CHARACTERIZATION OF TRAITS CONTRIBUTING TO GAINS IN MAIZE GENETIC IMPROVEMENT UNDER DROUGHT AND HEAT STRESS

MASTER OF SCIENCE DEGREE IN FOOD SECURITY AND SUSTAINABLE AGRICULTURE (PRODUCTION)

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APPROVAL FORM

The undersigned certified that they have supervised and recommended to Bindura University of Science Education for acceptance of dissertation entitled ‘**Characterization of traits contributing to gains in maize genetic improvement under drought and heat stress**’ submitted in partial fulfilment of a Master of Science Degree in Food Security and Sustainable Agriculture in Production.

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Signature: ……………………………….

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DECLARATION

I hereby declare that the research project entitled "**Characterization of traits contributing to gains in maize genetic improvement under drought and heat stress**" submitted to Bindura University of Science Education, Department of Agricultural Economics, Education and Extension is a record of an original work done by me under the guidance and supervision of **Mr. Mutsengi (BINDURA UNIVERSITY) and Dr. Mainassara (CIMMYT ZIMBABWE)** and this work is submitted in partial fulfilment of the requirements for the award of a Master of Science Degree in Food Security and Sustainable Agriculture. The results embodied in this thesis have not been submitted to any University or Institute for the award of any degree or diploma.

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DEDICATION

To my blessed mother, **(Loveness Mtsilizah)** my source of inspiration.

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ABSTRACT

Drought and heat stress are important limitations to maize growth and sustainable agriculture worldwide. These two environmental factors influence physiological processes, crop growth, crop development and yield processes. The study was carried to (i) to assess the genetic gain in yield and other secondary traits in CIMMYT hybrids released from 1999 to 2016 for their tolerance to drought stress (ii) to assess the genetic gain in yield and other secondary traits in CIMMYT hybrids released from 1999 to 2016 for their tolerance to heat stress (iii) to examine the transpiration response to a drying soil of different maize hybrids under varying VPD regimes. Genetic gain within the International Maize and Wheat Improvement Centre (CIMMYT) breeding program were estimated using time series of maize (*Zea mays* L.) hybrids. The experiments were planted using a 15*15 alpha (0.1) lattice design. A total of 72 of the best-performing hybrids from local trials from 1999 to 2016 were selected to form an era panel and evaluated in 2 trials in Zimbabwe – (Chiredzi). Treatments included heat stress and managed drought stress. Genetic gain was estimated as the slope of the regression of grain yield on the year of hybrid release. Grain yield was significantly different for the hybrids at $P \leq 0.01$. The hybrids significantly varied for traits Anthesis date, Anthesis-silking interval, Plant height, Ear height, Senescence 1 at $P \leq 0.001$. No significant differences were recorded for Ears/plant and Senescence 2. Heritability estimates under heat stress and managed drought stress conditions ranged from medium to moderately high indicating that the secondary traits can be directly selected for, to improve the hybrids. The same CIMMMYT hybrids were also grown in a pot experiment at CIMMYT Zimbabwe, Harare station to observe how they manage transpiration under varied Vapor pressure deficit regimes and also varying water deficit conditions. The experiment was laid out in completely randomized deign. 75 hybrids were planted with 3 replications. Understanding response of transpiration in maize hybrids contrasting for drought tolerance under different VPD levels and varying moisture depletion is needed as it enables identification of hybrids that tolerate moisture stress and elevated temperatures. Newly released hybrids exhibited high ability to conserve water under limited water conditions as compared to the old hybrids. The experiment was done when the ambient air had mean of 15.75% Relative Humidity and 28.73℃. In both (Vapor pressure deficit and soil drying) experiments hybrids CZH1261, CZH132119 and CZH15013 consistently appeared in the best 10 ranked hybrids that conserve water, whilst hybrids CZH01008, CZH15212 and CZH15467 consistently appeared in the least ranking group for not being able to conserve water.

Key Words: Transpiration - Vapor pressure deficit - Drought stress - Heat stress - Genetic gain

LIST OF ABBREVIATIONS

%	Percent
°C	Degrees Celsius
AD	Anthesis date
ANOVA	Analysis of variance
ASI	Anthesis Silking Interval
CIMMYT	International Maize and Wheat Improvement Center
CTD	Canopy Temperature Depression
EPP	Ears per plant
ET	*Exserohilum (Helminthosporium) tursicum*
FAO	Food and Agriculture Organization
FTSW	Fraction of transpirable soil water
GLS	Grey leaf spot
GYD	Grain yield
ha	Hectare
IPCC	Intergovernmental Panel on Climate Change
K	Potassium
Kg ha^{-1} yr^{-1}	Kilogram per hectare per year
LSD	Least significant difference
META-R	Multi-environment trial analysis using R-statistical package
MSV	Maize streak virus
MT	Metric tonnes
N	Nitrogen
NTR	Normalized transpiration ratio
OPV	Open-pollinated variety
P	Phosphate
SEN	Senescence
SSA	Sub-Saharan Africa
TR	Transpiration
VPD	Vapor pressure deficit
WFP	World Food Programme
WTA	Water to add
ΔG	Genetic gain

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CHAPTER 1

1.0 BACKGROUND OF THE STUDY

1.1 Significance of maize in sub-Saharan Africa

Maize (*Zea mays* L.) is a staple food to more than 1.2 billion people in sub-Saharan Africa (SSA) and Latin America. Maize occupies the second position after wheat in area (180 million hectares of land under production) and is considered the most important cereal crop in terms of quantity produced worldwide (FAO, 2015). It is widely cultivated staple crop that plays an important role in ensuring food security of SSA, (FAO, 2013). Maize occupies over 35 million hectares of sub-Saharan Africa's estimated 200 million hectares of land and produced under diverse climatic and socio-economic conditions, (CIMMYT, 2016). (van Ittersum *et al.,* 2016) reported the existence of a yield gap between the actual farm yields and the yield potential of the currently grown cultivars, which causes the SSA region not to be self-sufficient, thereby relying on imports to meet its cereal demand.

Maize is the primary staple food crop for close to 98% of the 12.7 million people in the country (CIA, 2012). Despite its importance, farmers in Zimbabwe harvest an average of 0.8 tons/ha which is compared against worlds' average of 5 tons/ha, (Cropfood, 2010). Current yields of maize in SSA are estimated at 1.8 MT/ha a figure which is far below the average potential that can be obtained by improved varieties and better crop management, even though some countries have been making significant productivity gains in recent years, (Abate *et al.,* 2017).

Yields recorded in recent and current years are barely enough to guarantee food security in the region, and often fall short of quantities required to meet population growth. The need to increase maize yields for food security in SSA is heightened by both climate and demographic changes. The population of sub-Saharan Africa is predicted to double by 2045, (United Nations / Department of Economic and Social Affairs, 2009) while climate predictions for the region show decreasing precipitation and increasing temperatures, (IPCC, 2007).

However, current maize production in SSA even with newly improved cultivars and good management is not sufficient to meet the growing demand in most countries and yields remain among the lowest in the world (Ray *et al.,* 2015) due to an array of biophysical and socioeconomic constraints (Shiferaw *et al.,* 2011).

1.1.1 Major abiotic stresses affecting maize productivity in sub-Saharan Africa

Maize, which is grown mainly under rain-fed conditions, is susceptible to increasing intensity and frequency of drought and heat stress due to climate change. This in turn increases chances of crop failure, negatively affecting livelihoods and food security status of millions of smallholder farmers and their families.

Heat and drought have multiple, negative impacts on crop yields, including reducing leaf photosynthesis and enhancing leaf senescence rates thus weakening global food security. Strong relationship exists between plant water status and temperature, which makes it difficult to separate contribution of drought and heat stress under field conditions, (Rivero *et al.,* 2007). Water shortage is the single one factor for plant growth that ultimately causes reduction in crop yield more than any other stress condition. Heat stress can also influence water relations of crops indirectly through faster depletion of soil water. Heat stress can deplete waster faster from the soil through combination of increased evaporation by influencing soil temperatures and transpiration by increased vapor pressure deficit, (Fahad *et al.,* 2017).

1.1.2 Impact of drought stress on maize productivity

Drought can be defined as the absence of rainfall or irrigation for a period of time sufficient to deplete soil moisture and injure plants. Drought is one of the major limitations under rain-fed systems with an estimated 40% of SSA's maize area facing occasional drought stress contributing to yield loss in the range of 10–25% (Fisher *et al.,* 2015). However, heat stress both alone and in combination with drought stress is increasingly becoming a constraint to maize production (Cairns *et al.,* 2012). Around 25% of the maize crop suffers frequent drought leading in loss of up to half the harvest (Cairns *et al.,* 2012). In addition, yield losses due to drought average 15% annually in sub-Saharan Africa (Badu-apraku *et al.,* 2014) even in areas where total rainfall is reasonably high. Total yield losses may be observed when severe drought occurs at the reproductive stages of plant development. The region has the lowest production levels at less than 2 tons/ha (Smale *et al.*, 2011) compared to the world average of 4.9 tons/ha as of 2012 (FAO, 2013). Millions of farms in southern Africa recently have been hit by El Niño, causing problems on sustaining the population across the region. This calls for urgent need to improve maize productivity in SSA for increased food security and to sustain economies.

1.1.3 Impact of heat stress on maize productivity

Heat stress is defined as the rise in temperature beyond a threshold level for a period sufficient to cause permanent damage to plant growth and development Heat stress has a number of negative effects on crop growth and can result in considerable yield losses in major cereals due to reduced photosynthesis (by damaging the stability of the thylakoid membrane structure and degrading chlorophyll, which reduces light energy absorption, transfer and photosynthetic carbon assimilation), pollen sterility, kernel abortion and earlier senescence resulting in lower maize grain number (Edreira *et al.,* 2011). Demand for maize grain is expected to expand by 50% in the developing world by 2050 (Msangi and Rosegrant, 2011) while, over the same period, mean global temperature is expected to increase by up to approximately 2 °C (Niang *et al.,* 2014). Statistical studies have indicated that daily maximum temperature greater than approximately 30 °C limit maize yields (Lobell *et al.*, 2011).

1.1.4 Progress and limitations that have been recorded in genetic crop improvement

Genetic gain studies have been usually made by comparing a historic set of cultivars with uniform management by many of researchers. Genetic gain is the amount of increase in performance achieved per unit time through artificial selection (Xu *et al.,* 2017) and can be defined as

$$R_t = \frac{ir\sigma_A}{y}$$

where R*t* is genetic gain over time, *i* is selection intensity, r is selection accuracy, σ_A is genetic variance, and y is years per cycle (Falconer and Mackay, 1996). This equation provides the framework for measuring breeding progress. Selection intensity is determined by the selection rate, that is, the proportion of the population selected from the total population (Xu *et al.*, 2017). Genetic gain is evaluated in era studies. An era study involves the evaluation of the best performing varieties from different years or points in time in a single trial in which the different varieties receive the same management, attributing differences to genetic differences (Hall and Richards, 2013). Era studies that compares older and newer hybrids are a common approach to understanding how genetic selection has contributed to economically important traits like grain yield in maize (Duvick, 2005; Wang *et al.*, 2014).

However, absolute yields in the region are still lower, reflecting the opportunity to further improve the yield potential of tropical maize, including in stress-prone environments. Yields both in the hybrid and OPV pipelines from regional trials showed no indication of reaching plateaus, confirming further gains can be made starting from the local hybrids currently under development from breeding CIMMYT breeding programme.

Furthermore, the impact of past selection of genetic gains for yield and secondary traits on developed CIMMYT hybrids under heat and drought stress is not as thoroughly documented in sub-Saharan Africa. Genetic gain needs to be further improved to meet the many challenges we are facing, mainly heat and drought stress. As current and expected future relative genetic gains are not sufficient to meet the projected demand for major crops by 2050, other opportunities and approaches need to be explored.

1.2 Problem statement

Heat and drought stress are undoubtedly becoming major constraints to maize productivity and food security globally. Heat tolerance was not previously a trait in African breeding programs. Relatively little research has been conducted on heat stress tolerance of maize as compared to other abiotic stresses. Breeding has been going on for a number of decades in Zimbabwe but it is not really known whether there is real improvement in the performance of CIMMYT maize hybrids. Research on genetic gains on yield and other secondary traits has not been quantified in Zimbabwe. If there is genetic gain, it is not known which traits are the drivers of the genetic gain in CIMMYT Zimbabwe breeding programmes, or at least there has been no correlation of traits to genetic gain. Demand from population growth for maize output is not met by current production. Resources for breeding have been reduced – now research requires obtaining higher genetic gains from less resources. Much work of maize genetic gains in Africa has focused on hybrids from West African countries and also OPVs.

1.3 Justification

Maize breeding has been ongoing for many years, but nevertheless there is need to optimize the current breeding strategies (Longin *et al.,* 2012) in order to improve productivity in the face of new challenges. The demand for food is set to increase in the coming years and development of high yielding hybrids will play a critical role in mitigating food shortages. A better understanding of growth and developmental responses of crop plants to drought and heat stress is of extreme significance as the growth and developmental traits can be utilized as indicators of drought and heat stress tolerance and improve crop productivity. Understanding the amount of genetic gain realized through past crop breeding efforts allows adoption of corrective methods and more efficient resource allocation that together result in an increase in the breeding programs efficacy, (Jewell *et al.,* 1994). Besides quantifying the progress obtained in a certain period, genetic gain analysis also enables aggregation of other information, which include comparison of the gains obtained with the use of diverse breeding strategies or in different environments, (Wilson *et al.*, 2014). Genetic gains have been done, but this research is trying to find what traits have contributed to these gains. If known, they are quickly incorporated in breeding programs to speed up genetic gains to cover population growth and decreasing water resources to meet crop production (demand gap).

1.4 Objectives

1.4.1 Main objective

The general objective of this study is to characterize traits contributing to gains in CIMMYT maize hybrids (released 1999-2016) and genetic improvement under drought and heat stress.

1.4.2 The specific objectives are:

- To assess the genetic gain in yield and other secondary traits in CIMMYT hybrids released from 1999 to 2016 for their tolerance to drought stress.
- To assess the genetic gain in yield and other secondary traits in CIMMYT hybrids released from 1999 to 2016 for their tolerance to heat stress.
- To examine the transpiration response to a drying soil of different maize hybrids under varying VPD regimes.

1.5 Research Hypotheses

- There is significant yield and secondary traits performance variation between the hybrids released by CIMMYT from 1999 to 2016 for their tolerance to drought stress.
- There is a significant variation in grain yield and key secondary traits that explain the observed yield gains in CIMMYT hybrids released from 1999 to 2016 for their tolerance to heat stress.
- There is significant transpiration response to a drying soil of different maize hybrids under varying VPD regimes.

1.6 Outline of Thesis

The thesis consists of six chapters. Chapter 1 discusses the overall background, significance of maize in Zimbabwe, regionally and globally and objectives of the study and hypotheses. Chapter 2 presents a literature review on previous related studies and topics related to this study. Chapter 3 This chapter presents the methodology and procedures followed by the researcher. It covers all the procedures and materials pertaining to crop management, experimental designs, description of sites and data collection and analyses. Chapter 4 and 5 manuscript results obtained are presented in table, graphical formats and discussed based on observed findings in comparison with other researchers' findings. Chapter 6 summary, conclusions and recommendation are presented drawn from chapter 3, 4 and 5 findings.

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CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Introduction

2.1.1 Maize production globally

Maize (*Zea mays* L.) plays a critical role in meeting the high food demand and is globally one of the most widely cultivated crops (Haarhoff and Swanepoel, 2018). Another study by (Alexandratos and Bruinsma, 2012) shows that global demand for cereals is expected to reach 3 billion tons in 2050, with majority of the demand coming from Asia and Africa.

Maize is facing limitations from biotic and abiotic stresses. Biotic stress includes (diseases, insects and weeds) whilst abiotic stress includes moisture stress, high and low temperature stress, salinity and nutrient stresses.

Global cereal demand is projected to outstrip genetic gains by 2050 (Ray *et al.*, 2015) while climate change threatens to reduce their impact (Challinor *et al.*, 2014). To accelerate yield improvement, physiological traits at all levels of integration need to be considered in breeding, (Gourdji *et al.*, 2013).

2.1.2 Maize production in Africa

300 million people in Africa depend on maize as their staple crop. The projected annual maize demand in SSA had been estimated to be 500 million tons by the year 2020, which will surpass the demand for both wheat and rice (Pinstrup-andersen, 2001). This is a result of a host of abiotic and biotic stress factors that affects the crop (FAO, 2013).

Maize is the principal staple in most countries within Eastern and Southern Africa, hence its availability and affordability is central to ensuring food security in the region. With more than 33 million ha harvested each year, maize occupies the largest land area of all staples in sub-Saharan Africa (SSA). More than 208 million households in SSA depend on maize for food security and it also acts as a source of livelihoods support. SSA accounts for more than 950 million people, which equates to about 13% of the global population. By 2050, this share is projected to increase to almost 22% or 2.1 billion, (Africa, 2016). Demand for maize grain is expected to expand by 50% in the developing world by 2050 (Msangi and Rosegrant, 2011) while, over the same period, mean global temperature is expected to increase by up to approximately 2°C (IPCC, 2014). The rapidly growing population in SSA has therefore necessitated the need to increase maize production in the region. Undernourishment has been a long-standing challenge as production is not sufficient to meet demand, with uneven progress across the region, (FAO, IFAD and WFP., 2015). Therefore; the development of maize varieties with enhanced performance under biotic and abiotic stresses continues to be an important objective (Quiroga *et al.*, 2017).

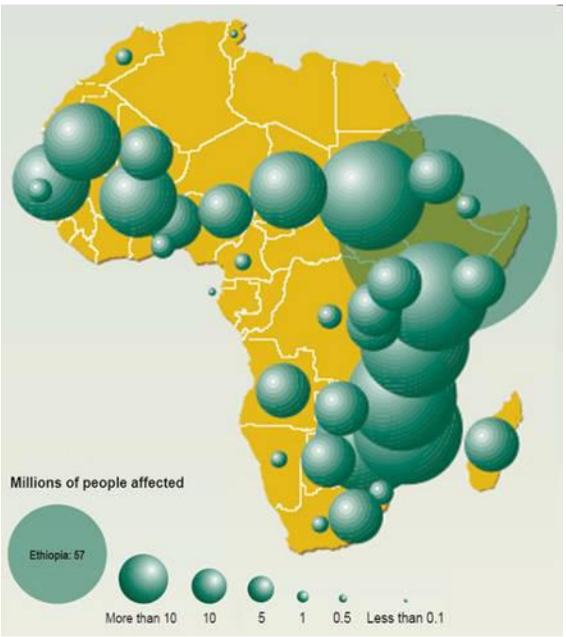


Figure 2.1: The Impact of Drought in the Tropics

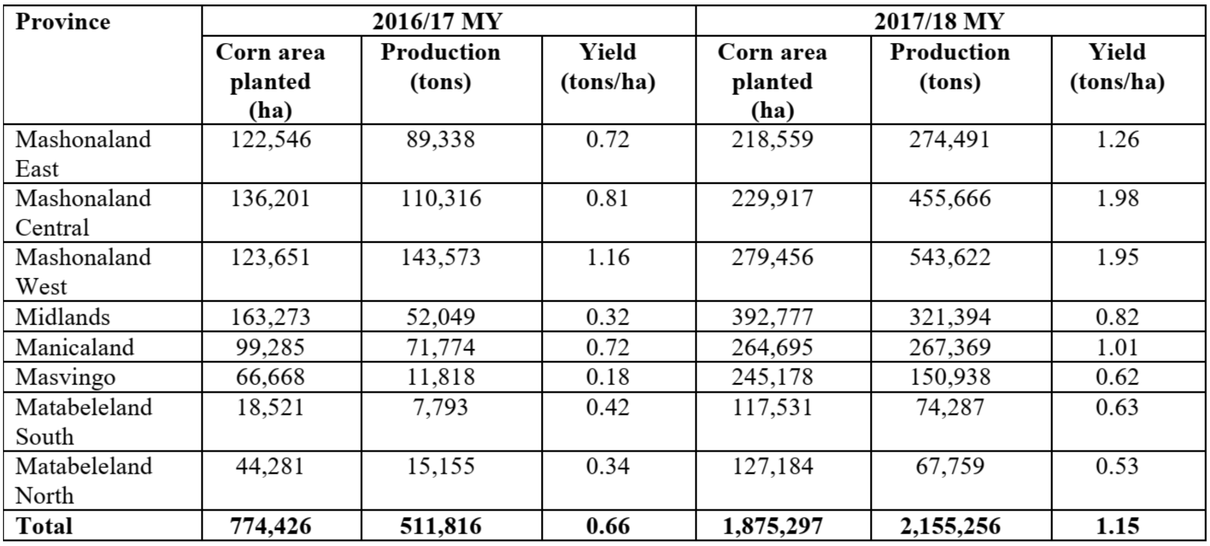
Source: (Prasanna, 2017)**.**

Abovementioned, Figure 2.1 gives an outline of how majority parts of Africa are being affected by occasional and frequent droughts. El Niño in Southern Africa, 2015-2016 affected food security of an estimate of 15.9 million people (Prasanna, 2017). El Niño is a climate cycle in the Pacific Ocean with a global impact on weather patterns. Drought is an unfortunate recurring theme in sub-Saharan Africa. Due to climate change, varieties on the market are now unable to withstand those high heat and prolonged dry spells especially the El Niño that was experienced during 2015/16 growing season hence low grain yields are received.

