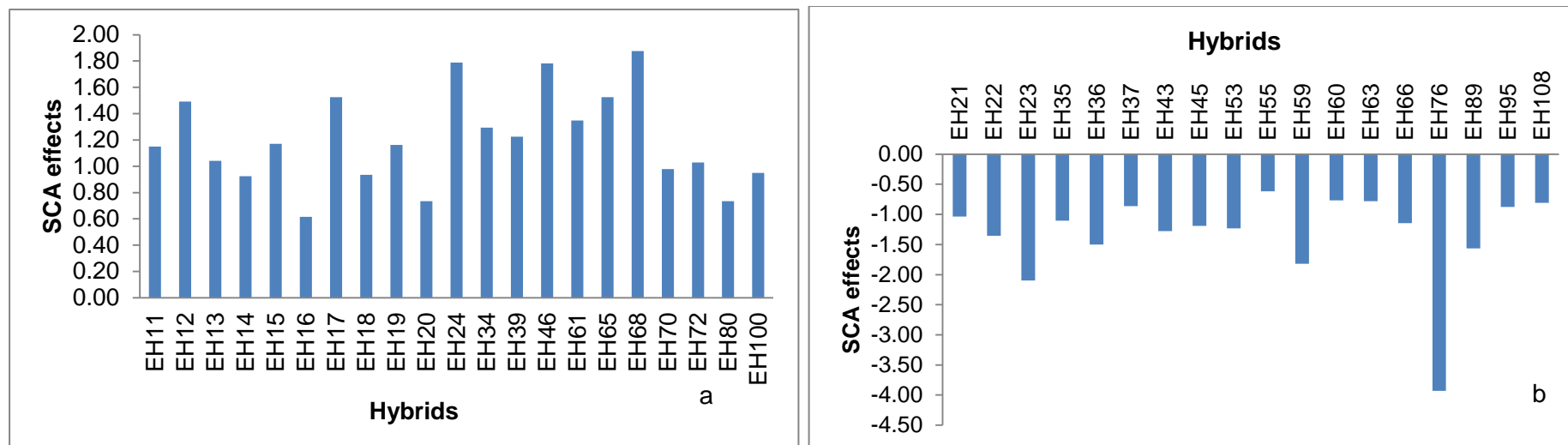
**Table 3.16 REML WALD statistics for general (GCA) and specific (SCA) combining ability for grain yield and secondary traits under the well watered conditions of Chisumbanje**

Fixed terms	d.f.	REML F-statistic								
		GY	DTA	ASI	EPP	LSEN	EP	SP	LP	PH
Reps	3	16.32*	7.01	5.17	3.56	1.01	9.32	0.98*	4.24	0.64
Crosses	71	18.11*	40.25*	10.34*	0.27*	2.98*	16.31*	1.97*	7.21*	0.98*
GCAf	7	14.28*	20.56*	14.52*	0.45*	2.14*	19.32*	3.21*	5.14*	1.57*
GCAm	8	11.74*	21.35*	7.21*	0.28*	3.25*	15.01*	2.58*	7.86*	1.87*
SCA	56	38.62*	19.21*	41.91*	2.14*	10.00*	11.31*	1.01*	1.12*	1.20*
GCA:SCA ratio and traits CVs										
GCAf:SCA		0.43	0.68	0.41	0.30	0.30	0.77	0.86	0.90	0.72
GCAm:SCA		0.38	0.69	0.26	0.21	0.39	0.73	0.84	0.93	0.76
CV%		14.61	5.02	8.21	3.12	9.41	2.41	11.98	10.10	3.10

GY= Grain yield ( $\text{t ha}^{-1}$ ); DTA = Days to anthesis; ASI = Ears per plant; LSEN = Leaf senescence; EP = Ear position; SP = Shelling percentage; LP = Lodge percentage; PH = Plant height (cm); Reps = Replications



**Fig. 3.8 General combining ability effects for grain yield ( $\text{t ha}^{-1}$ ) of (a.) eight female lines and (b.) nine male lines under well watered conditions. UT = Unknown tolerance; DT = Drought tolerance**



**Fig. 3.9** Specific combining ability for grain yield (t ha<sup>-1</sup>) of (a.) 21 of 72 hybrids with significant positive and (b.) 18 of 72 hybrids with significant negative effects under well watered conditions. EH = Experimental hybrids

### 3.3.6.2 Water stressed conditions

The  $GCA_f$ ,  $GCA_m$  and  $SCA_{fxm}$  effects and the interaction effects with the environment  $GCA \times E$ ,  $SCA \times E$ , were significant ( $P < 0.05$ ; Table 3.17) for GY and for many of the secondary traits. Significant  $GCA \times E$  and  $SCA \times E$  indicated that the magnitude of the GCA and SCA of inbred lines was dependent on the environment i.e. the GCA and SCA effects were not consistent over the two water stressed environments CHIS-WS and SAVE-WS. The effects for  $SCA \times E$  were not significant for DTA, LP and PH ( $P > 0.05$ ). The  $GCA_f:SCA$  ratio ranged from 0.32 (DTA) to 0.85 (PH) while the  $GCA_m:SCA$  ratio ranged from 0.22 (EPP) to 0.87 (EP). All traits including GY with a  $GCA:SCA < 1$  (both female and male) were influenced predominantly by non-additive gene action. However, PH and EP had relatively higher  $GCA_m:SCA$  and  $GCA_f:SCA$  ratios, that is, closer to one suggesting the increased contribution of additive gene action in these traits relative to the others. The CVs for the various traits for the combined analysis across the two water stressed environments were generally higher compared to the well-watered environments, ranging from 5.41% (EP) to 19.60% (GY). The female lines DT1 (0.02), DT3 (0.07), DT10 (0.07) and DT2 (0.21) had positive  $GCA_f$  effects for GY. The lines DT7 (-0.03), DT6 (-0.21), and DT9 (-0.29) had negative  $GCA_f$  effects with DT9 being the highest negative value (Fig. 3.10a). The male lines UT6 (0.05) UT7 (0.15), UT3 (0.19), P\_CNTRL (0.27) and UT2 (0.39) had positive  $GCA_m$  effects for GY. The male lines UT1 (-0.17), UT4 (-0.21), UT8 (-0.23) and N\_CNTRL (-0.41) and had negative  $GCA_m$  effects with N\_CNTRL having the lowest  $GCA_m$  (Fig. 3.10b). The hybrid EH24 (1.70; DT8xP\_CNTRL), had the best SCA effect for GY as was the case under WW conditions. This was followed by EH61 (1.52) and EH93 (1.22) (Fig. 3.11a). The hybrids EH46 and EH68 which had high SCA effects for GY under WW conditions had lower SCA effects under WS conditions. Hybrid EH68 did not appear amongst the 16 crosses that had significant positive SCA effects for GY under WS (Fig 3.13). Similarly to WW conditions, EH76 (-1.46) had the highest negative SCA effects for GY under WS conditions followed by EH54 (-1.06) and EH108 (1.04) (Fig. 3.11b).