2.1.3 Maize production in Zimbabwe

Maize is the fundamental staple crop and main source of carbohydrates, therefore, the most important grain crop in Zimbabwe (Kapuya *et al.*, 2013). It is the main source of livelihood for over 70% of Zimbabwe's population, either directly through production or indirectly through value addition (Anseeuw *et al.*, 2012). However, most parts of Zimbabwe have been receiving low maize yields.

Table 2.1: Maize area planted, production and yield for the 2016/17 MY and 2017/18 MY



Province	2016/17 MY			2017/18 MY		
	Corn area planted (ha)	Production (tons)	Yield (tons/ha)	Corn area planted (ha)	Production (tons)	Yield (tons/ha)
Mashonaland East	122,546	89,338	0.72	218,559	274,491	1.26
Mashonaland Central	136,201	110,316	0.81	229,917	455,666	1.98
Mashonaland West	123,651	143,573	1.16	279,456	543,622	1.95
Midlands	163,273	52,049	0.32	392,777	321,394	0.82
Manicaland	99,285	71,774	0.72	264,695	267,369	1.01
Masvingo	66,668	11,818	0.18	245,178	150,938	0.62
Matabeleland South	18,521	7,793	0.42	117,531	74,287	0.63
Matabeleland North	44,281	15,155	0.34	127,184	67,759	0.53
Total	**774,426**	**511,816**	**0.66**	**1,875,297**	**2,155,256**	**1.15**

Source: (Ministry of Agriculture - Grain and feed annual report, 2017).

Table 2.1 above shows average yield in Zimbabwe per province for the past two growing seasons. Yields obtained averaged 0.8 tons/ha which far less to meet food security of the country. Yields in Masvingo, Matebeleland are mostly affected by heat and drought as the areas are situated in agro-ecological zones that receive low rainfall to sustain crop production.

2.2 Major abiotic stress factors affecting maize production

Combined heat and drought stress is generally more detrimental than either stress alone (Pradhan *et al.*, 2012) and is much harder to control in field trials. The reduced precipitation and changed rainfall patterns are causing the frequent onset of droughts around the world (Lobell *et al.*, 2011). Severe droughts cause substantial decline in crop yields through negative impacts on plant growth, physiology, and reproduction (Barnabás *et al.*, 2008). These patterns are predicted to result in significant price rises by 2050: more than 50% for maize, without accounting for climate change (Ringler and Zhu, 2015).

2.2.1 Drought and maize production

Drought is the most important abiotic stress factor for maize production in both the temperate and tropical environments and annual average yield losses to drought are estimated to be 15% or even more of potential yield on a global basis (Fischer and Edmeades, 2010). It hinders the plant growth and development, and limit the crop productivity more than any other ecological component depending on the genotype, duration and intensity, and plant developmental stage (Anjum *et al.*, 2011). Yield losses due to drought average 15 percent annually in sub-Saharan Africa (Badu-Apraku and Lum, 2010).

Grain yield losses could be higher if drought occurs at the flowering and grain filling periods (Farooq *et al.,* 2011). In addition, drought duration and intensity are also totally unpredictable (Bashir *et al.,* 2016). Furthermore, drought interferes with growth, nutrient and water relations, photosynthesis, assimilate partitioning and ultimately cause a significant reduction in crop yields (Farooq *et al.,* 2009).

Water stress can affect soil chemical, physical, and biological activities that are essential for plant and soil health. Drought affects nutrient uptake by crops, as water is the major medium for moving nutrients into plants as a result of water uptake. The increase in soil temperature associated with lack of soil moisture has an impact on microbial activities and nutrient processing, both of which are important for plant use for biomass and grain production, (da Silva *et al.,* 2011). Furthermore, drought can cause nutrient deficiencies, even in fertilized soils, due the reduced mobility and absorbance of individual nutrients, leading to a lower rate of mineral diffusion from the soil matrix to the roots. Under water deficit conditions, a reduction in stomatal conductance constitutes one of the first strategies used by plants to diminish the transpiration rate, (CARR, 2009). Exposure to stress may induce alterations in photo-biological processes, resulting in stomatal restrictions regarding the supply of carbon dioxide and limitations to non-stomatal components, with harm to the reaction centres of photosystems I and II (PSI and PSII), thereby compromising photosynthesis efficiency, (Angelopoulos *et al.,* 2007).

Under heat and drought stress, with stomata closed and transpiration reduced, leaves will begin to wilt, and if conditions persist, plant leaves can age prematurely, negatively affecting photosynthesis and yield. In addition, reductions in plant water status would result in loss of turgor, thus leading to reduced cell division rather cell expansion, inhibition of leaf expansion and also leading to early or partial stomatal closure, (Blum, 2011). Reduced leaf expansion leads to decreased transpiration, lowering intercepted radiation (lowers photosynthetic capacity), ultimately decreasing biomass production, (Reddy *et al.,* 2003).

Traits such as number of ears per plant (reduced barrenness), anthesis and silking dates and the anthesis-silking interval are influenced by drought stress and have proved useful when selecting for drought tolerance in maize (Campos *et al.,* 2006).

2.2.2 Heat stress and maize productivity

High temperature has become a global concern because it severely affects the growth and production of crops. Heat stress is a major cause of yield loss and numbers and duration of heat events are projected to increase in the future. However, evidences are emerging in recent years that indicate the negative effect of high temperature on the performance of maize. A recent study showed that each degree day spent above 30°C reduced the final yield of maize by 1% under favourable growing conditions and 1.7% under drought stressed environments (Lobell *et al.*, 2011). The most important effects of elevated temperatures on maize yield reduction include shortened life cycle (rapid accumulation of heat units thus physiological maturity is reached early), reduced light interception, and increased sterility especially on pollen (Stone and Nicolas, 1995); (Cairns *et al.*, 2013). Furthermore, some studies showed that high temperatures at the time of flowering could reduce pollen viability (Porter and Semenov, 2005), cause kernel abortion (Edreira *et al.*, 2011) and reduce the number of seeds or number of kernels (Wheeler *et al.*, 2000). Therefore, mainstreaming heat stress tolerance in elite sub-tropical maize germplasm has emerged as an important breeding objective.

2.3 Selection based on yield and secondary traits under drought and heat stress

Secondary traits are plant characters other than grain yield that provide additional information on how the plant performs under a given environment (Lafitte *et al.*, 2007). The low heritability of maize grain yield under drought necessitates their use. An ideal secondary trait should be genetically variable, genetically correlated with grain yield in the target environment, should have a high level of heritability, be simple and cheap, non-destructive and fast to assay, be stable throughout the measurement period, and not associated with any yield penalty under non- stress conditions (Badu-Apraku and Akinwale, 2011).

In maize breeding for drought tolerance, the secondary traits that have been used include Anthesis-Silking interval, number of ears per plant, the number of kernels per ear, plant and ear height, leaf area, extent of leaf rolling, osmotic adjustment, leaf chlorophyll concentration, stomatal conductance, canopy temperature, hydraulic conductivity, abscisic acid concentration, ear growth and barrenness, stay-green characteristic, erect upper leaves, tassel size, and root architecture (Badu-Apraku and Akinwale, 2011). ASI and EPP were identified by (Lafitte and Edmeades, 1997) as the most reliable secondary traits among a number of secondary traits ranked on the basis of heritability, association with grain yield, cost, genetic variability, and time required for recording.

2.3.1 Significance of secondary traits to breeding

Grain yield is the primary and most important trait targeted in maize improvement (Cairns *et al.*, 2012) except in a few cases where maize is bred for silage biomass or nutritional quality. Unfortunately, grain yield heritability is highly variable and its selection is confounded by inconsistencies under variable environments (Cairns *et al.,* 2013). Studies on hybrids and inbred lines have concluded that yield is a sum of contributions by several traits having different contributory effects (Mohammadi *et al.,* 2011). It is therefore important to understand the nature of relationship that exists between grain yield and the secondary traits. The understanding of the relationship can help in devising an effective method to achieve high breeding gain in yield. Secondary traits are plant characteristics other than grain yield that provide additional information about how the plant performs under a given environment (Lafitte *et al.,* 2007). The relationship between yield and secondary traits has been exploited in breeding for increased yield in hybrids. Most of the important secondary traits such as ear prolificacy, plant height, flowering and anthesis days, ear length and grain moisture content have been reported to have high heritability and have been used in improving yield (Souza *et al.,* 2009). Several studies have demonstrated the importance of secondary traits by evaluating their genetic correlations with grain yield (Betrán *et al.,* 2003). Given the poor heritability of grain yield under drought stress conditions, genetic progress is hard to achieve via direct selection. Secondary traits use improves the selection response by focusing on direct effects of drought and avoiding confounding factors such as additional stresses (e.g. soil fertility, micronutrient deficiency and pathogens) that also determine final grain yield (Monneveux *et al.,* 2014).

Physiologically, genetic gains are associated with improved efficiency in grain production, increased tolerance to abiotic and biotic stresses, reduced tassel size, more upright leaves and lower grain protein percentage following changes in the genetic make-up. Stress tolerance comes from attributes such as stay green traits, tolerance to heat and drought stress, tolerance to cool and wet conditions, reduced barrenness under abiotic stress, tolerance to high plant density and reduced root and stalk lodging, which improves yield (Duvick, 2005).

2.3.1.1 Senescence

Modern hybrids have longer periods of growth due to stay green genes, (Bruce *et al.,* 2002). Delay of leaf senescence, also known as stay-green character, has been identified as an important component in the genetic improvement of several crops to promote stress tolerance and yield gain. The use of stay-green character in breeding programs may result in significant genetic progress for attributes such as high yield, industrial quality, disease resistance and tolerance to abiotic stresses, (Luche *et al.,* 2015). Leaf senescence affects the plant’s ability to fill the grains by reducing the grain filling duration. Plants that remain green in stress environments have the ability to have their chlorophyll active even in stress environments and therefore they can continue photosynthesizing even in stress environments as opposed to those that undergo necrosis and chlorosis.

2.3.1.2 Kernel weight

Individual kernel weight is an important trait for maize yield determination. In addition, maize grain yield can be described as a function of the number of harvested kernels and their individual weight which are both highly affected during the critical period around silking (Borrás and Otegui, 2001). Kernel weight (KW) is a highly heritable trait (Prado *et al.,* 2013) varying markedly among genotypes. Weight is an indication of the dry weight that would have been accumulated by a grain, high dry weight accumulation in stressed environments reflects resilience against the stress.

2.3.1.3 Anthesis-silking interval

Flowering in maize is a crucial trait in breeding for drought tolerance. ASI which is defined as the duration between male and female flowering in maize, has been used successfully to select drought tolerant maize. A short ASI has been correlated with improved performance of maize varieties under water limiting conditions. When maize flowers under drought, there is the delay in silking and the period between male and female flowering increases giving rise to a long anthesis silking interval (ASI), (Bänziger *et al.,* 1999). Plants with a large ASI under drought are often barren, or have few grains per ear as any further rise in temperature causes tassel blasting thus reduces the pollen viability and exposed silks may also desiccate rapidly and become non-receptive to pollen germination resulting in poor seed set and reduced grain yield, (Khodarahmpour *et al.,* 2011).

Drought stress may delay silk emergence until pollen shed is nearly or completely finished. Strong evidence associates an increased ASI with reduced grain yields in maize under drought stress. Tassels normally will shed pollen earlier (protandry) but the silks will not be ready for the pollen. There will be poor fertilisation hence very low to no grain formation. This asynchrony between male and female flowering dates is strongly associated with grain yield decreases in maize under drought conditions (Parajuli *et al.,* 2018).

2.3.1.4 Plant height

Reduction in plant height and ear height in maize has been a target of many breeding programs for a number of reasons, such as reducing lodging (Badu-Apraku *et al.,* 2017), improving standing ability of plants and reducing the amount of energy channelled towards maintenance of vegetative growth. Plant height is essential traits in maize breeding that plays significant role in plant lodging. It is one of the most heritable and easily measured traits in maize that is associated with grain yield. Semi-dwarf plants are desired, because such plants are more resistant to lodging.

2.3.1.5 Ear height

Ear height will indirectly increase yield through reduction in lodging, hence an optimum level for ear height is always desirable. Reducing the ear height below that level will decrease the yield as it becomes exposed to the rodent's and other pest attack in open field.

2.3.1.6 Ears per plant

Decreased number of ears per plant (EPP) may occur due to failure of fertilization (due to large ASI), or increased rate of kernel abortion due to water stress (Borrás *et al.,* 2003).

2.4 Breeding progress in maize breeding in the past years

Maize yields have risen continually wherever hybrid maize has been adopted, starting in the U.S. Corn belt in the early 1930s. Plant breeding and improved management practices have produced this gain jointly. On average, about 50% of the increase is due to management and 50% to breeding, (Duvick, 2005). The two tools interact so closely that neither of them could have produced such progress alone.

Genetic gains may have to bear a larger share of the load in future years. However, the climate will not make this task of increasing production easier. Drought is already prevalent in many maize-growing regions, and it is only expected to become more severe in the future (Harrison *et al.,* 2014). For breeders, the goal is to develop stress tolerant, productive cultivars. Grain yields have increased consistently since the advent of hybrid maize because of improved stress tolerance (Duvick, 2005). However, breeding gains achieved in any of the traits are only important if they are followed by breeding gains in grain yield (Vukosavljev *et al.,* 2011) which is the main objective of many breeding programs. Since heritability of traits varies with environment and genotype (Shimelis and Shiringani, 2010), breeding gains should be measured under prevailing environmental conditions and against specific objectives of a breeding program.

Early maize varieties were mainly low yielding landraces and open pollinated varieties (OPVs) contributed to the early improvements in maize yields (Duvick, 2005). Since the advent of hybrids significant gains have been realised. Many breeding programs now aim to improve the hybrids in one or more traits which contribute to yield increase. Several researchers have reported different genetic gains in grain yield and secondary traits in maize (Badu-apraku *et al.,* 2014; (Beyene *et al.,* 2015). The differences reported show that genetic gains are variable under different conditions and with different maize populations.

Genetic improvements can be arbitrarily classified as those that promote greater efficiency in grain production, and those that provide increased tolerance to abiotic and biotic stresses.

Several researchers concur to that maize yields have changed over the years. About 50% of the yield increases realised in the US since the 1930s have been attributed to genetic improvement accompanied by morphological changes in yield related traits (Duvick, 2005). The traits mostly targeted for change are those which contribute to efficient biomass accumulation and grain filling.

To meet the projected 100% increase in global demand for food, feed, and fiber by 2050 (Tilman *et al.*, 2011), linear progress will need to be increased. On the other hand, the genetic gain that has been achieved in breeders' experimental stations have not been realized in farmers' fields. Changes in climatic patterns, arable land, and water availability now provide additional challenges for ensuring yield stability across diverse environments (Monneveux *et al.*, 2013) and for closing the yield gap.

Identification and measurement of secondary traits associated with grain yield provides a guide to specific mechanisms that contribute to grain yield under drought. Ideally, secondary traits should be correlated with grain yield under stress, highly heritable, easy to measure, and stable over time. Genetic gains in grain yield of hybrids have been accompanied by changes in other traits.

2.5 Genetic gains history in maize

Globally

Under high input conditions, gain for maize grain yield has been estimated at 94.7 kg ha^{-1} yr^{-1} in China (Ci *et al.*, 2011), 132 kg ha^{-1} yr^{-1} in Argentina (Luque and Cirilo, 2006), 80 kg ha^{-1} yr^{-1} in Canada (Duvick, 2005), and 65 to 75 kg ha^{-1} yr^{-1} in the United States (Duvick, 2005). Under drought stress, the rate of genetic gain in the United States was estimated at 73 kg ha^{-1} yr^{-1} for mild drought stress (Duvick, 1997), 146 kg ha^{-1} yr^{-1} when drought stress was imposed at the flowering stage, and 76 kg ha^{-1} yr^{-1} when drought stress was imposed during mid-grain-filling stage (Campos *et al.*, 2004). In tropical maize, (Edmeades *et al.*, 1999) previously estimated a genetic gain of 144 kg ha^{-1} yr^{-1} under flowering stage drought stress in a recurrent selection program.

Regionally

Only a few such studies have been conducted in SSA (Kamara, 2015); (Badu-apraku et al., 2014). (Badu-apraku *et al.*, 2014) estimated genetic gain in OPVs in West and Central Africa. Using 50 OPVs separated into three eras (1988–2000, 2001–2006, and 2007–2010), (Badu-Apraku *et al.*, 2013) estimated a genetic gain in grain yield of 1.3% yr^{-1} under optimal

conditions and 1.1% yr^{-1} under managed drought stress. Genetic gain in grain yield per year in this study was higher than reported in West and Central Africa by (Ifie *et al.*, 2015) 109.4 compared with 40 kg ha^{-1} yr^{-1} under optimal conditions and 32.5 kg ha^{-1} yr^{-1} for managed drought and 22.7 kg ha^{-1} yr^{-1} for random drought stress compared with 13.5 kg ha^{-1} yr^{-1}.

Locally

Over the past 10 years, genetic gain in grain yield in the CIMMYT ESA hybrid maize breeding program was estimated at 109.4, 32.5, 22.7, 20.9, and 141.3 kg ha^{-1} yr^{-1} under optimal, managed drought, random drought, Low-N, and MSV-infested research conditions, respectively. To date, genetic gains in maize hybrid breeding locally has not been quantified. A recent study by (Masuka *et al.*, 2017) estimated genetic gain in CIMMYT's maize breeding program in ESA during 2000 to 2010, with particular emphasis on gains in grain yield under drought stress and a companion paper looking at genetic gain in OPVs in ESA over the same period (Masuka *et al.*, 2017) under optimal conditions, managed drought and random stress-prone environments.

2.5.1 Way forward to meeting current and future production needs

To maintain global food security, with the added challenge of climate change, there is an increasing need to exploit existing genetic variability and develop cultivars with superior genetic yield potential and stress adaptation. Genetic improvement is essential to achieve increments in maize grain yield components. Furthermore, the use of genetics to improve drought tolerance and provide yield stability is an important part of the solution to stabilizing global maize production. Studies indicate that about 25% of losses due to drought can be eliminated by genetic improvement in drought tolerance (Campos *et al.*, 2006).

Evaluating breeding gains allows breeders to understand the potential of a genotype and assess any unexploited genetic potential (Ci *et al.*, 2011). Investigation of breeding gains also helps in evaluating progress of current breeding program, prediction of possible course of action in future and selection of a breeding strategy (Campos *et al.*, 2006).

Therefore, breeding gains or improvement in any of the secondary traits would be expected to contribute to final yield. However, the final gain in yield depends on the genotype and correlations between the trait and grain yield (Bello and Olaoye, 2012).

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CHAPTER 3

To assess the genetic gain in yield and other secondary traits in CIMMYT hybrids released from 1999 to 2016 for their tolerance to drought stress.

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Abstract

Drought stress is an abiotic factor affecting growth and yields of crop plants and one of the most important limiting factors for maize growth and productivity in Zimbabwe, sub-Saharan Africa and world at large. Maize is a sensitive crop to drought, particularly at the reproductive stages of development. Improving drought tolerance in maize has become a major priority in maize breeding programs so as to increase grain yield over years. In this study we evaluated the genetic gain for yield and other secondary traits in CIMMYT varieties developed and released from 1999 to 2016 under drought stress conditions in Zimbabwe (Chiredzi during the 2018-19 season). 72 hybrids and 3 commercial checks were evaluated using a 15*15 alpha lattice. Newer varieties yielded better under drought compared to the older varieties. The study indicated a genetic gain of 35.03 kg ha^{-1} $year^{-1}$. Drought stress trial recorded significant differences in Grain Yield $P \leq 0.01$, Anthesis date, Anthesis-silking interval, Plant height, Ear height, Senescence 1 at $P \leq 0.001$. No significant differences were recorded for Ears per plant and Senescence 2 under drought stress. Positive genetic gains were observed for grain yield and secondary traits: Anthesis-silking date, Plant height, Ear height, Ears per plant under drought. The results indicated that hybrids with strong drought tolerance are CZH1261, CZH16340, CZH142020, CZH15603 and CZH15002.

Keywords: Drought stress - Abiotic stress - Genetic gain - Grain yield - Ears per plant.

3.1 Introduction

Drought stress has emerged as a common problem worldwide which can reduce maize crop productivity, (Bibi *et al.,* 2015). Drought is a meteorological term commonly defined as a period without significant rainfall (Jaleel *et al.,* 2009). Drought affects food production and food security. Maize grown in semi-arid tropical environments often faces a multitude of abiotic stresses such as drought (Cairns *et al.,* 2013). The growing concerns for food security and the sustainability of agricultural resources underlies the urgency and importance of tackling drought, which remains the single most important factor threatening the survival of people in the developing world. In addition, studies by (Jones and Thornton, 2003; Tesfaye *et al.,* 2016) projected that climate change could decrease rain fed maize yields by more than 12% in SSA. Maize is more sensitive to drought during the reproductive phase and traits most affected are anthers and silks (Meseka *et al.,* 2018). Delay in appearance of silk under drought stress conditions is responsible for increased anthesis-silking interval which is very critical index for efficient completion of reproductive growth stage. Traits such as number of ears per plant (reduced barrenness), anthesis and silking dates and the anthesis-silking interval are influenced by drought stress and have proved useful when selecting for drought tolerance in maize (Campos *et al.,* 2006).

3.1.1 Drought tolerant maize in sub-Saharan Africa

The aim of breeding for drought tolerant varieties is to enable farmers under dry land production to harvest at least 1 t ha^{-1} under drought stress to ensure household food security (Rovere et al., 2010). Frequency and severity of drought is predicted to increase due to climate change and deforestation, reducing global food production (Cairns *et al.,* 2012). The frequency of drought is predicted to increase to up to about 40% in some parts of SSA (Rovere *et al.,* 2010). Drought tolerant varieties will help curb predicted increase in chances of a failed season due to drought. One of the main objectives of CIMMYT's global maize programme in SSA is breeding for tolerance to drought, improving food security and farmers' livelihoods in Africa. Like in every breeding programme, there is need to evaluate the progress. By evaluating genetic gain, breeding strategies and objectives can be evolved based on the findings. This study will establish if there has been any genetic gain with respect to drought in CIMMYT hybrids released by the ESA maize programmes in from 1999 to 2016.

The aim of this study was to evaluate the genetic gain for yield and other secondary traits in CIMMYT hybrids developed and released from 1999 to 2016 under drought conditions in Zimbabwe. The objectives of the study were: i) To evaluate yield performance of hybrids released by CIMMYT released from 1999 to 2016 ii) To identify traits discriminating between the CIMMYT hybrids released from 1999 to 2016 iii) To estimate the contribution of these traits to the hybrids yield gains.

3.2 Brief description of the study site

This study was conducted in Chiredzi (21° 0' 58"S, 31° 34' 17E"). Chiredzi is in agro-ecological region IV. The study was a managed drought stress trial which on average received less than 450 mm of rain per annum. This amount of rainfall is either not sufficient to sustain maize production or is characterised by mid-season droughts that may coincide with critical stages such as ear determination, flowering and grain filling, resulting in reduced yield or total crop failure in maize. Chiredzi Research Station is characterised by red clayey loam soils.

3.3 Experimental design layout

The experimental design was a 15*15 alpha-lattice (Patterson and Biometrika, 1976) with 72 hybrids from different eras that were be replicated three times. The study included 3 local checks (SC513, SC403 and PAN413). The hybrids were coded CZH (CIMMYT Zimbabwe hybrids) which are early to intermediate maturing hybrids. The maize hybrids were assigned to eras as follows:

- **Era 1 = 1999-2002** (CZH99014, CZH99015, CZH99019, CZH00011, CZH00013, CZH00020, CZH01006, CZH01008, CZH02004, CZH02006 and CZH02008).

- **Era 2 = 2003-2006** (CZH03002, CZH03005, CZH03006, CZH04001, CZH04005, CZH04007, CZH0524, CZH0526, CZH0527, CZH0528, CZH0615, CZH0623 and CZH0629).

- **Era 3 = 2007-2010** (CZH0728, CZH0733, CZH0735, CZH0836, CZH088, CZH089, CZH0928, CZH0932, CZH0935, CZH0946, CZH1032, CZH1033 and CZH1036).

- **Era 4 = 2011-2016** (CZH1122, CZH1123, CZH1134, CZH1243, CZH1258, CZH1261, CZH1270, CZH132117, CZH132118, CZH132119, CZH132163, CZH132169, CZH142020, CZH142055, CZH142056, CZH142060, CZH15002, CZH15013, CZH15017, CZH15183, CZH15185, CZH15188, CZH15212, CZH15343, CZH15467, CZH15572, CZH15575, CZH15603, CZH16048, CZH16333, CZH16334, CZH16335, CZH16340, CZH16374 and CZH16389).

The number of hybrids per year ranged from 2 to 5 (in 1999 and 2014) and 7 to 15 (in 2015 and 2016). The hybrids were selected based on their superior performance in local trials in Zimbabwe between 1999 and 2016.

3.4 Trial management

Within this study environment refer to the drought imposed experimental conditions. Trial at the Chiredzi Research Station was planted on the 18th of July 2018. All hybrids were planted in 2 rows plots spaced 0.75 m apart and 0.25 m between plants. The trials were initially planted at two seeds per hole then later thinned to one plant per station at four weeks after crop emergence to achieve a population density of 53,333 plants ha^{-1}. Trial received basal fertilizer application rate 400kg/ha Compound D (7% N, 14% P, 7% K) at planting. Trail was top-dressed with two split applications of Ammonium Nitrate (34.5% N). First application (200kg) was at four weeks after crop emergence and the remaining 200kg at eight weeks after crop emergence. The managed drought stress trial was conducted during the dry season with irrigation applied at the beginning of the season to establish a good plant stand and withdrawing irrigation starting from 2 weeks before flowering to ensure that drought stress to coincide with the reproductive stage (Bänziger *et al.,* 2000). As a guideline, 7mm/hour for 6 hours was applied after planting to facilitate germination and thereafter 5mm/hour for 10 hours' irrigation cycle was administered depending on crop water needs.

3.5 Data collected

The traits that were recorded in this study are:

- **Grain yield** (GY) (All plants were hand harvested and shelled grain weight was measured. Grain weights was adjusted to 12.5% moisture using CIMMYT Fieldbook software content and 80% shelling percentage to calculate grain yield.).
- **Anthesis date** (AD) (measured as number of days after planting when 50% of the plants shed pollen); silking date, SD (measured as number of days after planting when 50% of the plants produce silk); anthesis-silking interval, ASI = SD – AD.
- **Senescence** (SEN) was estimated by visual notation and/or by counting the number of green leaves below the ear (Bänziger *et al.,* 2000). A score of 1-10 was used where 1 was when 10% of leaves below the ear, senesced and 10 was when 100% of the leaves below the ear senesced (Bänziger et al., 2000).
- **Plant height** (PH) measured as height between the base of a plant to the insertion of the first tassel branch of the same plant of 6 alternating plants in the plot. This was measured using LEICA DISTO gadget, taking the distance from the ground up to the flag leaf. Plant height was collected soon after flowering.
- **Ear height** (EH) was measured using LEICA DISTO gadget, taking the distance from the ground up to the ear height.
- **Ear number per plant** (EPP) was determined by dividing the total number of ears harvested by the number of plants in the plot at harvest.

To estimate the annual genetic gain achieved in grain yield and changes produced on associated agronomic traits, the mean values of each trait for each hybrid will be regressed against the year of release. The year of release shall be expressed as the number of years since 1999, the period when coordinated maize hybrid genetic improvement program started.

3.5.1 Data analysis methods

Analysis of variance was performed on plot means for grain yield and other measured secondary traits under drought stress using PBIB.test procedure of **R software version 3.5.2**.

Data was processed and analysed for variance in Fieldbook using the restricted maximum likelihood

(REML) method in the mixed model and using the built-in R-analysis in **Fieldbook (CIMMYT, 2012)**.

The best linear unbiased predictors (BLUP's) were computed for managed drought stress using the **Meta R statistical package**. The least significant difference (LSD) was used to determine significant differences among treatments at the 0.05 probability level.

3.6 Results

3.6.1 Analysis of variance of hybrid performance under managed drought stress trials

Table 3.1: Analysis of variance for grain yield and secondary traits under managed drought showing the top 10 and bottom 10 ranking hybrids

RANK	ENTRY	HYBRID	GYD	AD	ASI	PH	EH	EPP	SEN1	SEN2	RELEASE
1	**45**	**CZH1261**	4.53 a	75.67 efghijklmno	0.00 ab	201.52 defghijklmnopqr	105.60 bcdefghijklmnop	0.89	0.85 ijklm	1.17	2012
2	**73**	**CZH16340**	4.44 ab	75.33 fghijklmno	0.33 ab	223.56 a	130.14 a	0.93	1.49 efghi	1.17	2016
3	**54**	**CZH142020**	4.18 abc	77.33 cdefghijk	2.33 ab	213.36 abcdefgh	115.58 abcdefgh	0.78	0.83 ijklm	1.16	2014
4	**67**	**CZH15603**	4.13 abcd	77.00 defghijkl	0.00 ab	198.02 fghijklmnopqrs	111.01 abcdefghijklmn	0.86	1.86 cdefg	0.83	2015
5	**57**	**CZH15002**	4.07 abcde	73.00 lmnop	4.33 a	206.30 abcdefghijklmno	90.93 mnopq	0.87	1.97 bcdef	1.50	2015
6	**36**	**CZH0935**	3.95 abcdef	75.33 fghijklmno	1.00 ab	201.77 defghijklmnopqr	110.96 abcdefghijklmn	0.86	0.83 ijklm	1.33	2009
7	**52**	**CZH132163**	3.89 abcdefg	76.00 defghijklmn	1.00 ab	201.28 efghijklmnopqr	95.56 hijklmnopq	0.87	2.17 bcde	1.01	2013
8	**37**	**CZH0928**	3.89 abcdefgh	73.67 jklmnop	0.67 ab	201.05 efghijklmnopqr	100.18 fghijklmnopq	0.83	0.34 lm	1.33	2009
9	**68**	**CZH15343**	3.88 abcdefgh	81.33 abc	1.00 ab	218.92 abcd	115.73 abcdefgh	0.94	0.67 jklm	1.16	2015
10	**17**	**CZH03002**	3.86 abcdefghi	78.33 cdefgh	-0.33 b	215.25 abcdef	123.81 abc	0.80	0.79 ijklm	1.32	2003
66	**62**	**CZH15185**	2.64 klmnopqrs	75.67 efghijklmno	3.00 ab	216.41 abcde	110.32 abcdefghijklmn	0.84	1.81 cdefg	1.33	2015
67	**28**	**CZH0735**	2.51 lmnopqrs	72.67 mnopq	1.00 ab	186.88 qrst	101.19 defghijklmnopq	0.77	0.82 ijklm	1.49	2007
68	**34**	**CZH0946**	2.50 mnopqrs	72.00 nopq	-0.33 b	175.00 tu	84.89 qr	0.77	1.66 defgh	1.84	2009
69	**15**	**CZH03005**	2.44 nopqrs	78.00 cdefghi	1.00 ab	211.45 abcdefghij	111.19 abcdefghijklm	0.80	2.15 bcde	1.33	2003
70	**30**	**CZH0733**	2.36 opqrs	76.00 defghijklmn	0.67 ab	196.51 ghijklmnopqrs	99.38 fghijklmnopq	0.68	0.85 ijklm	1.33	2007
71	**75**	**CZH16048**	2.32 pqrs	78.00 cdefghi	0.00 ab	199.72 efghijklmnopqr	107.43 bcdefghijklmno	0.77	2.36 bcd	1.16	2016
72	**8**	**CZH00013**	2.28 qrs	74.00 ijklmnop	-0.33 b	199.31 efghijklmnopqrs	97.52 fghijklmnopq	0.81	0.84 ijklm	1.17	2000
73	**33**	**CZH088**	2.26 rs	68.67 q	1.67 ab	162.83 u	85.83 pqr	0.81	0.30 lm	1.34	2008
74	**11**	**CZH01008**	2.26 rs	84.00 a	0.00 ab	184.70 rst	103.87 bcdefghijklmnopq	0.69	1.15 ghijk	1.49	2001
75	**21**	**CZH0524**	2.14 s	74.67 hijklmnop	-0.67 b	203.00 cdefghijklmnopq	88.43 opqr	0.75	1.31 fghij	1.34	2005
	check	**SC403**	3.42 bcdefghijklmnc	75.33 fghijklmno	1.00 ab	213.03 abcdefgh	111.45 abcdefghijkl	0.84	1.51 efghi	1.33	1998
	check	**SC513**	3.02 defghijklmnopq	76.00 defghijklmn	1.67 ab	207.77 abcdefghijklmno	115.22 abcdefghi	0.77	1.83 cdefg	1.18	1997
	check	**PAN413**	2.80 ghijklmnopqrs	80.00 abcd	1.00 ab	194.78 ijklmnopqrs	97.76 fghijklmnopq	0.78	1.50 efghi	1.16	1998
		Mean	**3.26**	**76.3**	**0.80**	**201.3**	**103.2**	**0.80**	**1.29**	**1.24**	
		Mse	**0.78**	**20.33**	**2.87**	**282.00**	**359.38**	**0.01**	**1.09**	**0.11**	
		LSD	**1.14**	**4.19**	**1.88**	**17.56**	**20.53**	**0.137**	**0.96**	**0.49**	
		h2	**0.54**	**0.68**	**0.55**	**0.66**	**0.57**	**0.257**	**0.07**	**0.00**	
		p	**	***	***	***	***	**ns**	***	**ns**	
		Min	**2.14**	**68.7**	**-0.67**	**162.8**	**69.27**	**0.657**	**0.16**	**0.83**	
		Max	**4.53**	**84.00**	**4.33**	**223.6**	**130.1**	**0.937**	**3.18**	**1.84**	

*** P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, ns non significance at P ≤ 0.05, LSD (0.05) Least significant difference at (P < 0.05). Means with the same letter in each column have no significant difference (P<0.05).**

3.6.1.1 Anthesis date (AD)

Analysis of variance revealed highly significant (P ≤ 0.001) differences for anthesis date among the hybrids tested (Table 3.1). Average days to mid-anthesis ranged from 68.67 days CZH088 from era (2007 to 2010) to 84 days CZH01008 from era (1999 to 2002), (Appendix 1). The trial mean was 76.3 days.

3.6.1.2 Anthesis-silking interval (ASI)

Statistical analysis revealed highly significant ($P \leq 0.001$) differences among the hybrids tested for anthesis silking interval (Table 3.1). Mean ASI for the trial was 0.80 days, which ranged from -0.67 days CZH0524 from era (2003 to 2006) to 4.33 days CZH15002 from era (2011 to 2016) (Appendix 1).

3.6.1.3 Ears per plant (EPP)

Results showed non-significant differences among the hybrids tested for ears per plant at $P \leq 0.05$ (Table 3.1). The average number of ears per plant ranged from 0.66, CZH99019 from era (1999 to 2002) to 0.94 CZH15343 from era (2011 to 2016), (Appendix 1). The mean number of ears per plant was 0.80.

3.6.1.4 Plant height (PH)

Analysis of variance revealed highly significant ($P \leq 0.001$) differences for plant height among the hybrids tested (Table 3.1). Plant height means for the different sites ranged from 162.8 cm CZH088 from era (2007 to 2010) to 223.6 cm CZH16340 from era (2011 to 2016), (Appendix 1). The trial mean 201.3 cm.

3.6.1.5 Ear height (EH)

Statistical analysis revealed highly significant ($P \leq 0.001$) differences among the hybrids tested for ear height (Table 3.1). Ear height means for the ranged from 69.27 cm CZH1123 from era (2011 to 2016) to 130.14 cm CZH16340 from era (2011 to 2016), (Appendix 1). The trial mean 103.2cm.

3.6.1.6 Senescence (SEN)

The hybrids significantly varied for trait Senescence 1 at $P \leq 0.001$ (Table 3.1). No significant differences were recorded for Senescence 2 (Table 3.1). Senescence1 recorded at 50% silking and two weeks after varied from 0.16 CZH99014 from era (1999 to 2002) and 3.18 CZH132169 from era (2011 to 2016), (Appendix 1). Mean trial for senescence1 was 1.29. Senescence2 was recorded later four weeks after 50% silking and varied from 0.83 CZH15603 from era (2011 to 2016) lowest and 1.84 CZH0946 from era (2007 to 2010), (Appendix 1) highest with mean trial recorded was 1.24.

3.6.1.7 Grain yield (GYD)

Results showed significant differences among the hybrids tested for grain yield at $P \leq 0.01$. The hybrids were ranked based trial average yield from the highest to the least yielding (Table 3.1). Average grain yield among the top 10 yielding hybrids ranged from 3.86t ha^{-1} CZH03002 from (2003-2006 era) to 4.53 t ha^{-1} CZH1261 from (2011 to 2016 era) (Table 3.1). The yield range among the 10 lowest yielding hybrids was lower than the top 10 yielders ranging from 2.14 t ha^{-1} CZH0524 from (2003 to 2006 era) to 2.64 t ha^{-1} CZH15185 from (2011 to 2016 era). Checks ranked at 42 (SC403 - 3.42 t ha^{-1}), 49 (SC513 - 3.02 t ha^{-1}), and 60 (PAN413 - 2.80 t ha^{-1}) for checks 1, 2 and 3 respectively released in 1998, 1997 and 1998 respectively.