**Table 3.17 Combining ability analysis for grain yield (t ha<sup>-1</sup>) and secondary traits across two water stress environments**

Fixed terms	n.d.f.	REML F-statistic								
		GY	DTA	ASI	EPP	LSEN	EP	SP	LP	PH
Reps	3	154.21*	97.02*	213.4*	7.68	108.09*	21.14	87.3*	42.31	21.36
Env	1	128.14*	70.02*	193.21*	10.98*	369.71*	62.30*	130.54*	113.20*	282.40*
Hybrids (H)	71	28.00*	45.31*	95.3*	2.64*	101.00*	3.10*	71.21*	3.11	21.30*
GCA for females (f)	7	6.71*	3.64*	71.21*	0.98*	65.24*	1.71*	6.01*	11.20*	112.21*
GCA for males (m)	8	5.74*	4.28*	69.85*	0.41	51.40*	1.84*	7.84*	13.24	108.51*
SCA (f x m)	56	16.24	15.23*	108.12*	2.86*	106.25	0.54*	5.01	8.00	40.25*
Hybrids.Env	71	15.30*	16.21*	11.30*	3.41*	78.11*	14.21*	49.62*	3.10*	6.51*
GCAf*Env	7	1.10*	4.12*	3.25*	0.74	40.13*	1.94*	32.10*	4.01	1.45*
GCAm*Env	8	0.94*	1.57*	7.60*	0.32*	35.14*	1.20*	34.98*	3.65*	2.00*
SCA*Env	56	10.47*	20.30	5.73*	1.42*	49.70*	10.78*	41.23*	5.36	6.00
GCA:SCA ratio and CVs of traits										
GCAf:SCA		0.45	0.32	0.57	0.41	0.55	0.86	0.71	0.74	0.85
GCAm:SCA		0.41	0.36	0.56	0.22	0.49	0.87	0.76	0.77	0.84
CV%		19.60	12.01	18.82	14.10	16.32	5.41	18.24	11.78	6.18

\*Significant at 5% significance level; GY= Grain yield (t ha<sup>-1</sup>); DTA = Days to anthesis; ASI = Ears per plant; LSEN = Leaf senescence; EP = Ear position; SP = Shelling percentage; LP = Lodge percentage; PH = Plant height (cm); Env = Environment; GCA = General combining ability, SCA = Specific combining ability; CV% = Coefficient of variation percentage; Reps = replications

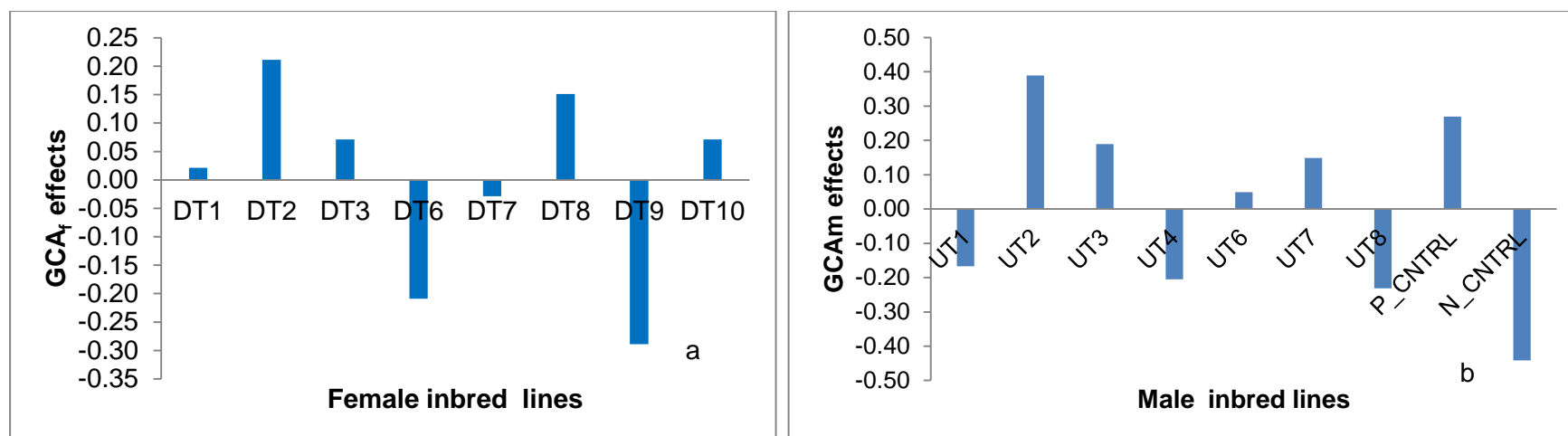


Fig. 3.10 General combining ability effects for grain yield ( $\text{t ha}^{-1}$ ) of (a.) eight female and (b.) nine male lines evaluated at two water stress environments. UT = Unknown tolerance; DT = Drought tolerance.

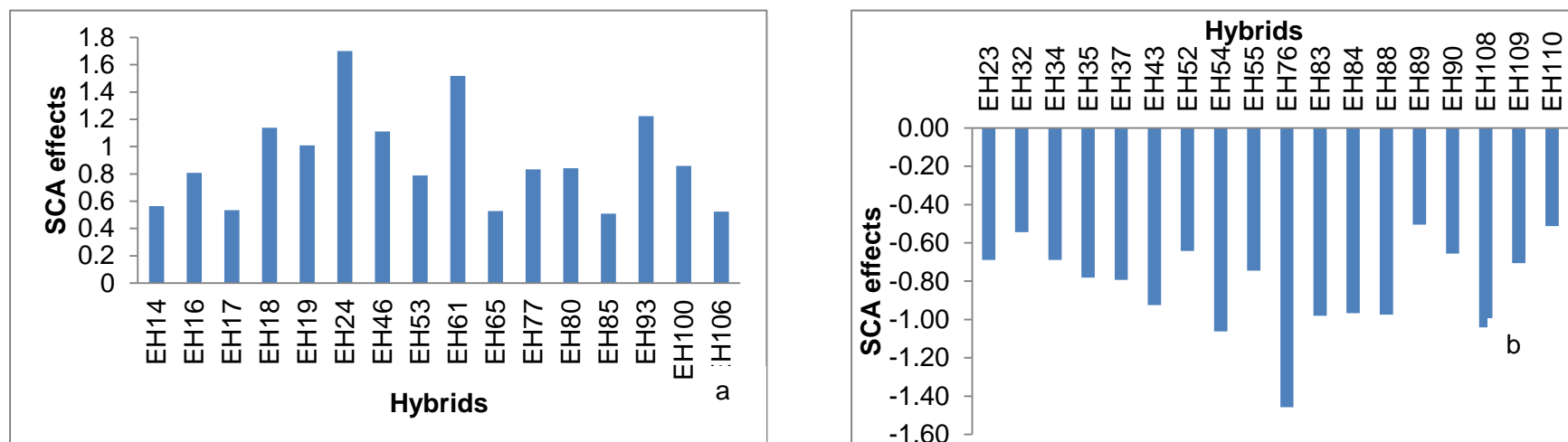


Fig. 3.11 Specific combining ability for grain yield ( $\text{t ha}^{-1}$ ) of (a.) 16 of 72 hybrids with significant positive and (b.) 18 of 72 hybrids with negative SCA effects at two water stress environment. EH = Experimental hybrids.