3.6.2 The yield performance of maize hybrids under managed drought stress

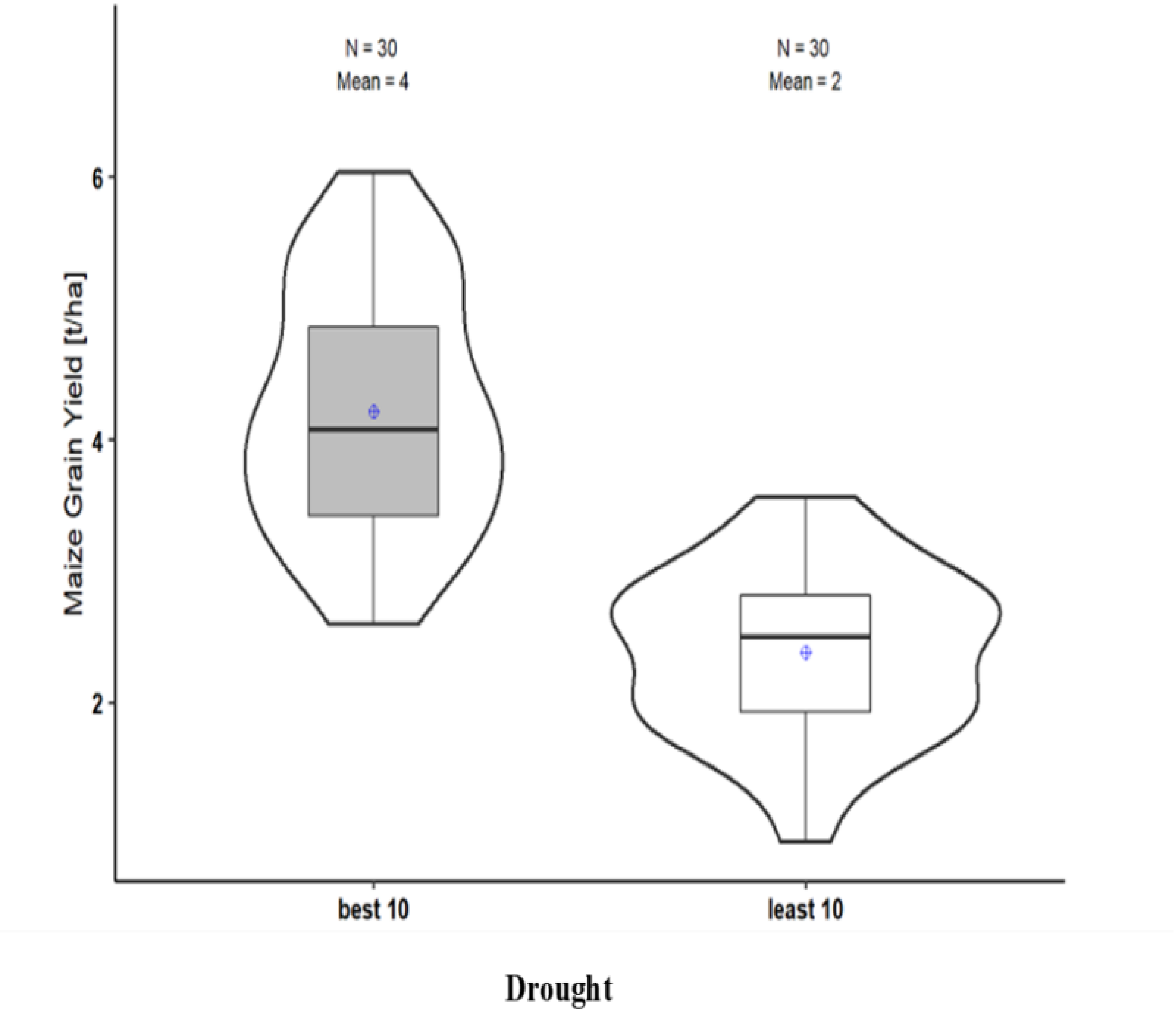


Figure 3.1: Comparison of best and least ranked hybrids for grain yield under drought and heat.

The best 10 ranked hybrids exhibited more concentration of yield around 3 t ha^{-1} to 4.2 t ha^{-1} on the box plot embedded with violin plot as shown in Fig 3.1. However, the least performing hybrids were concentrated more around 1.8 t ha^{-1} to 2.4 t ha^{-1}.

3.6.3 Genetic gain in yield and secondary traits under drought stress

The study showed significant genetic gain in hybrids developed by the CIMMYT ESA drought tolerance breeding programme over the 16-year period from 1999 to 2016.

3.6.3.1 Genetic gain in grain yield under drought stress

$$\boldsymbol{GYD = -67.12530 + 0.03503 * Release}$$

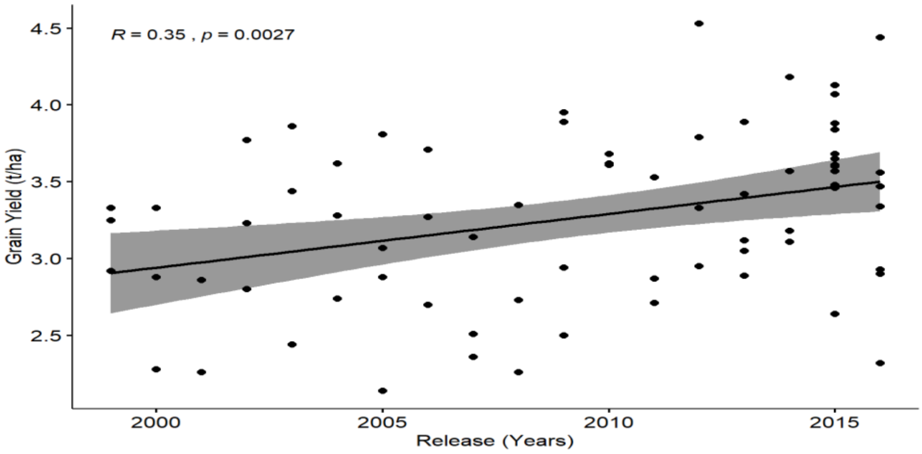


Figure 2.2: Genetic gain in yield in CIMMYT hybrids released from 1999 to 2016 tested under managed drought stress.

Genetic gain in grain yield against time of release showed net positive gains of 35.03 kg ha^{-1} year^{-1} (Figure 3.2). The new hybrids yielded better than the old hybrids.

3.6.3.2 Genetic gain in days to mid-anthesis under drought stress

$$\boldsymbol{AD = 142.41867 - 0.03292 * Release}$$

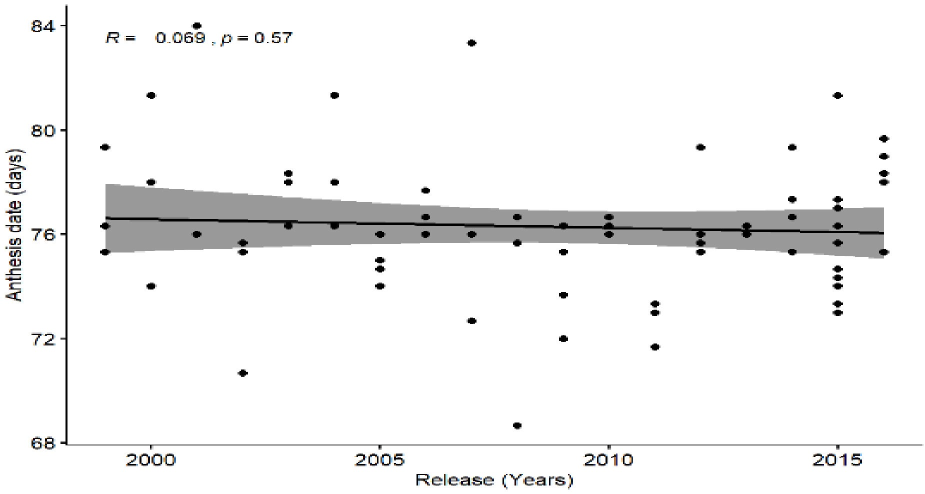


Figure 3.3: Genetic gain in days to mid-anthesis in CIMMYT hybrids released from 1999 to 2016 tested under managed drought stress.

There was no change in number of days to mid-anthesis over the 17 years ($P \leq 0.57$), (Fig 3.3). Newer and older hybrids flowered almost within the same days.

3.6.3.3 Genetic gain anthesis silking interval under drought stress

$$\boldsymbol{ASI = -61.21229 + 0.03086 * Release}$$

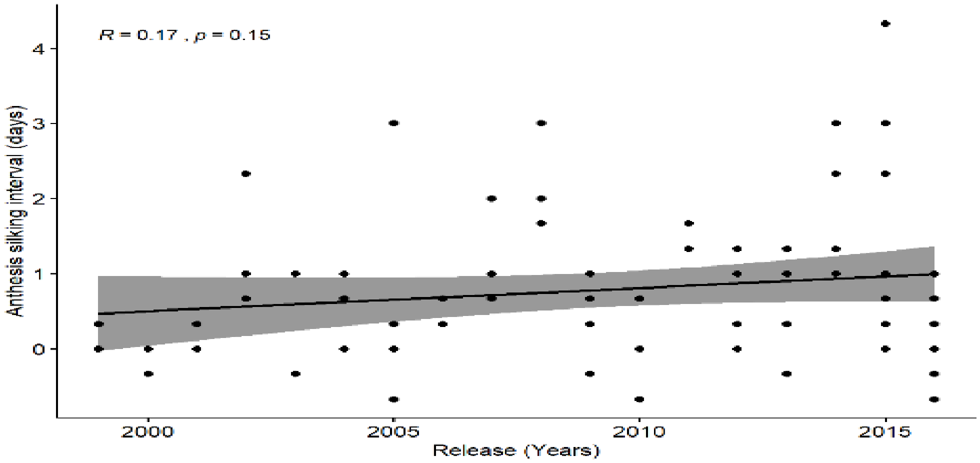


Figure 3.4: Genetic gain in anthesis silking interval in CIMMYT hybrids released from 1999 to 2016 tested under managed drought stress.

ASI showed a trend to increase at a rate of 0.03 d year $^{-1}$ (Figure 3.4) but the change was not significant ($P \leq 0.15$).

3.6.3.4 Genetic gain in plant height under drought stress

$$\boldsymbol{PH = -724.4652 + 0.4607 * Release}$$

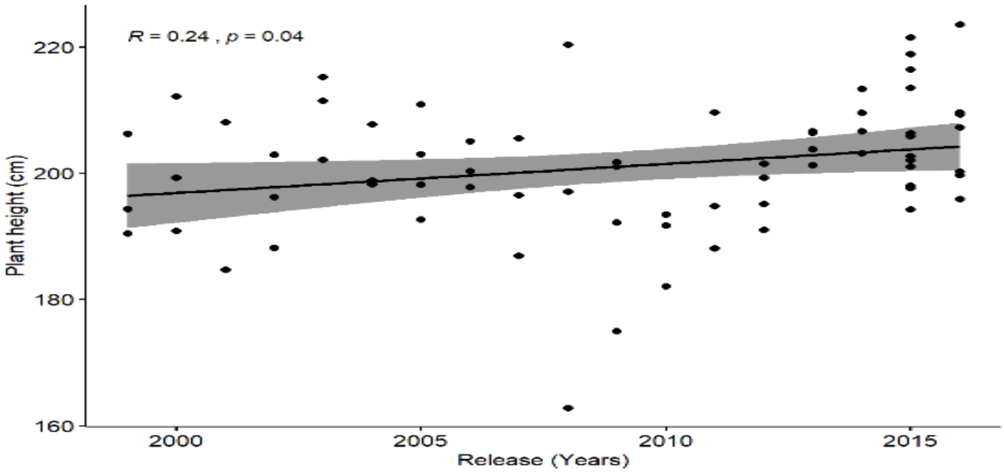
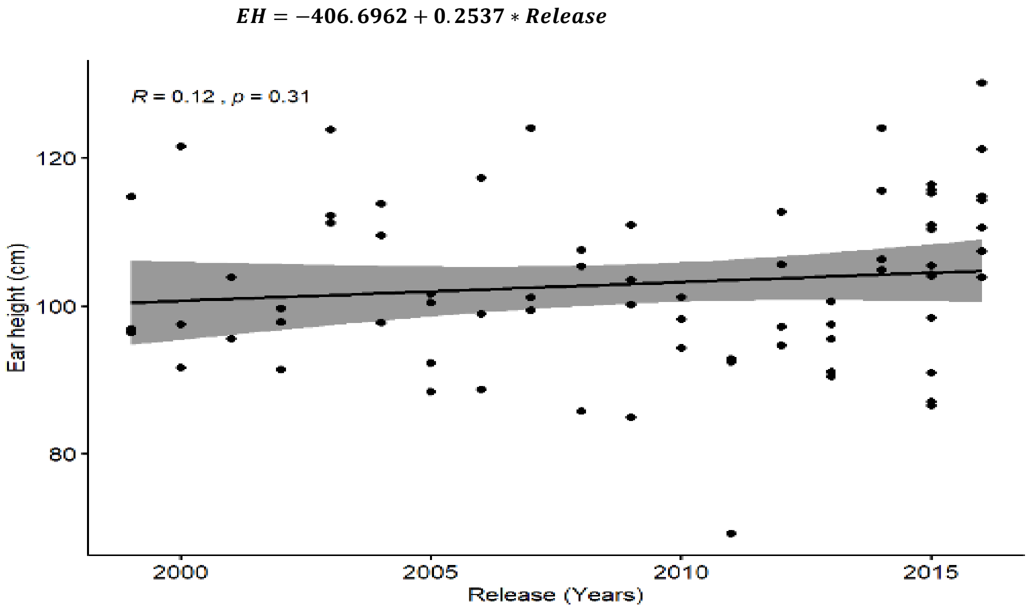


Figure 3.5: Genetic gain in plant height in CIMMYT hybrids released from 1999 to 2016 tested under managed drought stress.

There was a significant change in plant height ($P \leq 0.04$ as it showed a trend of increasing over the 17-year period at an estimated rate of 0.46 cm year^{-1}, (Fig 3.5).

3.6.3.5 Genetic gain in ear height under drought stress

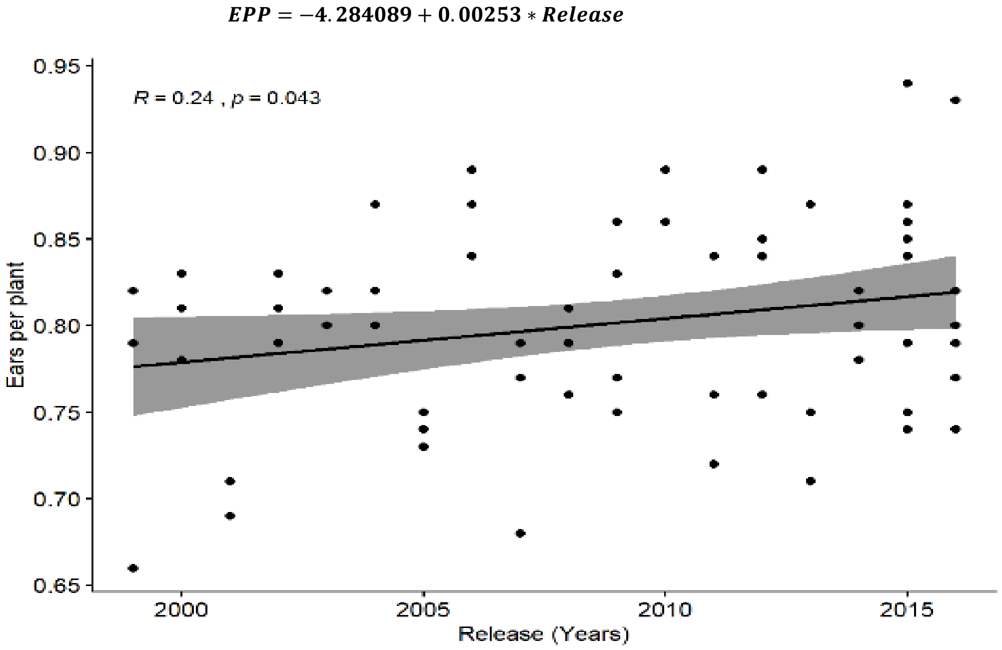


$$\boldsymbol{EH = -406.6962 + 0.2537 * Release}$$

Figure 3.6: Genetic gain in ear height in CIMMYT hybrids released from 1999 to 2016 tested under managed drought stress.

There was no significant change in ear height over the 17 years at P ≤ 0.31 but showed a trend to increase at a rate of 0.25 cm year^{-1}, (Fig 3.6).

3.6.3.5 Genetic gain in ears per plant under drought stress



$$\boldsymbol{EPP = -4.284089 + 0.00253 * Release}$$

Figure 3.7: Genetic gain in ears per plant in CIMMYT hybrids released from 1999 to 2016 tested under managed drought stress.

From 1999 to 2016 the number of ears per plant increased at a rate of 0.003 year^{-1} (Figure 3.7) at $P \leq 0.04$.

3.6.3.6 Genetic gain in senescence1 under drought stress

$$\boldsymbol{SEN1 = -66.52516 + 0.03374 * Release}$$

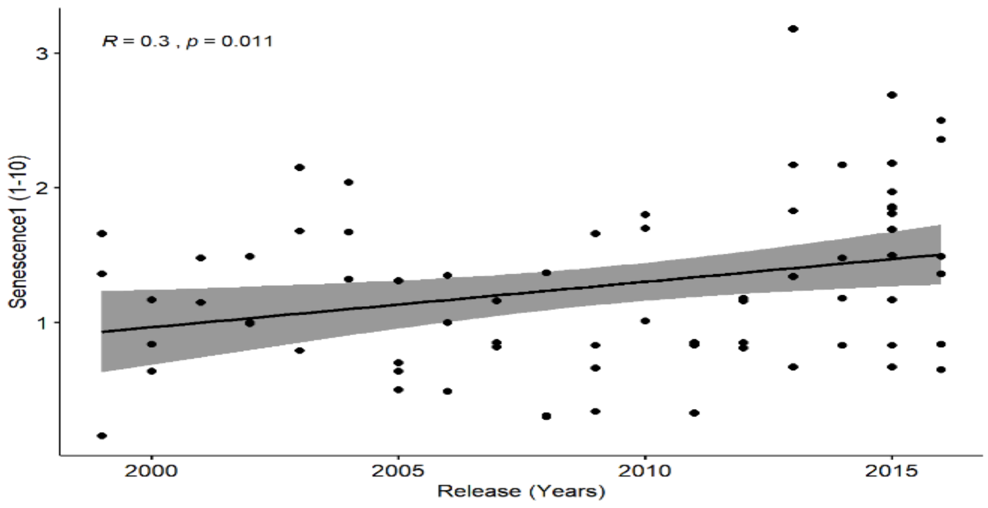


Figure 3.8: Genetic gain in senescence 1 in CIMMYT hybrids released from 1999 to 2016 tested under managed drought stress.

There was a significant change in senescence 1 over the 17 years at $P \leq 0.01$ and showed a trend to increase at a rate of 0.034 scores year-1 (on a 0-10 scale) as shown in Figure 3.8.

3.6.3.7 Genetic gain in senescence2 under drought stress

$$\boldsymbol{SEN2 = 13.042904 - 0.005874 * Release}$$

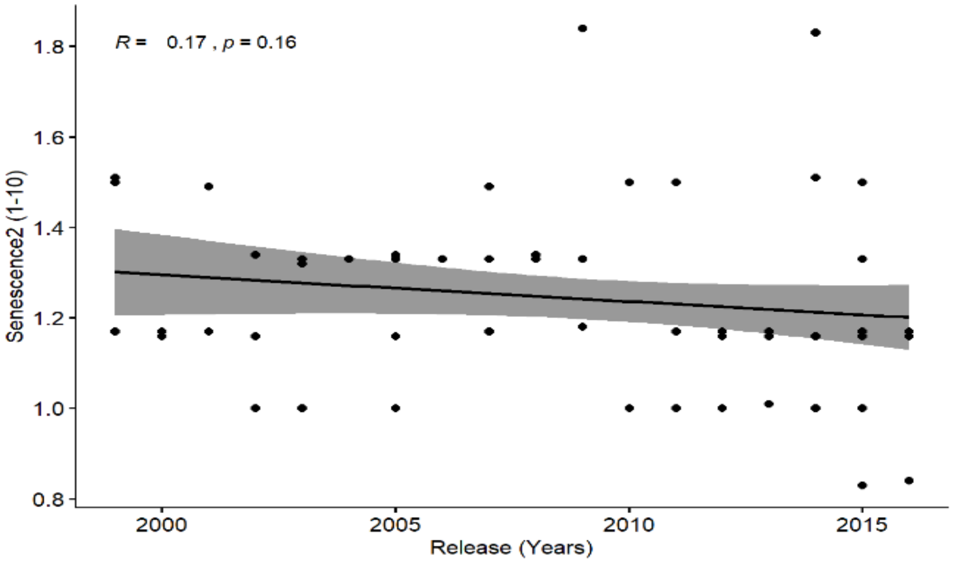


Figure 3.9: Genetic gain in senescence 2 in CIMMYT hybrids released from 1999 to 2016 tested under managed drought stress.

From a regression analysis of senescence 2 against time of release there was no significant change (P ≤ 0.16) and showed a trend to decrease at a rate of 0.006 scores year-1 (on a 0-10 scale) as shown in (Fig 3.9).

3.6.4 Correlation of grain yield and secondary traits under drought stress

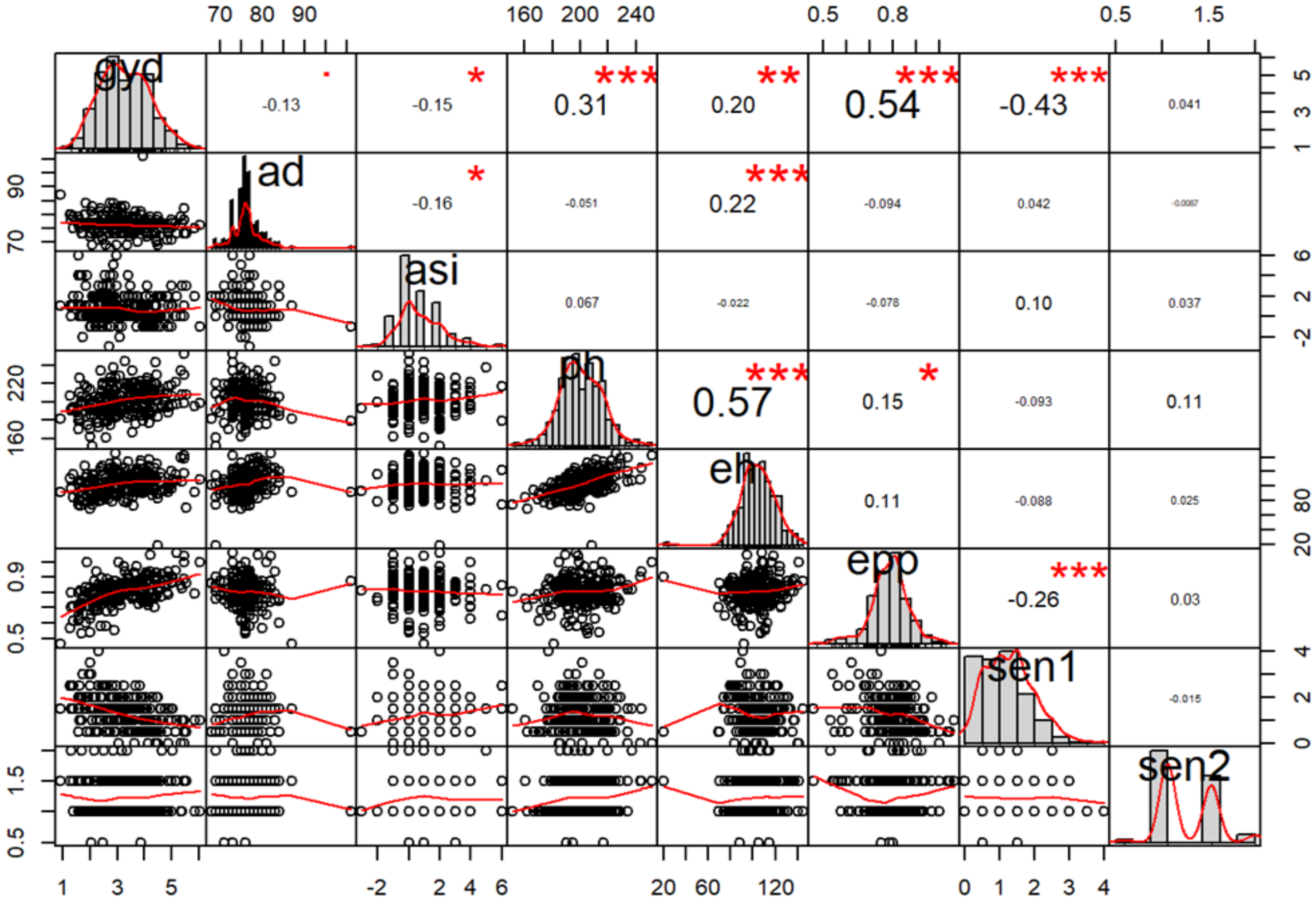


Figure 3.10: Genetic correlation matrix of grain yield and secondary traits under managed drought stress

The pairwise correlations among the 8 traits. The traits of GY, AD and ASI, PH, EH, EPP, SEN1, SEN2 are abbreviated from grain yield, anthesis date and anthesis-silking interval, plant height, ear height, ears per plant, senescence1, senescence2 respectively. The numbers with one or more star(s) represent the Pearson correlation coefficients at different significances (*: 0.05; * *: 0.01; * * *: 0.001) and the word size of them indicate the correlation level. The blank boxes indicate that there was no significant correlation for the corresponding traits.

A correlation matrix is a table showing correlation coefficients between variables. Each cell in the table shows the correlation between two variables. Fig 3.10 shows that grain yield was high positive correlation with plant height and ears per plant. In general, the GY was negatively and significantly correlated with ASI (-0.15) and senescence1 (-0.43) under drought stress. However, correlations were not observed between GY and AD. The study exhibited changes in yield which was associated with some physiological and phenological changes. Positive correlations were obtained for majority of traits. However, Anthesis date recorded negative correlations with Anthesis-silking interval (-0.16). Strong positive relationships were obtained from correlations of Grain yield and Ears per plant; Grain yield and Plant height; Grain yield and Ear height; Anthesis date and Ear height; Plant height and Ear height. Anthesis-silking interval is considered one of the most important traits in maize that affects the success of grain production.

Table 2.2: Drought stress heritability summary

LOCATION	STATISTIC	BLUP_GYD	BLUP_AD	BLUP_ASI	BLUP_PH	BLUP_EH	BLUP_EPP	BLUP_SEN1	BLUP_SEN2
Drought	Heritability	0.54	0.68	0.55	0.66	0.57	0.26	0.07	0
Drought	Genotype Variance	0.15	4.48	0.5	66.35	70.28	0	0.01	0
Drought	Residual Variance	0.38	6.44	1.24	103.1	159.53	0.01	0.3	0.09
Drought	Grand Mean	3.26	76.31	0.8	201.31	103.24	0.8	1.29	1.24
Drought	LSD	0.78	3.42	1.37	13.92	15.51	0.07	0.25	0
Drought	CV	19.03	3.33	138.43	5.04	12.23	10.38	42.73	24.12

Heritability percentage was categorized as low when less than 40%, medium, 40 – 59%, moderately high, 60-79% and very high, 80% and above as indicated by (Rosmaina *et al.,* 2016). Under drought stress, moderately high heritability ($h_2>0.60$) was exhibited for the following traits: anthesis date and plant height. (Table 3.2). Medium heritability ($h_2>0.50$) was estimated for grain yield, anthesis-silking interval and ear height. Low heritability estimates were observed for ears per plant and senescence.

3.7 Discussion

Among abiotic stresses, drought has been recognized as the major abiotic constraint to maize yields in most production regions. In addition, yield generally tend to decrease under stressful conditions and this is shown from the mean yields under the drought stress environment.

3.7.1 Ears per plant

Consistent with the literature, the study indicated that there were variations among hybrids for secondary traits such as number of ears per plant. The number of ears per plant is a parameter that is highly correlated to grain yield because grain yield is a function of number of ears, number of kernel rows, number of kernels per row and kernel weight. Ears per plant has been used as a secondary trait for selection under stress in drought breeding (Weber and Melchinger, 2012). (Mostafavi *et al.,* 2013) stated ear prolificacy to be highly significant and to have a positive correlation with grain yield in maize. In this study ear proliferation helped recently produced hybrids to outcompete the old hybrids in terms of grain yield. Grain yield is the key trait in maize-breeding programmes. However, for it to be improved to a greater extent, the contribution of other allied traits, such as the number of ears per plant must be considered.

Significant differences among the secondary traits' values were more pronounced under drought stress because of the contrasting nature of the environmental conditions. Ears per plant showed significant differences at (P<0.043) under drought stress, and this trait was reported by (Mhike, 2012) to be effective when selecting for hybrids with tolerance to drought.

Drought stress during, kernel development is responsible for 20–30 % yield losses which are mainly due to under sized kernels (Abdelgawad *et al.,* 2014). This indicates that most of the experimental hybrids were superior for drought stress tolerance. In agreement to our findings (Duvick, 1996; Duvick and Cassman, 1999) asserted that better tolerance to drought stress was obtained in new varieties compared to older varieties released in different eras in the USA. The positive correlation between EPP and GY was expected because grain yield is a primary dependent variable of EPP under drought conditions.

Furthermore, results from this study corroborates with findings by (Betran *et al.,* 2013) which reported significant association for ears per plant and senescence with grain yield under drought stress conditions.

3.7.2 Ear and Plant height

This study found that ear and plant height were significantly correlated with grain yield under drought stress, indicating that taller plants with high ear placement were better yielding compared to shorter plants with lower ear placement. This might be attributed to the high dry matter accumulation function carried out by the high number of leaves possessed in the case of tall plants. Similar results were also observed by (Singha, 2000) that grain yield is positively associated with plant height. Increased plant height provides more green area for increased photosynthetic activities and assimilates needed for grain filling (Asghar and Muhammad 2010). In addition, (Prasai *et al.,* 2015) found highly significant differences among 14 genotypes in early maize genotypes for plant height which strongly support this result.

3.7.3 Senescence

Second senescence recorded three to four weeks after flowering was relatively slower in the new hybrids compared to the old hybrids and negatively under drought stress trial. Drought stress could also reduce the leaf chlorophyll contents, which on the other hand may hamper the photosynthetic efficiency and plant growth. Reduced senescence was reported to increase dry matter accumulation in the new varieties (Duvick, 2005b). This should have contributed towards grain yield improvement in the drought stress trial. Stay green allows more photosynthesis and provides an extended grain filling period.

3.7.4 Anthesis silking interval

Stress during the pollination and silking period often reduces yield potential. One of the best indicators of how plants respond to stress during flowering is the Anthesis - silking interval, ASI. The phenotypic analysis also showed that GY was negatively and significantly correlated with ASI under each evaluation condition, this corroborates with the previous observation (Ribaut *et al.*, 2009). It indicated that ASI is an appropriate secondary trait to facilitate the selection on GY. Short ASI is required by breeders as it helps maize crop yield more due to improved synchronization of male and female plants, thus reducing barrenness helping increase yield.

Drought stress adversely affected grain yield and days to flowering, possibly due to its adverse effects on pollen production, or ovule fertility, leading to premature embryo abortion and reduced grain weight (Saini and Westgate, 1999). Under abiotic stress, silking is delayed (Bänziger *et al.*, 2000). ASI was negatively correlated with grain yield and this could have contributed to poor synchronisation, poor pollination, poor grain set and poor grain yield. Similar observations were reported by (Cheikh and Jones, 1995; Rattalino Edreira *et al.*, 2011, Beyene *et al.*, 2013). reported that abiotic stresses has direct effects on reproductive developments which results in prolonged anthesis-silking interval, reduced duration of grain filling and low kernel set. Furthermore, when soil water deficit occurs before flowering, silk emergence out of the husks is delayed while anthesis is largely unaffected, resulting in an increased anthesis-silking interval (Westgate *et al.*, 2000). Drought stress reduces the photosynthesis and translocation of photosynthetic assimilates followed by reduced grain filling.

Results for ASI from the study are in agreement with findings of (Odiyo *et al.*, 2014) who reported relatively higher increase (144%) in ASI due to moisture stress relative to optimum condition in drought-tolerant germplasm adapted to West Africa. The high yield reduction under stress environments could be partly explained by the wider ASI under stress. (Magorokosho *et al.*, 2004) reported selection of reduced anthesis- silking interval as more effective than grain yield under drought stress. Delay of silking whilst pollen is shed leads to a long anthesis-silking interval which correlate highly to setting of kernels (Westgate *et al.,* 2000).

3.7.5 Grain yield

The extent of yield loss is dependent on severity of drought stress, field environment and the maize hybrids under study. Drought stress trial reduced grain yield by 35.06% (Fig 3.1). This is supported by findings from (Bänziger *et al.,* 2000) who reported that when severe stress levels which affect both flowering and grain filling stage is exposed to the genotypes, a yield reduction of 30-60% of that realized under optimum conditions is expected. The average grain yield of the top ten experimental hybrids under drought stress was higher than the commercial checks under the management conditions.

3.7.6 Heritability summary amongst grain yield and measured secondary traits

Medium heritability was detected for grain yield under drought and heat trials (Table 3.3). This indicates that grain yield is a complex trait that is strongly influenced by environment during selection thus slow progress is expected during selection. Contrasting reports on the magnitude of heritability for grain yield has also been reported: low heritability (Bello and Olaoye, 2012; IQBAL, 2009) and high heritability (Beyene *et al.,* 2013; Wannows, 2015). Differences in the heritability values among researchers can be attributed to differences in environments used during the studies. Low heritability estimates for ears per plant and senescence were detected. This indicates that the traits may be influenced by environmental factors that mask genetic effects during selection (quantitative traits). Therefore, they are difficult to directly select for in breeding for the genetic gain hybrids. Further breeding gains for these traits can be obtained by increasing genetic variance, and improving quality of experiments to minimise error during assessment.

3.8 Conclusions

In this study drought significantly contributed to yield reduction. The new hybrids yielded better than the old hybrids. The study revealed considerable amount of variation for yield among the tested genetic gain hybrids. The extent of yield loss was dependent on severity of drought stress, field environment and the maize hybrids under study. Changes in yield which was associated with positive correlations were obtained for majority of traits. Strong positive significant relationships were obtained from correlations of Grain yield and Ears per plant; Grain yield and Plant height; Grain yield and Ear height; Anthesis date and Ear height; Plant height and Ear height. Furthermore, from regression analysis conclusion can be made that majority of the secondary traits also contributed to genetic gain obtained in grain yield over the years, except for no gain that was recorded for anthesis silking interval.

3.9 Recommendations

The results indicated that hybrids with strong drought tolerance are CZH1261, CZH16340, CZH142020, CZH15603 and CZH15002, these can be further grown in different environments to determine their tolerance stability. In order to develop promising genotypes, it is essential to know the different traits particularly associated with grain yield, which is the most ultimate objective in any breeding program. Traits positively correlating highest correlation and good heritability with grain yield such as ear height, plant height, ears per plant and senescence can be chosen as superior characteristics to help improve maize grain yield.

3.9.1 Acknowledgments

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CHAPTER 4

To assess the genetic gain in yield and other secondary traits in CIMMYT hybrids released from 1999 to 2016 for their tolerance to heat stress.

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Abstract

Maize is a sensitive crop to heat stress, particularly at the reproductive stages of development. Higher temperatures near anthesis have a large negative effect on maize grain yield. Improving heat tolerance in maize has become a major priority in maize breeding programs so as to increase grain yield over years. This study evaluated the genetic gain for yield and other secondary traits in CIMMYT varieties developed and released from 1999 to 2016 under heat stress conditions in Zimbabwe (Chiredzi during the 2018-19 season). The 72 hybrids and 3 commercial checks were evaluated using a 15*15 alpha lattice. The hybrids significantly varied for traits Grain yield, Anthesis date, Plant height, Ear height, Ear height at $P \leq 0.001$ under heat stress. No significant differences were recorded for traits Ears per plant. Positive genetic gains were observed for grain yield and secondary traits: Anthesis-silking date, Plant height, Ear height, Ears per plant. The study showed an estimated net genetic gain in yield of 39.36 kg ha^{-1} $year^{-1}$ over the 17-year period from 1999 to 2016. This was accompanied by some changes in secondary traits including an average decrease in anthesis-silking interval of -0.02 days' $year^{-1}$ and a trend of reduced in barrenness corresponding to an increase of 0.002 ears $plant^{-1}$ $year^{-1}$. The results indicated that hybrids with strong drought tolerance were CZH15575, CZH0527, CZH15017, CZH16389 and CZH15572, and should be tested widely.

Keywords: Anthesis date - Genetic gain - Secondary traits - Heat stress – Grain yield

4.1 Introduction

Maize grown in semi-arid tropical environments often faces a multitude of abiotic stresses such as drought and heat (Cairns *et al.*, 2013). The growing concerns for food security and the sustainability of agricultural resources underlies the urgency and importance of tackling drought, which remains the single most important factor threatening the survival of people in the developing world. While drought has been destabilizing maize yield in many parts of predominantly rain fed SSA, heat stress is becoming more important as the climate changes (Edmeades, 2013). Projected temperature increases will be higher in the drought-prone areas (Niang *et al.*, 2014) indicating that drought stressed areas will also face severe heat stress under the future climate. Drought stress usually goes along with high temperature and hence drought and heat tolerant crops will play an increasingly important role in hotter and drier production environments. (Lobell *et al.*, 2011) asserts that for every day in excess of 30ºC maize crop records yield losses of 1% and 1.7% under optimum and drought stress conditions respectively.

Heat stress can be defined as temperatures above a threshold level that results in irreversible damage to crop growth and development and is a function of intensity, duration and the rate of increase in temperature (Cairns *et al.,* 2013). High temperatures can cause an array of morphological, anatomical, physiological and biochemical changes within maize. The most significant factors associated with maize yield reduction include shortened life cycle, reduced light interception and increased sterility (Stone, 2001). Heat stress in the flowering and grain filling periods due to elevated temperatures drastically affect crop productivity. It is predicted that maize yield might be reduced up 70 % due to increasing temperatures (Khodarahmpour *et al.,* 2011). Now day's heat stress is one of key abiotic stress with high potential impact on maize crop growth and development and eventually on productivity.

4.1.1 Heat tolerant maize in sub-Saharan Africa

The vast majority of heat stress research has been conducted on temperate maize germplasm for high production areas. In addition, limited breeding progress has been made in the development of improved maize germplasm with specific tolerance to elevated temperatures. CIMMYT, together with partners under the CGIAR Research Program on Maize (MAIZE), have developed and continue to work on breeding heat-tolerant maize varieties through its breeding program in sub-Saharan Africa since 2011. Heat tolerance was not previously a trait in African breeding programs.

One of the main objectives of CIMMYT's global maize programme in SSA is breeding for tolerance to heat, improving food security and farmers' livelihoods in Africa. Like in every breeding programme, there is need to evaluate the progress. By evaluating genetic gain, breeding strategies and objectives can be evolved based on the findings. This study will establish if there has been any genetic gain with respect to heat in CIMMYT hybrids released by the Eastern and Southern Africa maize programmes from 1999 to 2016.