### 3.3.7 Heritability estimates for grain yield and secondary traits

Narrow-sense heritability estimates for GY and secondary traits were calculated using GCA and SCA variance components from the WW environment and mean of the WS environments (see method in Chapter 2). Heritability estimates varied between WW and WS conditions (Table 3.18). Grain yield had  $h^2$  of 71.3% and 45.7% under WW and WS conditions, respectively. Under WW, DTA, EPP and EP had  $h^2$  of 76.2, 86.4 and 85.7%, respectively, whereas under WS,  $h^2$  was 60.2%, 75.4% and 65.1%, respectively. Heritability for ASI was lower under WW (48.4%) than WS (51.6%) conditions. Heritability for LSEN was 25.8 and 23.4% under WW and WS, respectively. Plant height had the highest  $h^2$  (88.6%) which decreased to 78.6% under WS. Lodging percentage (LP) had the lowest  $h^2$  under both WW (22.1%) and WS (18.4%). The heritability estimate for ASI under WW of 48.4% was higher than the 51.6% under WS.

**Table 3.18 Narrow-sense heritability estimates of measured (and derived) traits under WW and WS conditions.**

	GY	EPP	DTA	ASI	LSEN	EP	SP	LP	PH
$h^2$ (WW)	71.3	76.2	85.7	48.4	25.8	85.4	31.2	22.1	88.6
$h^2$ (WS)	40.7	60.2	75.4	51.6	23.4	65.1	21.3	18.4	68.6

MS = mean square, WW = well watered, WS = Water stress, GY = grain yield, EPP = ears per plant, DTA = days to anthesis, ASI = Anthesis silking interval, LSEN = leaf senescence, EP = Ear position, SP = shelling percentage, LP = lodging percentage, PH = Plant height (cm).

## 3.4 Discussion

According to Bänziger *et al.* (2006), “...the challenge of developing abiotic stress tolerant crop varieties has generated an immense amount of literature....but most practical breeding efforts remain focused on increasing productivity under favourable conditions where genetic variance, heritability and therefore breeding progress for grain yield are greatest...”. The main objective of this study was to evaluate the drought tolerance of  $F_1$  hybrids by comparing their performances under WW and WS conditions. Comparison of hybrid performance under WW and WS was important to test to establish whether hybrid productivity is the same under both conditions. Across environments analysis permitted the characterization of genotypic stability and adaptability, whereas combining ability the analysis of GCA and SCA made it possible to identify superior lines and hybrid crosses.

## Effect of drought stress on mean grain yield

The experimental protocol used (withdrawing water before flowering) revealed significant differences in average yield between WW and WS environments. The conditions established by Bänziger *et al.* (2000) were met in this study, namely, drought stress was considered severe when average yield is below 60% of the WW environment. The percentage yield reduction varied from the one WS environment to the other. In this study, CHIS-WS and SAVE-WS produced 33.7 and 58.1% of the WW environment, respectively. As drought stress was imposed two weeks before flowering until two weeks after silking, the yield reduction could have been due to the abnormal development of reproductive organs and subsequent pollination and kernel development (Schussler and Westgate, 1995; Edmeades *et al.*, 2000; Edmeades, 2013). Many studies attribute yield reduction under drought conditions to both poor kernel set and grain filling. This, however, depends on the severity, timing and duration of the drought stress. Drought at flowering (fertilisation) primarily affects kernel number, whereas after flowering, it affects kernel size and final kernel mass (Bolaños and Edmeades, 1996; Bänziger *et al.*, 2000; Edmeades *et al.*, 2000; Barker *et al.*, 2005; Edmeades, 2013). Grant *et al.* (1989) reported complete barrenness when stress was imposed from just before tasseling to the beginning of grain filling.

Mean grain yield per environment is obtained by calculating the mean yield of all the test genotypes evaluated in that environment. The significant differences among hybrids in this study indicated the presence of adequate genetic diversity in the material chosen. Mean GY ranged from 0.17 (EH76) to 3.49 t ha<sup>-1</sup> (EH24) under WS conditions whereas, under WW conditions, GY ranged from 1.0 (EH76) to 6.81 t ha<sup>-1</sup> (EH68). Overall, across the three environments, the best experimental hybrid was EH24 (4.87 t ha<sup>-1</sup>), whereas the worst was EH76. Dobermann *et al.* (2003) termed the difference between HYP and actual GY under limiting factors the “yield gap”. The limiting factors can be divided into biotic and abiotic stresses. The data in Table 3.9 represented the extent to which drought stress reduced the grain yield of the experimental hybrids relative to their HYP. According to Barker *et al.* (2005), yields obtained under WW conditions can be used as a reasonable predictor of hybrid performance under moderate stress levels, but only when  $Y_{WS}/Y_{WW} = 70-90\%$ . When yields under stress are less than 60-70% of  $Y_{WW}$ , it is more efficient to select hybrids under the target stress environments. In this study, only two (EH38 and EH53) hybrids had  $Y_{WS}/Y_{WW}$  above 70% (Table 3.9). When  $Y_{WS}/Y_{WW}$  is <60%, Barker *et al.* (2005) suggest that the level of water stress the test genotypes were exposed to could be classified as intermediate or severe stress. If this suggestion is true, then drought stress in the present study could be generally classified as severe. This is supported by Bänziger *et al.* (2000) who regard

drought to be severe when average yield is between 1-3 t ha<sup>-1</sup>; the yield range recorded in this study.

### **Relationship between secondary traits and grain yield**

The use of secondary traits in selection has been widely suggested because heritability of GY is reportedly reduced under drought conditions while that of some secondary traits remains higher (Bänziger and Lafitte, 1997; Chapman *et al.*, 1997; Edmeades *et al.*, 1997; Chapman and Edmeades, 1999; Bänziger *et al.*, 2000; Andjelkovic *et al.*, 2008; Kumar *et al.*, 2008; Monneveux *et al.*, 2008; Cairns *et al.*, 2012; Mhike *et al.*, 2012; Ziyomo and Bernardo, 2013). The findings of these authors were supported by this study. The heritability estimates of secondary traits were generally higher than that of GY under stress conditions (Table 3.18). In particular, ASI, EPP and LSEN have been regarded as the most important secondary traits for selection under drought stress (Bänziger *et al.*, 2000; Moser *et al.*, 2006; Monneveux *et al.*, 2008). This study revealed that EPP was more positively and significantly correlated with GY than the other two traits under both WW and WS conditions. According to Ngugi *et al.* (2013), this strong positive correlation is expected because GY is primarily dependent on EPP. But it could equally be argued that one well filled large ear could give higher yield than two poorly filled ears.

Maturity measured as DTA was negatively and significantly correlated with GY under WS. A negative correlation between these two traits under WS was also reported by Ngugi *et al.* (2013). The positive correlation between GY and DTA under WW conditions implied that the late maturing hybrids were able to achieve their yield potentials whereas under WS, prolonged exposure to stress conditions may have reduced GY of the late maturing hybrids. Bänziger *et al.* (2000) stated that the goal of drought stress imposed at flowering is to achieve an ASI of 4 to 8 days and an EPP of 0.3 to 0.7; this was achieved in this study (Figs. 3.5 and 3.6). Water stress increased the ASI in some hybrids by up to 12 days more than stated by Bänziger *et al.* (2000) (Fig. 3.5). The ASI increased mainly as a result of delayed silking. For these hybrids (e.g. EH50 with ASI of 11 days), the only source of pollen could have been the neighbouring hybrids. According to Bassetti and Westgate (1994), plants produce viable pollen within seven days after the commencement of pollen shed. Several studies have related high yield with short ASI under drought (Moser, 2004; Xi *et al.*, 2009). As a result, Bolaños and Edmeades (1993) postulated that a short ASI under water stress could be used as a measure of drought tolerance.

The flowering period in maize has been regarded as a crucial stage in breeding for drought tolerance because of the devastating effects of drought when it coincides with this stage of maize development (Ngugi *et al.*, 2013). The significant positive correlation between PH and

GY (under WW) suggest that tall plants gave better yields compared to shorter plants. This could, perhaps, be attributed to the higher dry mass accumulated by longer leaves and stem tissue that may be possessed by tall plants. Conversely, the correlation between PH and GY under WS was weak and non-significant. It is expected that under drought stress, plants may not grow tall and therefore the vegetative material required for biomass accumulation is reduced. The results of this study imply that tall plants had improved capacity to support kernel growth. The information on the correlations among traits is crucial in improving the efficiency of breeding programmes. Positively associated traits could be linked or positioned closely together on the same chromosome or could be controlled by pleiotropic genes<sup>1</sup> (Bocanski *et al.*, 2009).

### **General and specific combining ability**

The analysis of GCA and SCA for GY was carried out to determine the nature and magnitude of gene action for yield under WW and WS conditions. The  $GCA_f$  and  $GCA_m$  and SCA effects for GY were significant under both WW and WS, suggesting the influence of both additive and non-additive gene action in the inheritance of GY and associated secondary traits under both conditions (Dabholkar, 1999). The presence of significant GCA and SCA effects under WW and WS conditions were also reported by Makumbi *et al.* (2011). Significant  $GCA_f$  and  $GCA_m$  was indicative of the genetic diversity of the parent lines used in this study. Significant GCA x environment effects suggests the need for selecting different parental lines for developing hybrids for specific target environments. Similarly, significant SCA x environment interaction implies the non-additive contribution to the performance of a specific cross between parent lines varies depending on the environment. Inbred lines such as DT2 and DT3 had the highest positive  $GCA_f$  effects for GY under both WW and WS conditions. Under WS, DT2 and DT8 had the highest  $GCA_f$  effects. The male lines UT2 and P\_CNTRL maintained high positive GCA effects under both WW and WS. Line UT8 had positive GCA and therefore desirable under WW conditions but produced negative GCA effects under WS. The lines with positive GCA effects across WW and WS indicate parental lines that are stable in their contribution of additive genetic effects and could be potential sources of stable additive gene action for drought tolerance. Hybrids with high positive SCA were also associated with high GY performance (for example, EH24, EH61 and EH46) greater than expected on the basis of the additivity of the GCA effects of their parents, whereas hybrids with negative SCA had performance lower than that expected on the basis of the additivity of the GCA effects of their parents. Hybrid EH24 (with parents DT8xP\_CNTRL) and EH61 (with parents DT3xUT1) had the best SCA effects under WS conditions. Conversely, EH76 and

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<sup>1</sup>When one gene (or gene cluster) has multiple effects

EH50 had the lowest yields of the hybrids. The GCA:SCA ratio values indicated that the magnitude of SCA, under both WW and WS, was generally higher than that of GCA, implying the greater influence of non-additive gene action in the inheritance of the measured traits. However, traits such as PH and EP, with GCA:SCA ratio close to 1 had an increased influence of additive gene action in their inheritance. Simple pedigree selection would be effective in improving these traits. These results are consistent with the study by Živanović *et al.* (2010). Dabholkar (1999) stated that when variance due to SCA is twice that of GCA, selection should be based on individual crosses. This condition was not met in its entirety; hence, both GCA and SCA should be used as selection indices.

### **Heritability estimates**

Apart from ASI,  $h^2$  estimates were lower under WS than WW conditions. The highest  $h^2$  estimates were recorded for PH, EP and DTA under WW conditions. This suggested that these traits were simply inherited and are readily passed from one generation to the next. It also implied that they are under genetic control and the environment did not greatly influence their phenotypic expression. The higher  $h^2$  under WW conditions also reveals the greater influence of additive gene in the expression of the phenotypic values for traits under WW conditions as was reported by Bocanski *et al.* (2009). This is corroborated by the GCA values obtained in this study. Even though  $h^2$  for these traits remained high under WS, the reduction under WS relative to WW conditions indicates that water stress reduces the influence of additive gene action relative to environmental effects in their phenotypic expression. Secondary traits had, comparatively, higher  $h^2$  than GY. With moderately high  $h^2$  under WW, which was reduced under WS, GY appeared to have been more influenced by the environment than other secondary traits. In this study, ASI, DTA and EPP are recommended for selection alongside GY.

### **Genotype by environment interaction, yield stability and adaptation**

Drought stress environments are often associated with fluctuations in hybrid performance, producing rankings that significantly differ from one experiment to the other (Bänziger *et al.*, 2000). The differential response of hybrids to changing environments is termed GxE interaction. Various studies have reported the limitations posed by GxE interactions on breeding and selection progress for target environments (Bänziger *et al.*, 2000; Tolessa *et al.*, 2013). However, a better understanding of the level and nature of GxE interactions serves as a decision tool for generating essential information on patterns of the stability and adaptability of hybrids, especially under drought stress. In addition, it also forms the basis for selecting for either general or specific adaptation.

The differential ranking of hybrids in this study (Table 3.7) has been reported to be common under drought conditions (Bänziger *et al.*, 2000). According to Barker *et al.* (2004), the negative correlation between rankings in unstressed and stressed environments (caused by the differential ranking of hybrids) is indicative of the need to breed for specific adaptation. It also points to the presence of GxE interactions of the crossover type. Hybrids that consistently rank above the general mean across environments (quantitative GxE interaction) are considered to be widely adapted and those that show considerable change in ranks from one environment to another are unstable. In this study, EH24 was generally adapted to all three environments whereas EH46, EH68 and EH61 were specifically adapted to CHIS-WS, CHIS-WW and SAVE-WS, respectively, and so they can be recommended for the environments they performed best in. The objective of breeding for general adaptation is to produce hybrids that combine good performance under both unstressed and stressed conditions. According to Asfaw *et al.* (2009), the best hybrid should combine high yield and stable performance across a range of production environments.