The aim of this study was to evaluate the genetic gain for yield and other secondary traits in CIMMYT varieties developed and released from 1999 to 2016 under heat conditions in Zimbabwe. The objectives of the study were: i) To evaluate yield performance of hybrids released by CIMMYT from 1999 to 2016 for heat stress tolerance ii) To identify traits discriminating between the CIMMYT hybrids released from 1999 to 2016 for their tolerance to heat stress iii) To estimate the contribution of these traits to the hybrids yield gains.

4.2 Brief description of the study site

The same study site was used as described in Chapter 3 section 3.2.

4.3 Experimental design

The same experimental design was a 15*15 alpha-lattice (Patterson and Biometrika, 1976) with 75 hybrids that were be replicated three times as described in Chapter 3 section 3.4. The study was conducted at the site described in Chapter 3 section 3.3. The same hybrids presented in Chapter 3 were analysed.

4.4 Trial management

Trials at the Chiredzi Research Station were planted on the 11th of August for heat stress. The genotypes were grown at the site and same agronomic practices presented in Chapter 3 section 3.4 were followed. The heat trial was irrigated from planting until physiological maturity. Irrigation scheduling was determined by the crop water requirements as dictated by the development of crop and evapotranspiration.

4.5 Data collected

Data was collected for measured traits as described in Chapter 3 section 3.5.

4.5.1 Data analysis methods

Data was processed and analysed as described in Chapter 3 section 3.5.1

4.6 Results

4.6.1 Analysis of variance of hybrid performance under heat stress trial

Table 3.1: Analysis of variance for grain yield and secondary traits under heat stress showing the top 10 and bottom 10 ranking hybrids

RANK	ENTRY	HYBRID	GYD	AD	ASI	PH	EH	EPP	RELEASE
1	**63**	**CZH15575**	6.54 a	70.61 pqrstuvwx	0.67	224.60 bcdefg	110.04 cdefghijklmnopq	0.88	2015
2	**23**	**CZH0527**	6.41 ab	73.01 fghijklmn	1.67	221.73 bcdefghijkl	123.60 abcdef	0.86	2005
3	**59**	**CZH15017**	6.32 abc	72.38 ghijklmnopq	-0.33	214.04 cdefghijklmnopqrs	112.58 bcdefghijklmn	0.88	2015
4	**71**	**CZH16389**	6.04 abcd	72.49 ghijklmnop	-1.33	207.23 ghijklmnopqrstuvw	112.13 bcdefghijklmn	0.86	2016
5	**66**	**CZH15572**	5.90 abcde	70.97 nopqrstuv	0.67	219.18 bcdefghijklmn	103.86 ghijklmnopqr	0.90	2015
6	**58**	**CZH15188**	5.86 abcdef	72.45 ghijklmnop	-0.67	213.62 cdefghijklmnopqrs	112.99 abcdefghijklm	0.87	2015
7	**36**	**CZH0935**	5.85 abcdef	70.76 opqrstuvw	0.00	210.00 efghijklmnopqrstuv	111.56 bcdefghijklmno	0.88	2009
8	**67**	**CZH15603**	5.83 abcdefg	72.50 ghijklmnop	-0.67	220.77 bcdefghijklm	121.31 abcdefgh	0.86	2015
9	**62**	**CZH15185**	5.81 abcdefgh	74.29 cdefgh	1.33	233.14 ab	126.49 abcd	0.85	2015
10	**37**	**CZH0928**	5.73 abcdefghi	70.03 stuvwxy	0.00	200.64 opqrstuvw	91.03 qr	0.92	2009
66	**4**	**CZH99015**	4.18 klmnop	71.50 klmnopqrstu	2.33	198.57 rstuvw	95.54 lmnopqr	0.85	1999
67	**75**	**CZH16048**	4.17 klmnop	73.30 efghijkl	1.33	212.54 defghijklmnopqrst	107.35 defghijklmnopq	0.78	2016
68	**7**	**CZH00011**	4.15 lmnop	69.27 vwxy	-0.33	208.26 fghijklmnopqrstuv	100.60 ijklmnopqr	0.79	2000
69	**8**	**CZH00013**	4.15 mnop	70.81 opqrstuvw	0.33	211.86 defghijklmnopqrstu	96.53 klmnopqr	0.76	2000
70	**10**	**CZH01006**	4.09 nop	71.54 klmnopqrstu	0.33	217.00 bcdefghijklmnopq	104.35 fghijklmnopqr	0.84	2001
71	**2**	**SC403**	**4.06 op**	**71.50 klmnopqrstu**	**0.33**	**224.14 bcdefgh**	**98.87 jklmnopqr**	**0.78**	**1998**
72	**43**	**CZH1134**	3.81 p	71.83 jklmnopqrst	1.67	209.02 fghijklmnopqrstuv	97.18 klmnopqr	0.77	2011
73	**3**	**PAN413**	**3.81 p**	**73.83 efghij**	**1.33**	**198.38 rstuvw**	**103.53 ghijklmnopqr**	**0.87**	**1998**
74	**44**	**CZH1258**	3.80 p	69.89 tuvwxy	1.67	224.12 bcdefghi	113.08 abcdefghijkl	0.85	2012
75	**33**	**CZH088**	3.76 p	65.26 B	1.67	175.86 y	92.55 opqr	0.85	2008
	check	**SC513**	**5.15 cdefghijklmno**	**73.13 efghijklmn**	**0.33**	**225.21 bcdefg**	**118.42 abcdefghi**	**0.87**	**1997**
		Mean	**5.02**	**71.98**	**0.45**	**211.3**	**106.62**	**0.85**	
		Mse	**1.05**	**14.20**	**2.78**	**376**	**309.00**	**0.004**	
		LSD	**1.24**	**2.15**	**2.02**	**18.02**	**19.36**	**0.10**	
		h2	**0.58**	**0.90**	**0.47**	**0.74**	**0.62**	**0.10**	
		p	*******	*******	******	*******	*******	**ns**	
		Min	**3.76**	**65.3**	**-1.67**	**175.98**	**86.18**	**0.75**	
		Max	**6.54**	**76.9**	**2.33**	**243.7**	**132.17**	**0.93**	

*** P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, ns non significance at P ≤ 0.05, LSD (0.05) Least significant difference at (P < 0.05). Means with the same letter in each column have no significant difference (P<0.05).**

4.6.1.1 Anthesis silking interval (ASI)

Hybrids were also significantly different (P < 0.01) for anthesis silking interval (Table 4.1) under heat stress. ASI ranked from the least -1.67 days CZH15212 from era (2011 to 2016) to highest 2.33 days CZH15002 from era (2011 to 2016), (Appendix 2).

4.6.1.2 Anthesis date (AD)

Analysis of variance revealed highly significant (P ≤ 0.01) differences for anthesis date among the hybrids tested (Table 4.1). Average days to mid-anthesis ranged from 65.26 days CZH088 from era (2006 to 2010) to 76.90 days CZH15343 from era (2011 to 2016), (Appendix 2). The trial mean was 76.3 days.

4.6.1.3 Ears per plant (EPP)

Results showed non-significant differences among the hybrids tested for ears per plant at $P \leq 0.05$ (Table 4.1). Ears per plant ranged from 0.75 CZH01008 from era (1999 to 2002) and 0.93 CZH0615 from era (2006 to 2010), (Appendix 2). Heat stress trial recorded mean of 0.85 ears per plant.

4.6.1.4 Plant height (PH)

Analysis of variance revealed highly significant ($P \leq 0.001$) differences for plant height among the hybrids tested, (Table 4.1). Plant height ranged from 175.98cm CZH088 from era (2006 to 2010) to 243.71cm CZH15343 from era (2011 to 2016), (Appendix 2). Trial average was 211.29cm.

4.6.1.5 Ear height (EH)

Statistical analysis revealed highly significant ($P \leq 0.001$) differences among the hybrids tested for ear height. Average Ear height ranged from 86.18cm CZH15183 from era (2011 to 2016) to 132.17cm CZH142020 from era (2011 to 2016), (Appendix 2). The trial average was 106.62cm.

4.6.1.6 The yield performance of maize hybrids under heat stress

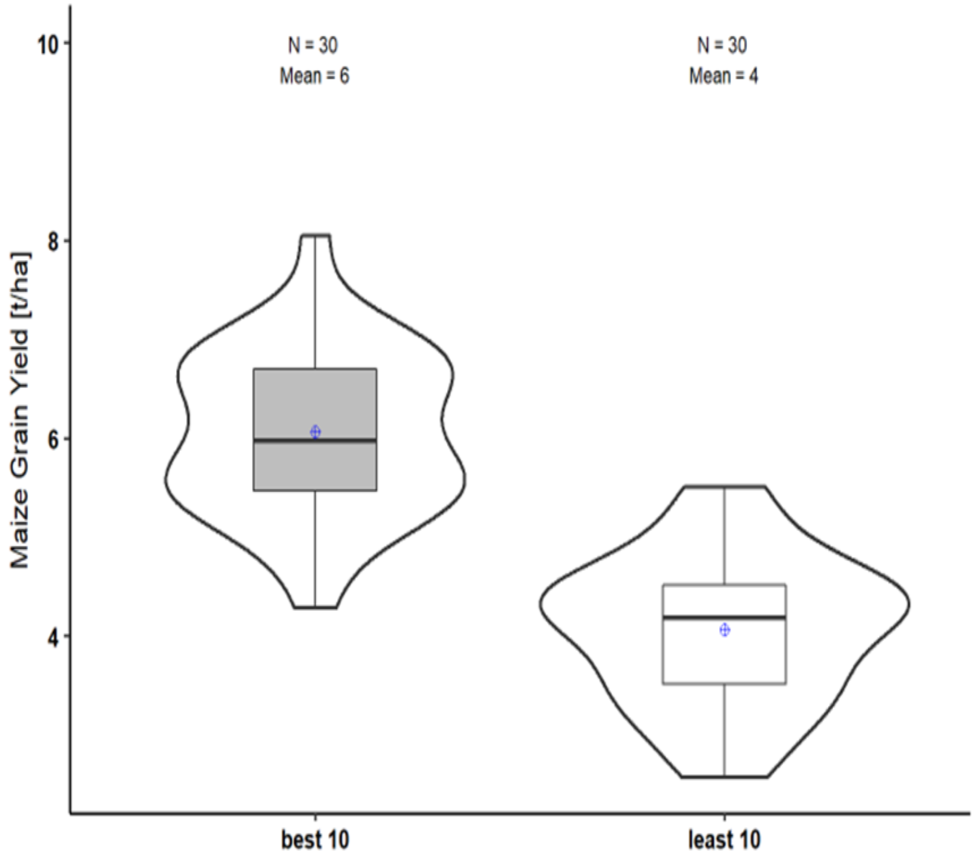


Figure 4.1: Comparison of best and least ranked hybrids for grain yield under heat

The above Fig 4.1 shows concentration of hybrids in terms of performance for grain yield in box plots embedded with violin plots. Best performing hybrids had yield concentrated from 5.80 to 5.9 t ha^{-1} whilst the least performing hybrids were concentrated from 4.06 to 4.18 t ha^{-1}.

4.6.1.7 Grain yield (GYD)

The hybrids significantly varied for traits Grain yield at $P \leq 0.001$. Grain yield ranged from around 3.76 t ha^{-1} CZH088 from era (2006 to 2010) to approximately 6.54 t ha^{-1} CZH15575 from era (2011 to 2016) under heat stress. Overall, the hybrids in the heat stress trial exhibited a mean grain yield of 5.02 t ha^{-1}. Checks ranked at 34 (SC513 - 5.15 t ha^{-1}), 71 (SC403 - 4.06 t ha^{-1}), and 73 (PAN413 - 3.81 t ha^{-1}) for checks 1, 2 and 3 respectively released in 1997, 1998 and 1998 respectively (Table 4.1).

4.6.2 Genetic gain in yield and secondary traits under heat stress

4.6.2.1 Genetic gain in grain yield under heat stress

$$\boldsymbol{GYD = -74.04152 + 0.03936 * Release}$$

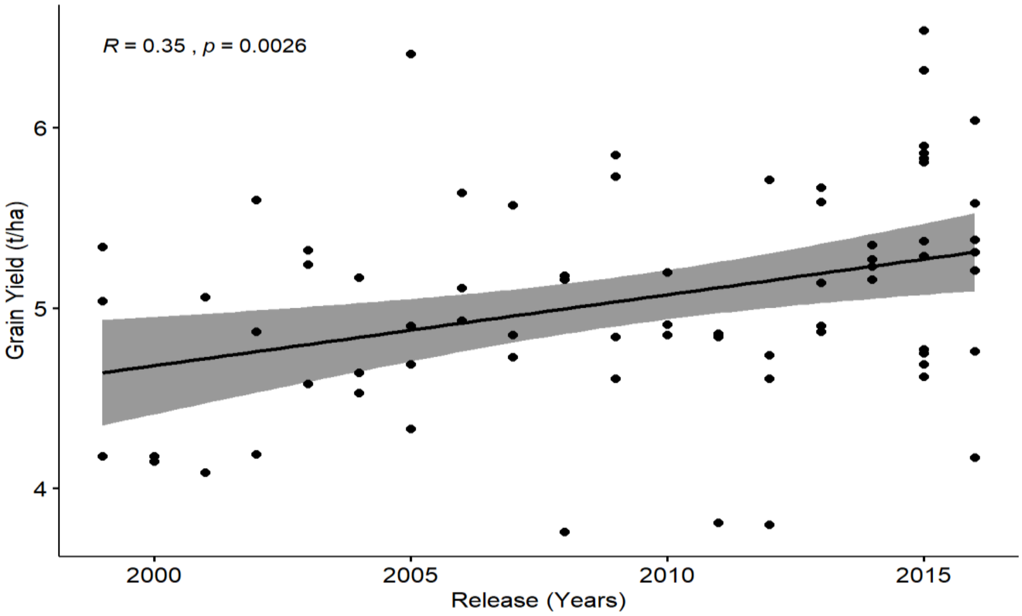


Figure 4.2: Genetic gain in yield in CIMMYT hybrids released from 1999 to 2016 tested under heat stress.

Under heat stress a genetic gain in yield of 39.36 kg ha^{-1} year^{-1} was recorded (Figure 4.2).

4.6.2.2 Genetic gain in days to mid anthesis under heat stress

$$AD = 53.84 + 0.009009 * Release$$

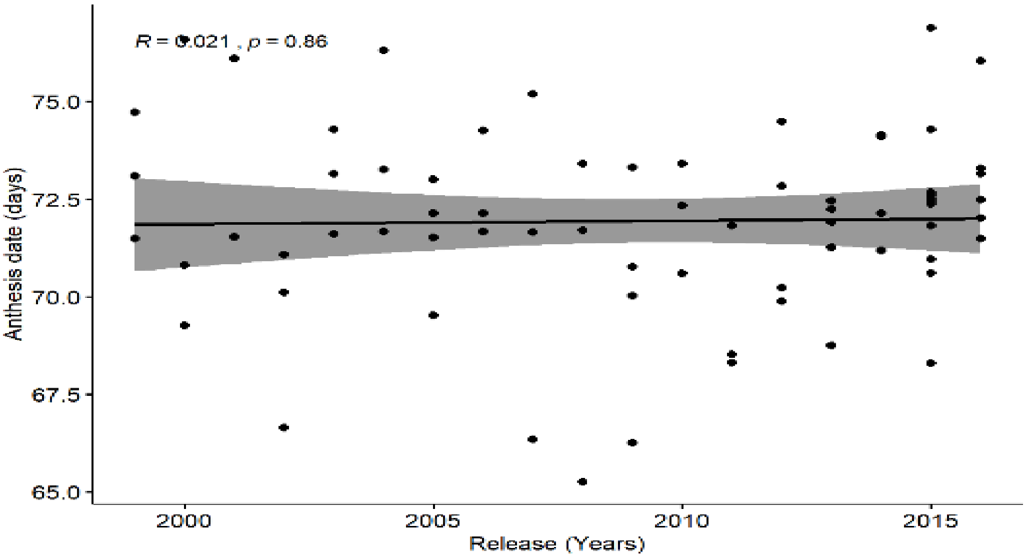


Figure 4.3: Genetic gain in days to mid anthesis in CIMMYT hybrids released from 1999 to 2016 tested under heat stress.

Days to mid-anthesis did not significantly change over the 17 years at P ≤ 0.05 but showed a tendency to increase at a rate of 0.001 days' year^{-1}. New hybrids flowered within the same range as the old hybrids but yielded better than the old hybrids.

4.6.2.3 Genetic gain in days to anthesis silking interval under heat stress

$$ASI = 36.70959 - 0.01805 * Release$$

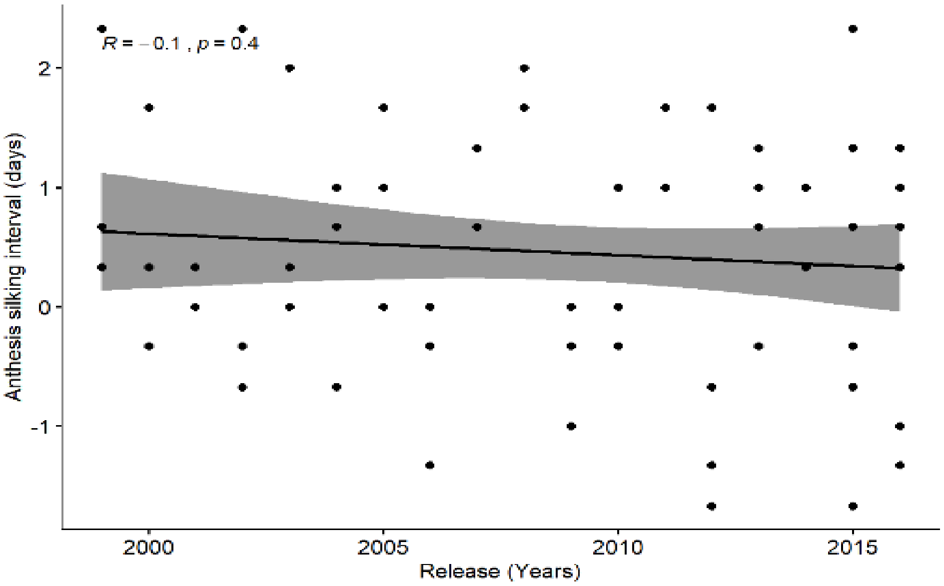


Figure 4.4: Genetic gain in days to anthesis silking interval in CIMMYT hybrids released from 1999 to 2016 tested under heat stress.

A net decrease of, on average, 0.02 days' year^{-1} was recorded for ASI under heat stress (Figure 4.4) at $P \leq 0.05$. New hybrids had relatively shorter ASI compared to the old hybrids.

4.6.2.4 Genetic gain in plant height interval under heat stress

$$\boldsymbol{PH = -417.3203 + 0.3128 * Release}$$

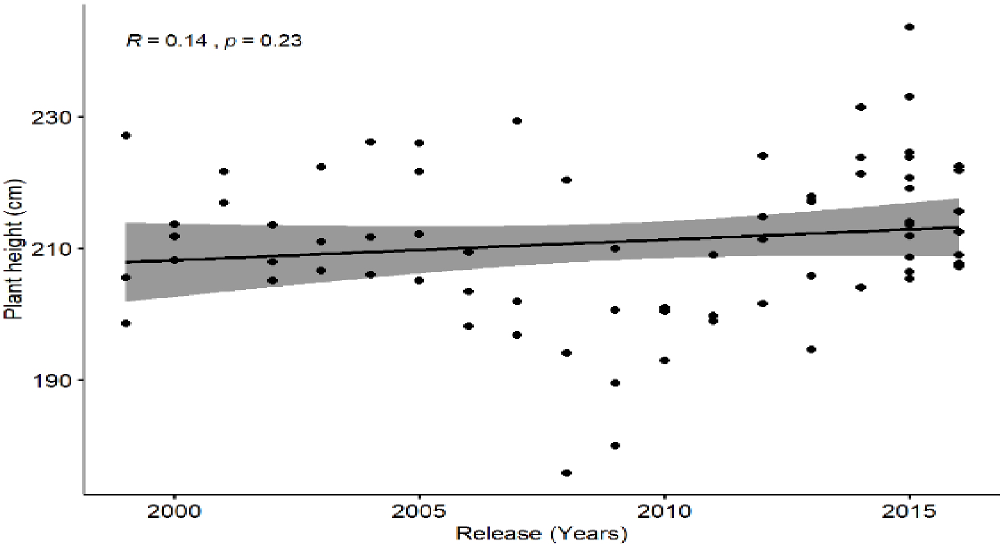


Figure 4.5: Genetic gain in plant height in CIMMYT hybrids released from 1999 to 2016 tested under heat stress.