Yield stability can be divided into two concepts, that is, static and dynamic (Lin *et al.*, 1986). Static (or biological) stability refers to the unchanged performance of hybrids regardless of any variation in environmental conditions, while dynamic (or agronomic) stability refers to a predictable response to the productivity level of the environments (Becker, 1981; Lin *et al.*, 1986). Hybrid EH24 appeared to express dynamic stability, producing 3.50 t ha<sup>-1</sup> and 6.82 t ha<sup>-1</sup> under stress and unstressed conditions, respectively (Table 3.9). This showed that the hybrid responded to increased water by producing more grain yield. On the other hand, even though EH38 was the top yielder (3.01 t ha<sup>-1</sup>) under WS at SAVE-WS, it was one of the lowest yielding hybrids (3.52 t ha<sup>-1</sup>) under unstressed conditions. The small difference in GY between stressed and unstressed conditions indicated that EH38 expressed static stability (that is, it did not respond to improved production conditions) would incur a yield penalty under WW conditions. This type of stability is generally regarded as undesirable (Becker, 1981).

### **Additive Main effects and Multiplicative Interactions**

The Additive Main effects and Multiplicative Interactions (AMMI) model advocated by Gauch (1988) was found to be adequate for explaining both the main effects (G and E) and interaction effects (GxE) in this study. The large total variation explained by the environments (67.76%) was indicative of the diverse nature of the three environments used, accounting for most of the variation for grain yield. Mean yield per environment was 1.41, 2.43 and 4.18 t ha<sup>-1</sup> at CHIS-WS, SAVE-WS and CHIS-WW, respectively.



The AMMI1 biplot gave a model fit of 67.76%. The displacement along the abscissa indicated differences in hybrid and environment main effects (or mean performance), whereas displacement along the ordinate indicated differences in the interaction effects. Most hybrids were clustered within the IPCA1 score of -0.5 and +0.5 and along the abscissa. Hybrids and environments on the same vertical line had similar yields and a hybrid or environment on the right side of the midpoint of the abscissa had higher yields than those on the left hand side. Among the hybrids, EH48 and EH46 exhibited high yields with higher main (additive) effects. Hybrid EH48 had a negative IPCA1 score whereas EH46 had a positive IPCA score. Hybrids such as EH18, which had an IPCA1 score of nearly zero, had small interaction effects and were considered stable. Similarly, environments CHIS-WS had a positive but very low IPCA1 score indicating that it interacted to a small extent with the hybrids. This environment was associated with low yields such as those produced by hybrids EH29 and EH31. Typically, breeders opt for high mean yield and stable performance across the target growing conditions (Tolessa *et al.*, 2013). As EH48 and E46 are specifically adapted to WW conditions they would be desirable if the target environments are at low risk of water stress, that is, supplemental irrigation is available in the event of low rainfall during critical growth phases. It has been reported to be practically impossible to assemble the genes responsible for superior performance in both stressed and unstressed environments (Annicchiarico, 2002). This would seem to support the objective of selecting for specific adaptation.

In the AMMI2 biplot, vectors from the origin to the environmental coordinates were presented. Environment CHIS-WS, with the shortest vector had the least interactions with the hybrids. CHIS-WW had the longest vector and hence interacted more than the two WS environments with the hybrids. The hybrids EH105, EH18, EH35 and EH37 that plotted close to the origin were not sensitive to environmental interaction and those distant from the origin were more sensitive to environmental effects and presented larger interactions. In the context of this study, three groups of hybrids can be categorised from the results: (i) hybrids that were specifically adapted to drought conditions (with IPCA1&2 scores greater than zero and of the same sign as CHIS-WS and SAVE-WS); (ii) hybrids that were specifically adapted to well watered conditions (with IPCA1&2 scores greater than zero and of same sign as CHIS-WW); and (iii) hybrids that were generally adapted (or stable) under both drought and well watered conditions (with IPCA scores close to zero). Gene expression of quantitatively inherited traits is influenced by various environmental factors such as rainfall, soil type, temperature, etc. According to Bänziger *et al.* (2000), selection under drought conditions could lead to the full expression of stress tolerance traits. From the AMMI analysis, the best

hybrids for CHIS-WW, CHIS-WS and SAVE-WS were EH68, EH46 and EH61, respectively. These hybrids should undergo further evaluation in a broader range of environments.

### 3.5 Conclusions

The main objective of this study was to evaluate the performance of F<sub>1</sub> maize hybrids under drought stress imposed during flowering and grain filling relative to their performance under WW conditions. The results revealed that the hybrids selected did not yield similarly under WS and WW conditions: they were high yielding under WW conditions, but with yield reductions of more than 50% under WS conditions.

The ranking of hybrids from one environment to another was inconsistent, revealing the presence of crossover GxE interactions. In some cases, hybrids that performed well under WW conditions also performed well under WS conditions (for example, EH24), but this was generally not the case. Hybrid performance fluctuated from WW to WS growing conditions. Therefore, as has been recommended by other studies, breeding for drought tolerance should be carried out under target drought conditions so that drought tolerance related genes can be fully expressed.

The analysis of GCA and SCA revealed that both additive and non-additive gene actions were responsible for the inheritance of GY, with SCA contributing more to the inheritance of GY. The greater involvement of SCA relative to GCA to the performance of the hybrid crosses suggests that significant progress can be made by selection based on the hybrids with the best SCA under both well watered and water stressed conditions. Inbred lines with desirable GCA effects under drought conditions can be used as potential drought tolerant donors e.g. DT2, DT3, DT8, P\_CNTRL and UT8. Hybrids EH24, EH61, EH18, EH93, EH46 and EH100 had both high SCA and yield performance. These hybrid crosses can be considered as drought tolerant hybrids for further evaluation and potential release.

Hallauer *et al.* (2010) previously stated that the estimates of GCA and SCA are relative to and dependent on the particular set of inbred lines included in the hybrids under test. In this context, the conclusions drawn in this study from the combining ability analysis of the parental lines are specific to the germplasm, the environments and the evaluation methodology used. Nevertheless, the results can be used to provide background information for future breeding endeavours. For instance, lines identified with desirable GCA and SCA effects can be used in future hybrid crosses.

Yields of F<sub>1</sub> hybrids fluctuated from one environment to another and therefore their rank orders were inconsistent across the environments, demonstrating the presence of GxE

interactions of the crossover type. Selections can be made from the following groups of hybrids that are: (i) specifically adapted to drought conditions; (ii) specifically adapted to favourable conditions (well watered); and (iii) generally adapted to both drought stressed and unstressed conditions. The worst performing EH in CHIS-WW, CHIS-WS and SAVE-WS were EH76 (0.93 t ha<sup>-1</sup>), EH76 (0 t ha<sup>-1</sup>) and EH (0.22 t ha<sup>-1</sup>), respectively, achieving yields below the worst CHs in the respective environments. The top three EHs in CHIS-WW, CHIS-WS and SAVE-WS were EH68 (6.82 t ha<sup>-1</sup>), EH46 (3.25 t ha<sup>-1</sup>) and EH61 (4.28 t ha<sup>-1</sup>). These can be selected as the top hybrids for cultivation in respective environments. Even though EH24 had a  $Y_{ws}/Y_{ww}$  of 52% (lower than EH38), its absolute performance under both WW and WS conditions was better than all hybrids, ranking second in both CHIS-WW and SAVE-WS and fifth in CHIS-WS. This demonstrated a desirable level of stability. Therefore out of all the experimental hybrids, EH24 (DT8xP\_CNTRL) was considered the most consistently best performing hybrid across well watered and water stressed conditions, achieving 30% more yield compared to the best commercial hybrid CH2.

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## Appendices 3

**Appendix 3.1 Field layout for 110 hybrids with numbers in the cells indicating plots or hybrid and the rows and columns within one replication**

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

**Appendix 3.2 A 8x9 factorial mating design for general and specific combining ability**

		Male lines								
		UT1	UT2	UT3	UT4	UT6	UT7	UT8	P_CNTRL	N_CNTRL
Female lines	DT1	DT1xUT1	DT1xUT2	DT1xUT3	DT1xUT4	DT1xUT6	DT1xUT7	DT1xUT8	DT1xP_CNTRL	DT1xN_CNTRL
	DT2	DT2xUT1	DT2xUT2	DT2xUT3	DT2xUT4	DT2xUT6	DT2xUT7	DT2xUT8	DT2xP_CNTRL	DT2xN_CNTRL
	DT3	DT3xUT1	DT3xUT2	DT3xUT3	DT3xUT4	DT3xUT6	DT3xUT7	DT3xUT8	DT3xP_CNTRL	DT3xN_CNTRL
	DT4	DT4xUT1	DT4xUT2	DT4xUT3	DT4xUT4	DT4xUT6	DT4xUT7	DT4xUT8	DT4xP_CNTRL	DT4xN_CNTRL
	DT5	DT5xUT1	DT5xUT2	DT5xUT3	DT5xUT4	DT5xUT6	DT5xUT7	DT5xUT8	DT5xP_CNTRL	DT5xN_CNTRL
	DT6	DT6xUT1	DT6xUT2	DT6xUT3	DT6xUT4	DT6xUT6	DT6xUT7	DT6xUT8	DT6xP_CNTRL	DT6xN_CNTRL
	DT7	DT7xUT1	DT7xUT2	DT7xUT3	DT7xUT4	DT7xUT6	DT7xUT7	DT7xUT8	DT7xP_CNTRL	DT7xN_CNTRL
	DT10	DT10xUT1	DT10xUT2	DT10xUT3	DT10xUT4	DT10xUT6	DT10xUT7	DT10xUT8	DT10xP_CNTRL	DT10xN_CNTRL



**Appendix 3.3 Percentage moisture for SAVE-WS measured using moisture probes**

Depth	Weeks						
	1	2	3	4	5	6	7
30cm	2.63	—	4.94	1.52	0.70	2.97	1.48
60cm	16.48	11.15	21.21	16.50	13.14	10.46	10.89
90cm	17.40	15.84	21.86	17.78	16.07	15.15	14.87
120cm	18.06	16.52	18.21	15.77	16.63	16.15	16.08
150cm	24.29	26.83	25.33	25.69	25.05	24.76	24.69
200cm	27.43	31.49	30.37	29.56	28.23	27.72	27.37

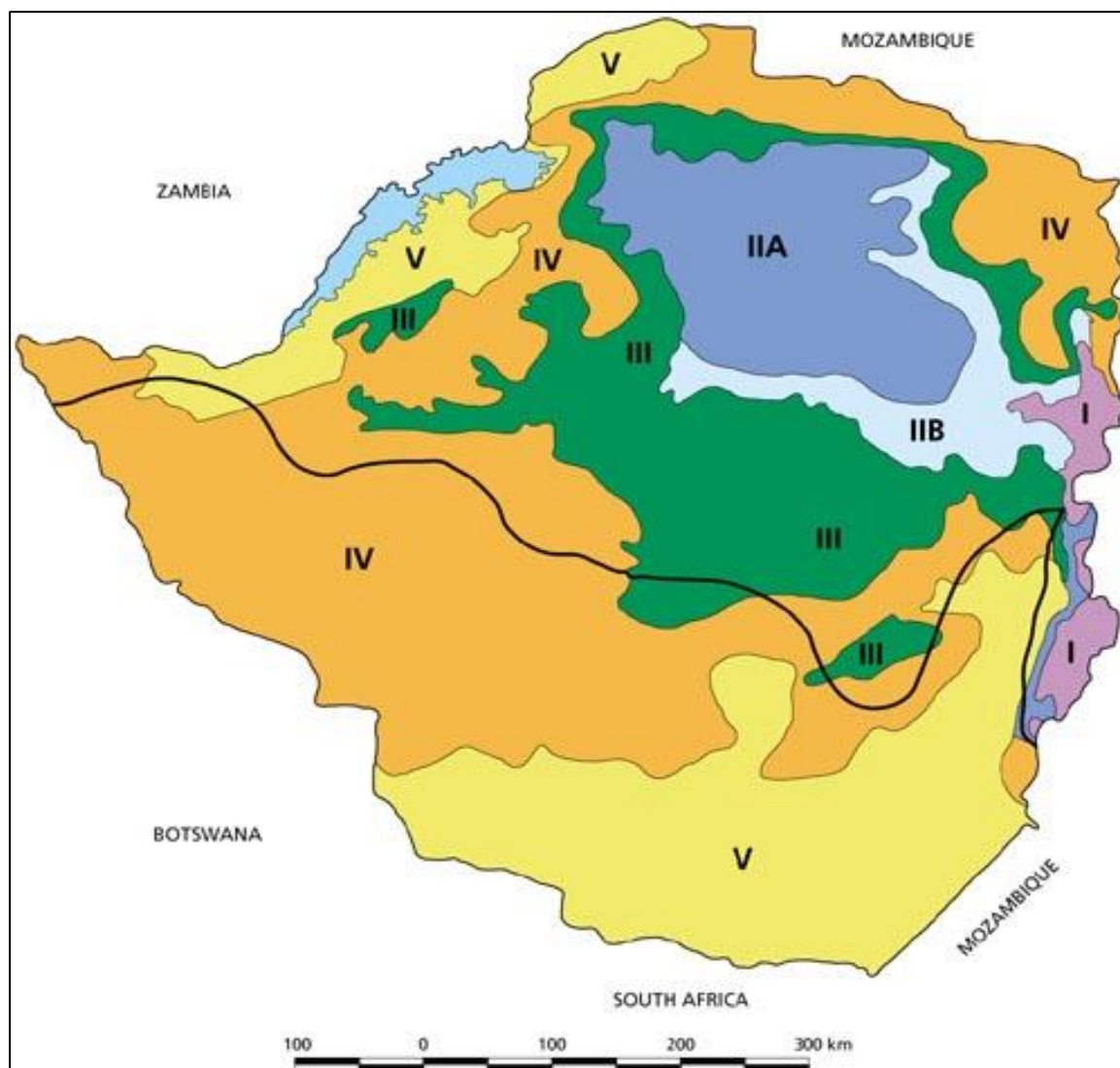
**Appendix 3.4 Variances and degrees of freedom from Bartlett's test**

Source of variation	Df	Chi probability
Environment	2	0.061
Environment	1	0.201

**Appendix 3.5 Spearman's rank correlation coefficient between Yws and Yww**

	$Y_{ws}$	$Y_{ww}$
$Y_{ws}$	1	
$Y_{ww}$	0.08	1

Appendix 3.5 Agro-ecological zones in Zimbabwe: experiments were conducted in region five.  
<http://www.fao.org/docrep/009/a0395e/a0395e06.htm>



## Chapter 4

### General thesis overview

This chapter provides a brief overview of the rationale of the study, objectives and the findings of the research. It also provides a summary of results and recommendations, and the challenges and opportunities associated with breeding for drought tolerance.