Plant height showed a trend to increase at an average rate of 0.31 cm year^{-1} but there was no significant change at $P \leq 0.05$.

4.6.2.5 Genetic gain in ear height interval under heat stress

$$\boldsymbol{EH = -179.4659 + 0.1424 * Release}$$

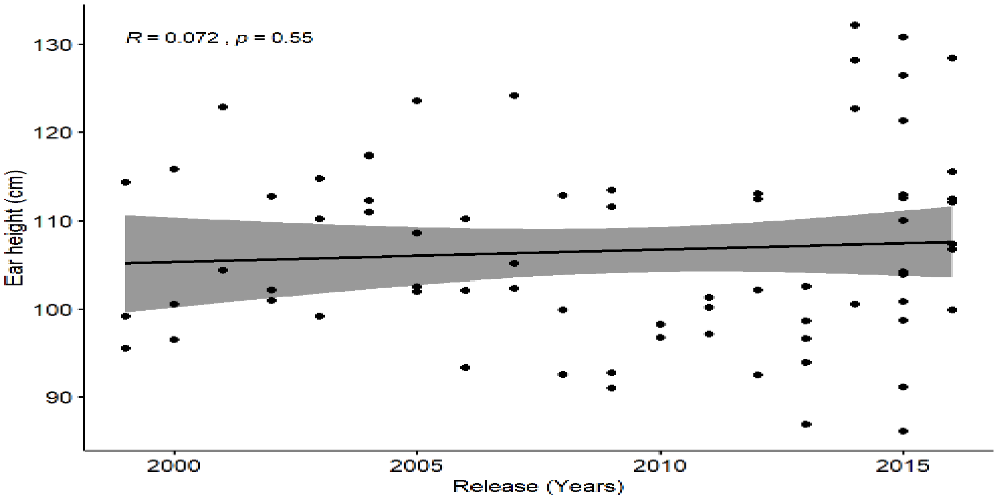


Figure 4.6: Genetic gain in ear height in CIMMYT hybrids released from 1999 to 2016 tested under heat stress.

Ear height did not significantly change over the 17 years at $P \leq 0.05$ but showed a tendency to increase at a rate of 0.14 cm $year^{-1}$.

4.6.2.6 Genetic gain in ears per plant interval under heat stress

$$EPP = -2.317367 + 0.001578 * Release$$

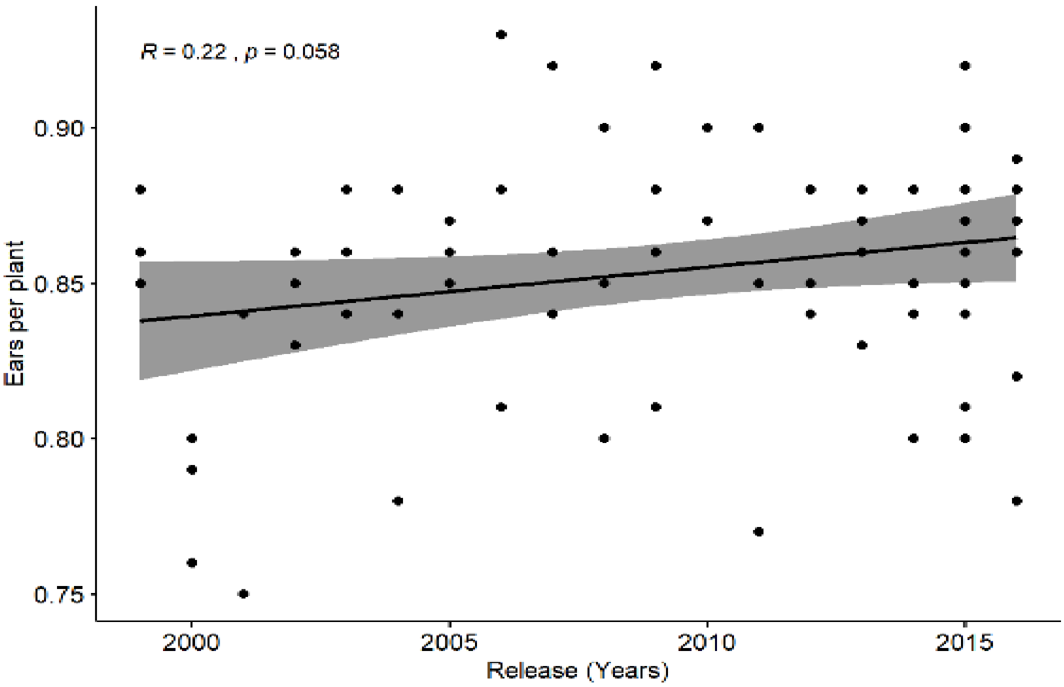


Figure 4.7: Genetic gain in ears per plant in CIMMYT hybrids released from 1999 to 2016 tested under heat stress.

New hybrids had a relatively higher number of ears per plant that increased at a rate of 0.002 (Figure 4.7) which is not significant at $P \leq 0.05$. New hybrids that yielded better than old hybrids had shorter ASI or improved synchronization at flowering and increased number of ears per plant.

4.6.3 Genetic gain in yield and secondary traits under heat stress

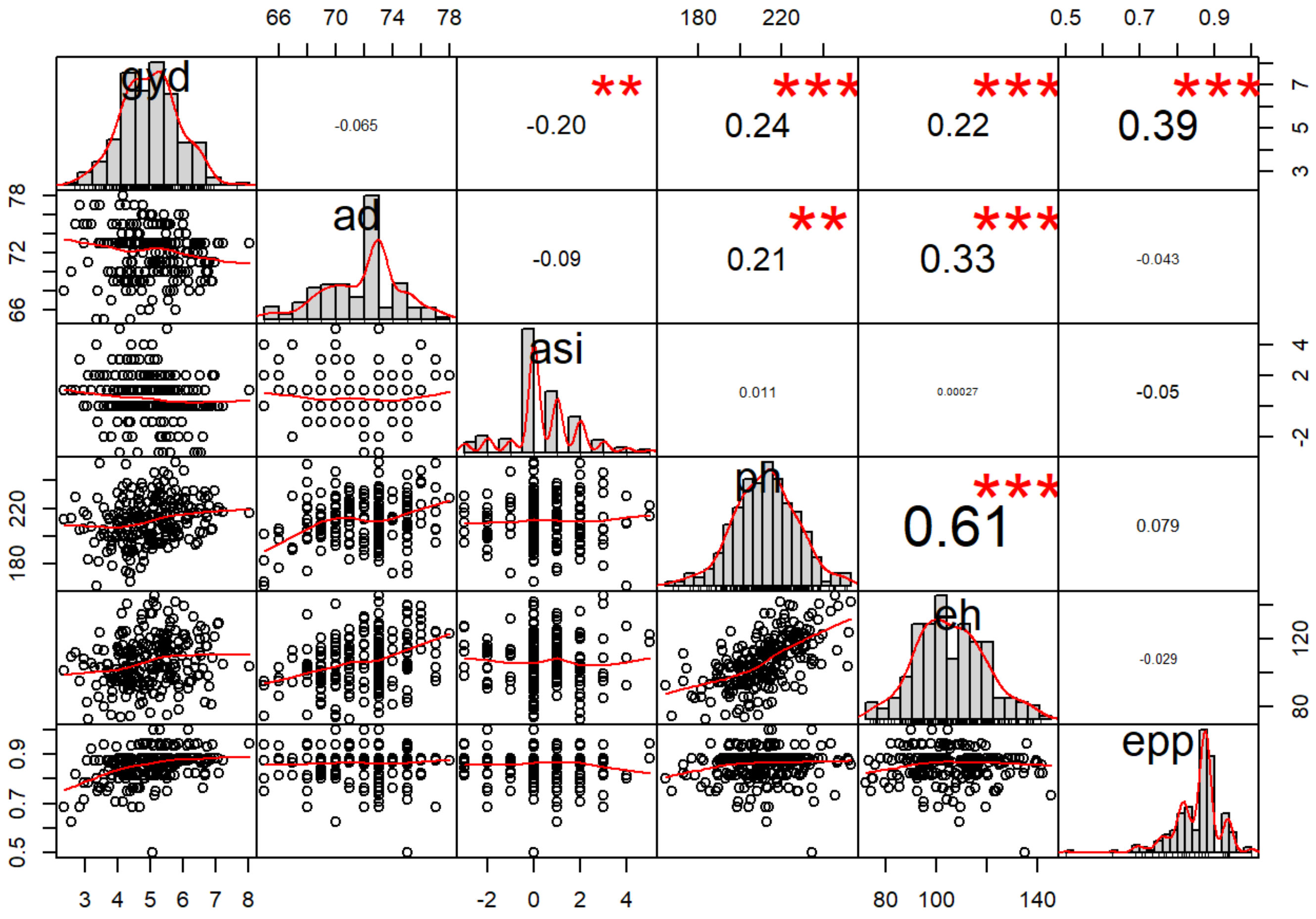


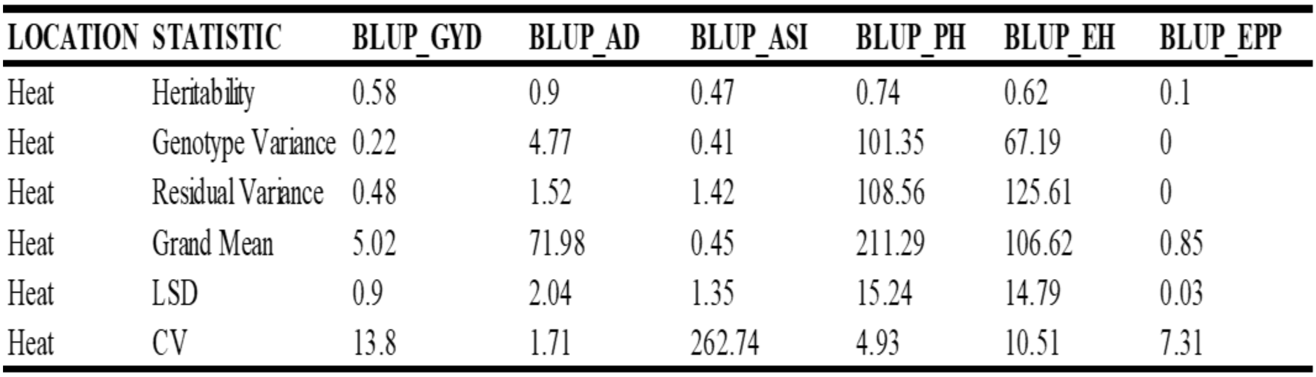
Figure 4.8: Genetic correlation matrix of grain yield and secondary traits under heat stress

The pairwise correlations among the 6 traits. The traits of GY, AD and ASI, PH, EH, EPP are abbreviated from grain yield, anthesis date and anthesis-silking interval, plant height, ear height, ears per plant respectively. The numbers with one or more star(s) represent the Pearson correlation coefficients at different significances (*: 0.05; * *: 0.01; * * *: 0.001) and the word size of them indicate the correlation level. The blank boxes indicate that there was no significant correlation for the corresponding traits.

Grain yield was higher in hybrids that flowered early, that had reduced barrenness, with shorter anthesis-silking interval and low senescence rates, with higher ears per plant, reduced plant and ear height under heat stress trial. Changes in yield which was associated with positive correlations were obtained for majority of traits. The study exhibited changes in yield which was associated with some physiological and phenological changes. Positive correlations were obtained for majority of traits. However, Anthesis date recorded negative correlations with Anthesis-silking interval (-0.09).

Strong positive significant relationships were obtained from correlations of Grain yield and Ears per plant; Grain yield and Plant height; Grain yield and Ear height; Plant height and Ear height (Fig 4.8).

Table 4.2: Heritability estimation for grain yield and secondary traits under heat stress.



LOCATION	STATISTIC	BLUP_GYD	BLUP_AD	BLUP_ASI	BLUP_PH	BLUP_EH	BLUP_EPP
Heat	Heritability	0.58	0.9	0.47	0.74	0.62	0.1
Heat	Genotype Variance	0.22	4.77	0.41	101.35	67.19	0
Heat	Residual Variance	0.48	1.52	1.42	108.56	125.61	0
Heat	Grand Mean	5.02	71.98	0.45	211.29	106.62	0.85
Heat	LSD	0.9	2.04	1.35	15.24	14.79	0.03
Heat	CV	13.8	1.71	262.74	4.93	10.51	7.31

Heritability percentage was categorized as low when less than 40%, medium, 40 – 59%, moderately high, 60-79% and very high, 80% and above as indicated by (Rosmaina *et al.*, 2016). Heritability estimates were high for anthesis date (0.90), moderate for ear height (0.62), plant height (0.74), medium for grain yield (0.58) and anthesis silking interval (0.47) while remaining ears per plant showed low heritability estimates, (Table 4.2).

4.7 Discussion

Genetic gain in maize is due to improvements in the plant system efficiency and stress tolerance (Duvick, 2005). High temperature stress causes adverse effect on plant development, physiological process and grain yield. The duration of the grain filling process is the longest physiological process during the reproductive stage, increasing the probability of experiencing high temperature during this stage.

4.7.1 Anthesis silking interval

Heat has devastating effects on silking. If plants are slow to silk, the bulk of the pollen may already be shed and gone. Continuous heat, over several days before and during pollen-shed, results in only a fraction of normal pollen being formed, probably because of the reduced sugar available. High temperatures, like drought stress, affect grain yield by reducing the number and weight of kernels (Cairns *et al.,* 2013). Under heat stress conditions the number of successful ovules fertilised is reduced (Schoper *et al.,* 1987) because pollen production and viability are compromised. The position of the tassel gives maximum exposure to heat stress, which damages the pollen, leading to lack of pollen viability (Magorokosho, 2006). In this study ASI was shorter in the new varieties compared to the old varieties. ASI has shown consistent correlation with yield in earlier studies (Campos *et al.,* 2006). This study showed that genetic variability for ASI exists under heat stress and therefore ASI can be used in selection for heat stress tolerance.

4.7.2 Anthesis date

The new varieties on average, used the same season length as the old hybrids. This means the hybrids can still grow in the same season as the old hybrids but yielded better. The results of these findings are in agreement with the work of (Campos *et al.,* 2006) which outlines that low phenotypic correlation between grain yield and days to mid-anthesis suggests genetic tolerance in the materials. Selection by breeders is now directed towards both yield and stress tolerance.

4.7.3 Plant height and Ear height

New plants became taller as compared with old hybrids. This might explain increase in grain yield over the years, as increase in plant height enabled the hybrids to have more photosynthetic surface area. Heat stress reduces internodal elongation, which results in reduced plant height. This help explains the reduction in yield of old hybrids as compared to the high yield newer ones.

4.7.4 Grain yield

High temperatures increase the respiration rate of the plant, causing it to use up or waste sugars for growth and development. Stress during pollination and silking could result in shorter ears, increased tip back and fewer kernels per ear. All of these contribute to less yield potential. Extreme heat stress (even in the presence of adequate soil moisture) can cause a reduction in plant stomatal conductance, which reduces plant transpiration rate, causing reductions in plant productivity and yield. If the heat stress is coupled with water stress, this would cause an increase in root clumping, which will decrease the efficiency of plant water uptake. The differences in grain yield from this study may be attributed by the difference in the genetic contribution of the different parents which produce the different hybrids (Heterotic) as supported by the work of (Birchler *et al.* 2003). In addition, the yield was reduced probably due to limited water for photosynthesis, rapid growth and shorter grain filling period which was induced by mild heat stress. This results in poor grain filling and therefore low grain weight. Higher yield in the hybrids could also have been attributed by the ability of the hybrids to reduce apical growth of the stem and diverting assimilates to the cob and grain filling. During grain filling, heat stress affects cell division, sugar metabolism and starch biosynthesis, thereby reducing dry matter accumulation in the grain (Monjardino *et al.,* 2005). High temperatures hasten grain filling and also reduce endosperm starch content, resulting in poorly filled grains with reduced mass. Therefore, genetic improvement of the heat tolerance in maize hybrids can help identify and select the best hybrids that may not necessarily escape the heat stress, but maintain normal metabolism, growth rate and grain yield stability under heat stress.

4.8 Conclusions

The net estimated genetic gain in yield in this study was 39.36 kg ha^{-1} year^{-1}. The new varieties yielded well despite the heat stress showing improvement in the CIMMYT breeding program through era evaluations. These hybrids were CZH15575, CZH0527, CZH15017, CZH16389 and CZH15572. High yield was associated with a reduction in ASI of -0.02 days' year^{-1} thus reducing barrenness. Genetic gain in grain yield was also contributed by its strong positive significant correlation with secondary traits: Grain yield and Ears per plant; Grain yield and Plant height; Grain yield and Ear height; Plant height and Ear height. In addition, regression analysis results conclude that majority of the secondary traits also contributed to genetic gain obtained in grain yield over the years as they showed trends of increasing from 1999 to 2016.

4.9 Recommendations

The results indicated that hybrids with strong heat tolerance are CZH15575, CZH0527, CZH15017, CZH16389 and CZH15572, these can be further grown in different environments to determine their tolerance stability. There is need to do further trials to confirm the performance of the genetic gain hybrids so as to identify heat tolerant hybrids and secondary traits contributing to gains in grain yield. Genetic gain hybrids tested should also be grown in further experiments as grain yield and other secondary traits showed they can still be improved as no signs of reaching plateaus were observed. Since heat stress is often associated with drought, there is need to do a combined trial on heat and drought so as to obtain the effects of these two as they cannot be extrapolated.

4.9.1 Acknowledgements

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CHAPTER 5

Genetic Gain Maize Hybrids Contrast for Terminal Drought Tolerance Under Different Vapor Pressure Deficit Regimes and Varying Soil Drying Environment.

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Abstract

Maize is an important crop in sub-Saharan Africa and many parts of the world. However, its production is being greatly affected by effects of terminal drought stress and increasing temperatures due to climate change. Water deficit stress is considered as one of the most important environmental factors that adversely affect maize production. A lack of water decreases the seedling survival rate and increases the post-pollination embryo abortion rate, ultimately leading to decreased yield. It has been hypothesized that limitation on transpiration at high VPD is a promising trait that could be incorporated into breeding programs to improve drought tolerance in maize so that soil water is conserved for use later in the growing season .75 genetic gain maize hybrids contrasting for yield were grown in pot experiment with three replications. The experiment was laid out in completely randomized deign with three replications. These hybrids ranged from years 1999 to 2016. CIMMYT Zimbabwe is developing and promoting genetic gain drought-tolerant hybrids, but their physiological drought-tolerance mechanisms are not well understood. This study aimed at (i) measuring the sensitivity of transpiration of different maize hybrids to different VPD levels (ii) measuring the sensitivity of transpiration of different maize hybrids under varying water deficit conditions (iii) understanding the morpho-physiological traits that confer drought tolerance under different water stress levels and varying Vapor pressure deficit. Results from this study exhibited that maize hybrids that contrast for yield in the field also contrast significantly in their response to transpiration under varying Vapor pressure deficit (VPD) regimes and limited water conditions. Hybrids showed increase in transpiration with significant differences observed at 3pm where VPD recorded the highest value of 3.32 (kPa). Hybrids were ranked to obtain the best 10 hybrids that conserve water and the least hybrids that lose water in both the VPD and the soil drying experiment. Based on transpiration stability across VPD and soil drying experiment, hybrids (CZH1261, CZH132119 and CZH15013) were observed to conserve water efficiently. The results from genetic gain analysis also showed that hybrids bred earlier conserve water efficiently than hybrids released in later years.