#### 4.1 Introduction

Maize is the principal staple food crop in Africa, but the limitation on yield posed by drought stress threatens the continent's food security. Maize is grown by both commercial large scale farmers and subsistence/small scale farmers. Most small scale farmers aim to produce enough for their own consumption and any surplus is sold or bartered. However, the production of maize by small scale farmers relies predominantly on rainfall; thus, they are more vulnerable to the yield reductions caused by dry spells. This, coupled with the use of open pollinated and poorly adapted maize varieties, has caused maize yields to remain as low as 1-2 t ha<sup>-1</sup>. Therefore, the development of maize hybrids that are drought tolerant is a high priority. Drought tolerant maize hybrids are required to achieve high and stable maize yields, especially for farmers who are located in the drier parts of the continent. Since it has been widely hypothesized that genetic variation for drought tolerance exist in current elite lines, the overall objective of this study was to develop F<sub>1</sub> hybrids from known drought tolerant lines and elite lines from CIMMYT and PANNAR PTY (Ltd) seed company, South Africa.

The main objectives were to:

- i. Characterise the performance of the F<sub>1</sub> single cross hybrids under random drought stress; and
- ii. Evaluate the drought tolerance of F<sub>1</sub> single cross hybrids under managed drought stress.

The specific objectives of the research were to:

- i. Determine the performance of F<sub>1</sub> maize hybrids under random drought stress (RDS) environments in South Africa and Zimbabwe
- ii. Evaluate the performance of F<sub>1</sub> maize hybrids under managed drought stress (MDS) in Zimbabwe
- iii. Establish the phenotypic association between yield and secondary traits of experimental hybrids under both RDS and MDS environments

- iv. Investigate GxE interactions and yield stability of F<sub>1</sub> maize hybrids under both RDS and MDS environments
- v. Investigate the general and specific combining ability (GCA and SCA) of parental lines

## 4.2 Outputs from the study

Average yields differed from one environment to the other depending on the level of stress. Drought stress caused a greater percentage of yield loss under MDS than RDS conditions. The ratio of the worst environment mean yield relative to the best environment mean yield (expressed as a percentage) was 57.50% under RDS (Greytown/Devonia; Chapter 2) and 33.7% under RDS and MDS (CHIS-WS/CHIS-WW; Chapter 3).

Grain yield (GY) and days to anthesis (DTA) had the highest significant positive correlation in the high potential environments (Cedara, Devonia and CHIS-WW) implying the greater relative importance of the association between these two traits than with and between the other traits. The negative correlation between GY and grey leaf spot (GLS) reinforces the well proven detrimental effects of the disease on yield and emphasises the imperative of co-selecting for tolerance to the disease and drought stress for those target environments in which both stresses are common. Overall, anthesis silking interval (ASI), DTA and ears per plant (EPP) were considered to be important secondary traits for co-selection with GY.

Narrow sense heritability of the traits evaluated in this study varied from one environment to another. The implication thereof was that the extent of the additive gene action in the phenotypic expression of the traits evaluated was influenced by the environments in which the experimental hybrids were evaluated. This in turn has the implication that selection for the traits has to be conducted in each target environment.

Combining ability analysis revealed the contribution of both additive and non-additive gene action in the inheritance of GY. Lines DT2, DT7, UT8, UT1 and UT4 had the highest GCA effects under RDS for GY whereas DT2, DT3, DT8, UT2 and UT8 had the best positive GCA under MDS. Overall, DT2 and UT2 were the best lines based on GCA effects for GY under both RDS and MDS conditions. Parental lines DT2 and DT7 produced two of the top yielding hybrids DT2xUT2 and DT7xP\_CNTRL under RDS, whereas DT3 and DT8 produced the top two yielding hybrids DT3xUT1 and DT8xP\_CNTRL) under MDS. The F<sub>1</sub> hybrid cross with the highest SCA effect for GY under RDS was EH79 (DT7xUT8) whereas under MDS it was EH24 (DT8xP\_CNTRL). The presence of significant GCAxE and SCAxE implies that selection based on combining ability should be conducted separately in each test environment. The F<sub>1</sub> hybrid

that combined both stability and yield performance under well watered and water stressed conditions was DT8xP\_CNTRL.

### **4.3 Limitations for breeding for drought tolerance**

Africa is a very diverse continent in terms of agro-ecological zones and this presents an enormous challenge to breeders as it is very difficult to breed for general adaptation and stability to the enormous range of abiotic and biotic stresses presented by the different production zones. Notwithstanding the challenges, the need to develop drought tolerant hybrids remains owing to the concerns about climate change, water scarcity in agriculture and the rising demand for food. The rise in global demand for food while water resources are diminishing entails that dryland productivity must increase (Blum, 2011). It is for this reason that breeders will not “turn a blind eye” on the subject of drought tolerance. In recent times, technologies such as marker-assisted selection (MAS) have been widely deployed in crops. This has led to development of molecular markers to create genetic maps, and exploitation of genetic linkages between markers and important crop traits. As reviewed in this study, this technology has led to considerable success in *Bacillus thuringiensis* (Bt) transformed maize (Hellmich and Hellmich, 2012) and Roundup Ready® crops (Funke *et al.*, 2006) for pest and weed control, respectively. However, there has not been a significant improvement on yield under drought conditions and breeding has largely relied on direct phenotypic selection for improved performance under drought stress environments (Fleury *et al.*, 2010). The selection of appropriate test environments that can replicate target environments is therefore an important factor in breeding for drought tolerance.

#### **4.3.1 Selection of test environments**

Generally breeders tend to carry out evaluation and selection of genotypes under favourable conditions and commonly it is assumed that the performance under favourable conditions would be replicated under unfavourable conditions (Barker *et al.*, 2004). However, this study and several others have shown that due to high genotype x environment interaction in stressed environments, genotypes often produce rankings that differ significantly from one environment to the other. This makes it difficult to select the best performing hybrids. Essentially, the initial processes of line development under favourable conditions may lead to the loss of the much useful genetic variation for stress tolerance (Bänziger *et al.*, 2000). When lost, this variation cannot be replaced later even when multi-location testing is implemented. It is, therefore, suggested that for best results, the process of inbred line development and evaluation of the

hybrid progeny should be conducted in target stress environments. However, heritabilities and genetic variances for GY in the target stress environments often decrease under abiotic stress as yield levels fall. With low yield levels, differences between genotypes are usually non-significant and the expected selection gains are less than under conditions where yields would be high. In this study, RDS and MDS environments were used for evaluation of hybrid progeny. However, the environments represented only a small sample of the target production environments that are prone to drought.

#### **4.3.1.1 Random drought stress environments**

In this method, genotypes are evaluated under randomly occurring stress conditions with an expectation that the crop will be exposed to random levels of stress. However, the interaction and the confounding effects of different levels (and types) of stresses will slow down breeding progress (Bänziger *et al.*, 2000). It is also challenging to quantify the stress levels therefore it becomes difficult to relate the plant's performance to a strictly defined stress mechanism.

#### **4.3.1.2 Managed drought stress environments**

At CIMMYT, most drought stress experiments are conducted during the winter season when rainfall probability is almost zero and water is applied through irrigation. Contrary to RDS environments, under managed stress, timing, intensity and uniformity of stress can be carefully controlled. However, winter experiments are not prevented from intercepting rainfall. Apart from the possibility of incident rainfall, the testing of genotypes (in the winter season) that are ordinarily planted in the summer season presents immense confounding effects. For example, maize is influenced by day length and heat units and the crop is commonly produced in summer. The ambient climatic (humidity, evaporative demand, temperature, etc.) conditions in winter are different i.e., days are shorter and nights are longer and colder than in summer. This implies that genotypes tested in the winter season are not subjected to the same abiotic and biotic stresses (such as high temperature and high diseases pressure) present in the normal summer production season. By extension, performance in the winter season may not be replicated under summer conditions. The use of rainout shelters has been suggested in some studies as a method to exclude 100% of rain from experiments while allowing other aspects of the environment to remain largely unaltered (Yahdjian and Osvaldo, 2002). Rainout shelters allow for control of the timing of drought stress. With this method, testing of genotypes can be conducted during the summer season. The main challenge, however, is that rainout shelters cannot be installed for large numbers of genotypes across many environments because of the

high costs of installation (Yahdjian and Osvaldo, 2002) and therefore, it becomes an impractical system.

#### **4.3.2 Induction of stress tolerance in maize**

For both RDS and MDS environments, the timing, duration and intensity (or severity) of stress are critically important (Barker *et al.*, 2004). Under MDS it is easier to control these three aspects than under RDS environments. The most critical period for drought stress in maize production is two weeks before flowering and two weeks after flowering. In this study the timing of the induction of stress could have been affected by the presence of different maturities groups of hybrids. As a result, hybrids may have been exposed to unequal levels of stress at flowering. Severe stress at this stage of maize development has detrimental effects on yield. Major stresses before silking cause ovule abortion and inhibition of ear development. If ears or silks develop, further stress after pollination leads to kernel abortion and reduced kernel numbers (Schussler and Westgate, 1995).

#### **4.3.3 Techniques for evaluating plants for drought tolerance**

Whether MDS or RDS environments are used, techniques to quantify stress so that plant performance is related to a defined level of stress are important. Soil water status gives an indication of how much water is available for uptake by the plant whereas plant water status can be used to evaluate the plant's need for water or how well it is adapted to its environment (Reicosky and Lambert, 1978; Cleary *et al.*, 2009). The unavailability of (effective) techniques to quantify soil and plant water status is a big challenge in evaluating plants for drought tolerance. Various techniques have been proposed but a few have come close to practical applicability. Techniques that measure soil water potential are regarded as being more reliable than those that measure volumetric water content (Cepuder *et al.*, 2008; Cleary *et al.*, 2009). The most common technique to measure water potential is tensiometry; however, it cannot provide readings of up to 1500 kPa (permanent wilting point). To quantify plant water status, the leaf porometer, GreenSeeker® and chlorophyll meter are being widely used. These techniques do not directly measure plant water status but a surrogate<sup>1</sup> which indirectly provides the level of water content in the soil or plant. The most reliable method is the Scholander pressure chamber because it allows direct measurement of plant water status (Scholander *et al.*, 1965). however, it is not a practical technique to evaluate large numbers of genotypes.

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<sup>1</sup> A parameter that indirectly provides a measure of soil water potential

#### **4.3.4 Choice of parent lines**

The main challenge faced in this study was the number of unsuccessful crosses which reduced the number of progeny from the factorial mating designs that could be analysed for performance, stability and combining ability. There were a number of likely causes of the lower than expected number of successful crosses:

- i. either male or female incompatibility;
- ii. either male or female sterility; and/or
- iii. unsynchronized flowering (protandry and protogyny)

Unsuccessful crosses led to incomplete factorial mating designs so the size of the mating designs were reduced to the nearest complete factorial matrix.

#### **4.4 Implications for breeding**

The superior inbred lines and hybrids identified in this study could be used in future breeding programmes as parents or released cultivars for drought stress environments. The identification of parental lines with good GCA effects and crosses between specific parental lines with good SCA effects for GY provides invaluable information for future drought tolerant breeding endeavours. The results indicated that both additive and non-additive gene action were important in the inheritance of GY. From these results, future work should focus on the validation of yield performance, stability and combining ability with other lines in these same and other test environments that replicate the conditions of target production environments. Also, future work should be based on evaluating an expanded gene pool of inbred lines with putative combining ability for drought tolerance. The range of environments simulating the drought conditions of target environments should be expanded. The repeatability of hybrid performance under specific drought stress conditions could be evaluated by: (i) reproducing the hybrids and testing them under defined MDS conditions; and (ii) crossing different tester lines to the potential drought tolerant donors.

The  $F_1$  hybrids and inbred lines with good performance could be used for three-way crosses and/or as parents for further inbred line development. Repeatability of the GCA effects of inbred lines will facilitate confirmation or establishment of heterotic groupings. As an extension to this study, genetic relationships determined using morphological and single nucleotide polymorphism (SNP) markers could also assist breeders in identifying divergent parents and for designing effective crossing programmes.



## 4.5 References

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## **Presentations emanating from this thesis**

### **Presentation 1**

Muyambo, C. 2013. Drought tolerance studies in maize: Literature overview

The presentation was done at PANNAR's annual research conference in Pretoria in October 2013. The content of the presentation was entirely my own and some of the thoughts and questions emanating from the conference were incorporated into this thesis.

### **Presentation 2**

Muyambo, C., Shanahan, P.E., and Brauteseth, E.M. 2014. Characterisations of the drought tolerance of F1 single cross maize (*Zea mays* L.) hybrids under random and managed drought stress (Abstract).

The presentation was done at the South African Plant Breeders Association (SAPBA) symposium in March 2014. The presentation contained original results from random and managed drought stress experiments of this thesis. The results presented were incorporated into Chapter 2 and 3 and the abstract was published in the 10<sup>th</sup> SAPBA booklet.