Keywords: Fraction of transpirable soil water - Transpiration - Vapor pressure deficit - Drought stress - Genetic gain

5.1 Introduction

Maize is one of the most important cereal crops in the world. It is relatively susceptible to drought. According to (Fahad *et al.*, 2017) drought is the most important abiotic stress that limits plant productivity and will only worsen in the future when constraints on water resources increase. In addition, drought stress limits maize growth and yield in many agricultural communities and will probably increase in the ongoing climate change. Drought can be defined by the absence of soil water to provide conditions for crops to grow as a consequence of precipitation being less than normal (UN/ISDR, 2009). Terminal drought stress is typical of end season where the crops grows and matures on a progressively depleting soil moisture profile. Terminal drought stress usually affects grain filling stage in cereals leading barrenness – reduced grain yield, and also reduced kernels weight. Maize's susceptibility to water deficit is more pronounced at the reproductive stage and causes the greatest reduction in grain yield when stress coincides with the irreversible reproductive processes. Vapor pressure deficit (VPD) is a measurement of humidity in a particular area. VPD determines the difference between the amount of moisture in the air and the amount of moisture the air can hold.

Genetic gain is the amount of increase in performance achieved per unit time through artificial selection (Xu *et al.,* 2017). Genetic gain is evaluated in era studies. An era study involves the evaluation of the best performing varieties from different years or points in time in a single trial in which the different varieties receive the same management, attributing differences to genetic differences (Hall and Richards, 2013). Temperature and vapour pressure deficit (VPD) are two important factors influencing stomatal conductance and transpiration, with stomatal conductance measuring the degree of stomatal opening and this can be used as an indicator of plant water status, (Giménez *et al.,* 2013). (Devi *et.al.,* 2018) asserts that a limitation on transpiration rate (TR) at high levels of atmospheric Vapor pressure deficit (VPD) is a plant mechanism that could be exploited to allow soil water conservation and to allow sustained crop growth during dry periods in the later part of the growing season.

Although roots can play a significant role in increasing crop yields (Gewin, 2010), limited attention has been paid to root characteristics in crop breeding, possibly because roots grow belowground and are difficult to investigate (White *et al.*, 2013). Over the past decades, improvement in maize's ability to enhance root growth has been the primary driving force for higher yields of newer hybrids (Duvick, 2005). Plant breeders have made considerable gains on 'aboveground' traits, but little attention has been paid on root characteristics.

Plant roots play an important adaptive role in drought prone environments. Although there have been many efforts to improve root traits to develop drought tolerant maize varieties but significant progress has not yet been made mainly due to difficulty in screening root traits. The root system is therefore generally considered as the most important organ with respect to improving crop adaptation to water stress (Vadez, 2014).

Adaptation to water limited environments becomes significant when genotypes that have restricted transpiration rate under high vapour pressure deficit (VPD) conditions so that soil water is conserved for use later in the growing season, (Gholipoor *et al.,* 2013). Erratic rains are being received in many parts of the world, bringing the need to characterize best maize hybrids that perform under varied Vapor pressure deficit (VPD) and transpiration, (Vadez *et al.,* 2008). FTSW define portion of the soil water that plants can take up to support transpiration. (Gholipoor *et al.*, 2013), asserts that under water limited environments hybrids with a higher FTSW threshold (fraction of transpirable soil water for transpiration) can be characterized as having conservative behaviour with regard to plant use. Soil moisture threshold (FTSW), where transpiration declines is extremely useful to understand and forecast maize hybrids behaviour in the face of a water deficit (Sinclair and Ludlow, 1986).

The overall objective of this study was to examine the transpiration response to a drying soil of different maize hybrids and lines under varying VPD regimes. Specific objectives were to: (i) measure the sensitivity of transpiration of different CIMMYT maize hybrids released from 1999 to 2016 to different VPD levels under well-watered conditions. (ii) measure the sensitivity of transpiration of different CIMMYT maize hybrids released from 1999 to 2016 under water deficit conditions. (iii) understand the morpho-physiological traits that confer drought tolerance under different water stress levels in the CIMMYT hybrids released from 1999 to 2016.

5.2 Materials and methods

5.2.1 Genetic material and study site

The study was conducted at CIMMYT research station (17°43′37.21″ S, 31°01′00.60″ E, and altitude 1489 m above sea level) in Harare, Zimbabwe.

The experiment was laid out in completely randomized deign. 75 hybrids were planted with 3 replications. 72 CIMMYT hybrids and 3 local checks from (SC513, SC403 and PAN413) contrasting in tolerance to drought stress were selected for the study from CIMMYT. Plants were grown in pots during October to November 2018 under outdoors conditions. The plants were grown in pots of 22cm height and 16cm diameter. The pots were filled with 2.9kg (± 0.2kg) of CIMMYT Zimbabwe greenhouse soil. Plants were kept well-watered at all times by increasing the frequency and amount of irrigation, applied by hand, during plant growth.

5.2.2 Transpiration measurement to varying VPD regimes

A measurement of leaf transpiration rate (g cm^{-2} h^{-1}) was done at 40 days after planting when the plants were at the late vegetative stage, in outdoor conditions. Vapour Pressure Deficit measurements were done for the first 2 consecutive days and these were used as control – when transpiration is still optimum in all pots. All pots were irrigated to field capacity 2 days before starting the experiment and allowed to drain overnight. They were bagged the following day with a transparent plastic bag wrapped around the stem to avoid soil evaporation (Zaman-Allah *et al.*, 2011). Measurements of transpiration under varied Vapour pressure deficit interval was performed by weighing the pots using a digital scale on 2-hour intervals throughout the day, starting at 7am and finishing at 5pm. To calculate atmospheric VPD, temperature and relative humidity was recorded every 30 minutes.

VPD was calculated from the corresponding air temperature and relative humidity recorded throughout the day.

$$\text{SVP (Pascals)} = 610.7 * 10^{\mathbf{7.5T/(237.3+T)}}$$

$$\text{VPD} = ((100 - \text{RH})/100) * \text{SVP}$$

where RH (%) is relative humidity and SVP is saturated vapour pressure (kPa) taken from a table of air temperature and RH data.

5.2.3 Plant growth and response to drought

All pots were irrigated to field capacity 2 days before starting the experiment and allowed to drain overnight. The first 2 days were used as control this is when transpiration is still optimum in all pots. They were bagged the following day with a transparent plastic bag wrapped around the stem to avoid soil evaporation. The soil in the three replications treatments was allowed to dry progressively over 1 week. Water was added to drying pots if needed to have only a maximum 80g (for the first 4 days) then reduced to 60g (the remaining 2 days) net loss of water each day. Any transpiration in excess of these maximum daily water loss allowed was added back to the pots (Vadez and Sinclair, 2001). Water to be added (WTA) was calculated as (Day1 weight – Day2 weight) – 60g (constant) using excel. Independent WTA was calculated for each pot. If WTA > 0 (positive) that figure was used as the amount of water to be added for the day in respective pots. If WTA ≤ 0 (negative) no water was added. All pots were weighed first using a digital scale at a constant day time (9am) interval for 7 days and the figures collected were recorded in Microsoft excel sheets. After recording and calculating WTA, required water was added to the respective pots for that day.

Transpiration data normalization calculated as (Transpiration rate of each day for Water stress / Average of Well-watered plants for VPD treatment), (Kholová *et al.*, 2010). Normalization done to eliminate the variations in transpiration rates among days as a result of weather variations. Normalization performed for each plant in drying soil experiment calculated as (Daily value of first normalization of each plant / Average of the first normalization value of that plant over the past 2 days of the experiment (when plants in the drying soil were still under well-watered conditions) (Kholová *et al.*, 2010). After harvest, the Fraction of Transpirable Soil Water (FTSW) was calculated. FTSW define portion of the soil water that plants can take up to support transpiration. FTWS of day (n) was calculated as (Pot weight of day (n) – Final pot weight) / (Initial pot weight – Final pot weight). The root: shoot ratio was calculated after removing the plants from the soil – (Dry weight for roots/dry weight for top of plant = root/shoot ratio), (Zhao *et al.*, 2018). Shoot was cut at the soil line and roots were washed off to remove any loose soil. Shoot fresh weights were measured at the moment of the harvesting. Maize roots and shoots (including stem and leaves) were separated and oven dried at 150 ºC. Roots and shoots were then separately weighed for each plant using a digital scale.

5.3 Data analysis

Analyses of variance (ANOVAs) were done with the statistical program package R version 3.5.2. To compare genotype means across treatment where the Tukey–Kramer test was then used for the analysis of differences between genotype means (letters in Table 1 and 2). Regression analysis of Transpiration (TR) response to increasing VPD was done using TR data of 3 replicate plants per genotype. TR response was plotted against each VPD using **R software**.

Split-line regression, which provides a break-point value where the slope of fitted regression changes as well as value of the slope was done using **GraphPad Prism version 8** to obtain NTR against FTSW graphs as described previously (Ray and Sinclair, 1998).

5.4 Results

5.4.1 The response of maize hybrids to different VPD regimes under varying time intervals.

Table 5.1: Means for maize hybrids and the top 10 best and 10 bottom performing hybrids when evaluated under non-stressed environments with varying VPD.

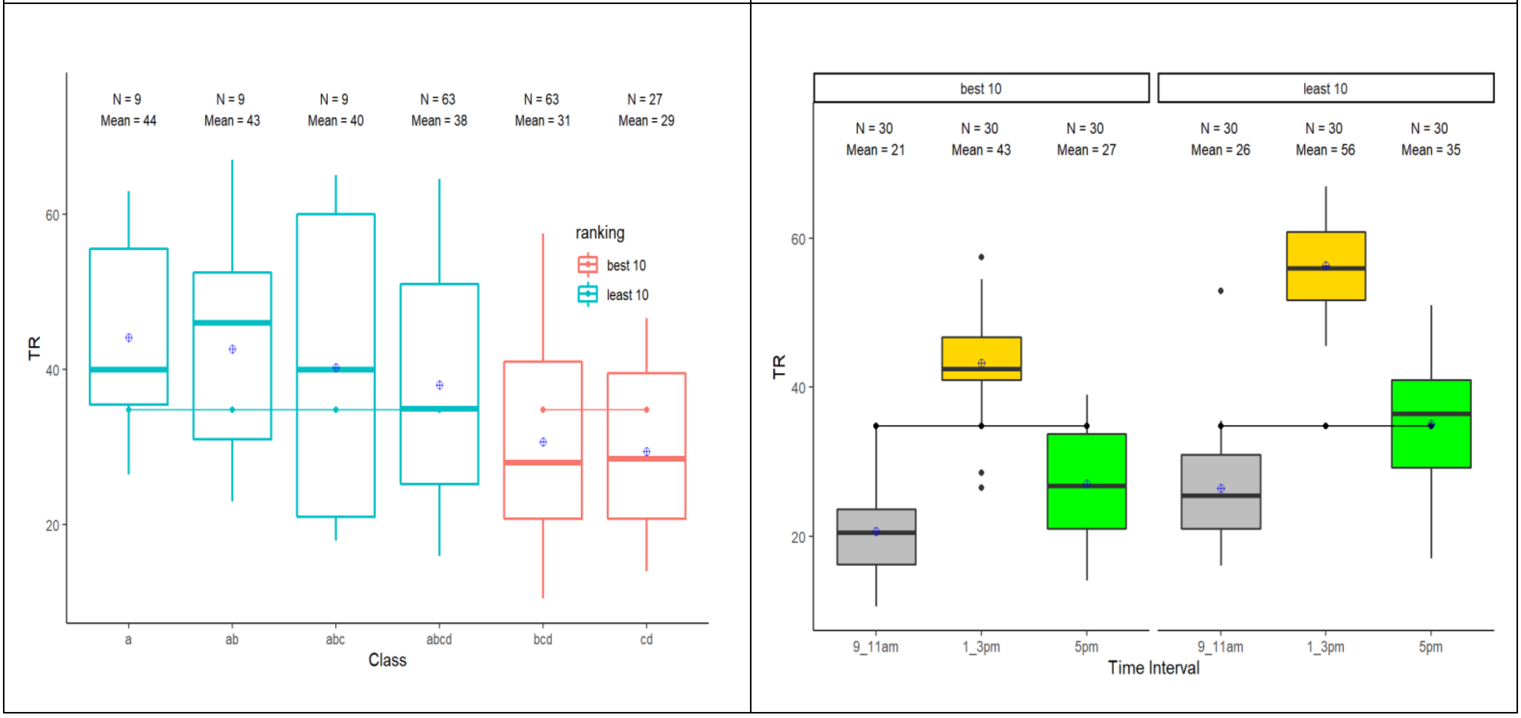
Class	Ranking	Entry	Hybrid	TR-9am	TR-11am	TR-1pm	TR-3pm	TR-5pm	Total Water Lost	Average
Top 10	1	55	**CZH142055**	12.33	23.33	43.33	**39.67^{b}**	28.33	147	**29.40^{cd}**
Top 10	2	61	**CZH15013**	15.83	28	36.33	**45.67^{ab}**	25.33	151.17	**30.23^{cd}**
Top 10	3	35	**CZH0932**	13.33	29.67	43.67	**40.33^{b}**	25.33	152.33	**30.47^{cd}**
Top 10	4	70	**CZH16334**	10	28	41.33	**44.67^{ab}**	30	154	**30.80^{bcd}**
Top 10	5	53	**CZH142056**	12.33	19.33	48.67	**46.67^{ab}**	28	155	**31.00^{bcd}**
Top 10	6	49	**CZH132119**	13	38.33	40.67	**42.33^{b}**	22	156.33	**31.27^{bcd}**
Top 10	7	45	**CZH1261**	11.67	24	41.33	**53.33^{ab}**	27.17	157.5	**31.50^{bcd}**
Top 10	8	22	**CZH0528**	14	29.67	44.67	**44.67^{ab}**	25	158	**31.60^{bcd}**
Top 10	9	44	**CZH1258**	15.33	32	39	**42.00^{b}**	29.67	158	**31.60^{bcd}**
Top 10	10	58	**CZH15188**	13.33	29	41	**45.33^{ab}**	29.67	158.33	**31.67^{bcd}**
Top 10 grand mean				13.12	28.13	42	**44.47**	44.47	154.77	**30.95**
Bottom 10	11	24	**CZH0526**	15	34.33	55.83	**52.50^{ab}**	32.33	190	**38.00^{abcd}**
Bottom 10	12	72	**CZH16335**	16.67	39.67	50.67	**51.67^{ab}**	34.33	193	**38.60^{abcd}**
Bottom 10	13	2	**SC403**	13.33	25.33	57	**62.67^{ab}**	35	193.33	**38.67^{abcd}**
Bottom 10	14	36	**CZH0935**	14.33	51	51.33	**50.00^{ab}**	28	194.67	**38.93^{abcd}**
Bottom 10	15	52	**CZH132163**	17	31.67	54.67	**54.33^{ab}**	38	195.67	**39.13^{abcd}**
Bottom 10	16	50	**CZH132118**	20	38	53.33	**55.00^{ab}**	31	197.33	**39.47^{abcd}**
Bottom 10	17	65	**CZH15467**	19.33	34.33	58	**55.67^{ab}**	33.33	200.67	**40.13^{abcd}**
Bottom 10	18	11	**CZH01008**	11.67	28	62.33	**62.33^{ab}**	38.67	203	**40.60^{abc}**
Bottom 10	19	64	**CZH15212**	18.33	41.33	57	**62.33^{ab}**	38.33	217.33	**43.47^{ab}**
Bottom 10	20	51	**CZH132169**	18.67	41.33	53.33	**67.33^{a}**	42	222.67	**44.53^{a}**
Bottom 10 grand mean				16.43	36.5	55.35	**57.38**	35.1	200.77	**40.15**

Different letters in columns indicate a statistical difference between hybrids in time interval transpiration at $P<0.05$ (Turkey mean separation).

There were observable differences in the transpiration response to Vapor pressure deficit between the 2 measured days of each hybrid. Table 5.1: shows fluctuations for total transpired water amongst 75 maize hybrids in the well-watered treatment over a duration of two days. Trial overall mean transpiration was (34.78 g cm^{-2} h^{-1}). Hybrid CZH142055 had the least transpiration mean of 29.40 (g cm^{-2} h^{-1}) as compared to hybrid CZH132169 which recorded the largest total water loss of 44.53 (g cm^{-2} h^{-1}) under 0.75 kPa and 2.89 kPa. All the best 10 ranked hybrids had transpiration below the overall mean, whilst all the least ranked hybrids had all their means above the overall mean. In the experiment it was observed that transpiration increased with increasing VPD levels and the highest transpiration rates were recorded at around 3pm. Transpiration differences in contrasting maize hybrids were statistically significant at $P< 0.001$ for both time intervals and hybrids.

Transpiration at 9am, 11am, 1pm and 5pm showed no significant differences but 3pm time interval showed very significant difference. This brought the need to group time interval as (9-11am; 1-3pm and 5pm) so as to clearly observe discriminations between the joint time intervals as presented in Fig 5.1. Overall, hybrids contrasting for response to transpiration in (Table 5.2) showed significant variations between the best grouped and least grouped in mean interval transpiration.

5.4.1.1 The response of maize hybrids to different VPD regimes ranked mean classes and time intervals.


a) Class vs Ranked Hybrids (TR)

b) Time Interval vs Ranked Hybrids (TR)

Figure 5.1: Combination of box plots for transpiration differences amongst varying VPD regimes.

** From Figure 5.1 above:

- Fig a) shows classes after mean separation. These mean separated classes for hybrids were compared against Transpiration (TR). Colour codes in a) **Green** indicates least conserving hybrids in relation to Transpiration and **Red** indicates the best water conserving hybrids under different VPD regimes.

- Fig b) shows time intervals measured compared with Transpiration (TR) of hybrids. Colour codes in b) **Grey** indicates times interval for period 9am to 11am; **Gold** indicates time interval for period 1pm to 3pm; **Green** indicates time interval for period 5pm under varying VPDs.

The red colour represents the best hybrids that conserve water under varying VPDs and the green colour represent the sensitive hybrids in plot **(a)**, **(b)**. The lines inside boxplot represent the average transpiration of the grouped hybrids. (Fig 5.1) also shows hybrids best ranked and least ranked in transpiration under different time intervals. The average transpiration for the two rankings was 35.55 (g cm^{-2} h^{-1}). In plot **(a)** the best hybrids that transpired less, average means ranged **(cd)** (30.03

g cm^{-2} h^{-1}), **(bcd)** (31.35 g cm^{-2} h^{-1}) whereas the least water conserving hybrids had average means of abcd) (38.99 g cm^{-2} h^{-1}), **(abc)** (40.60 g cm^{-2} h^{-1}) and **(ab)** (43.47 g cm^{-2} h^{-1}) and **(a)** (44.53 g cm^{-2} h^{-1}) respectively. In plot **(b)** shows gradual increase in transpiration from the VPD experiment. 9am interval recorded the least mean averages on both the best ranked and least ranked in transpiration, with 1pm and 3pm recording peak transpiration values and 5pm mean averages for both ranked hybrids started decreasing gradually. Hybrids CZH142055 from era (2011 to 2016), CZH15013 from era (2011 to 2016) and CZH0932 from era (2007 to 2010) showed the best characteristics to conserve water under varying vapor pressure deficit, whilst CZH01008, CZH15212 and CZH132169 from eras (1999 to 2002) and (2011 to 2016) respectively.

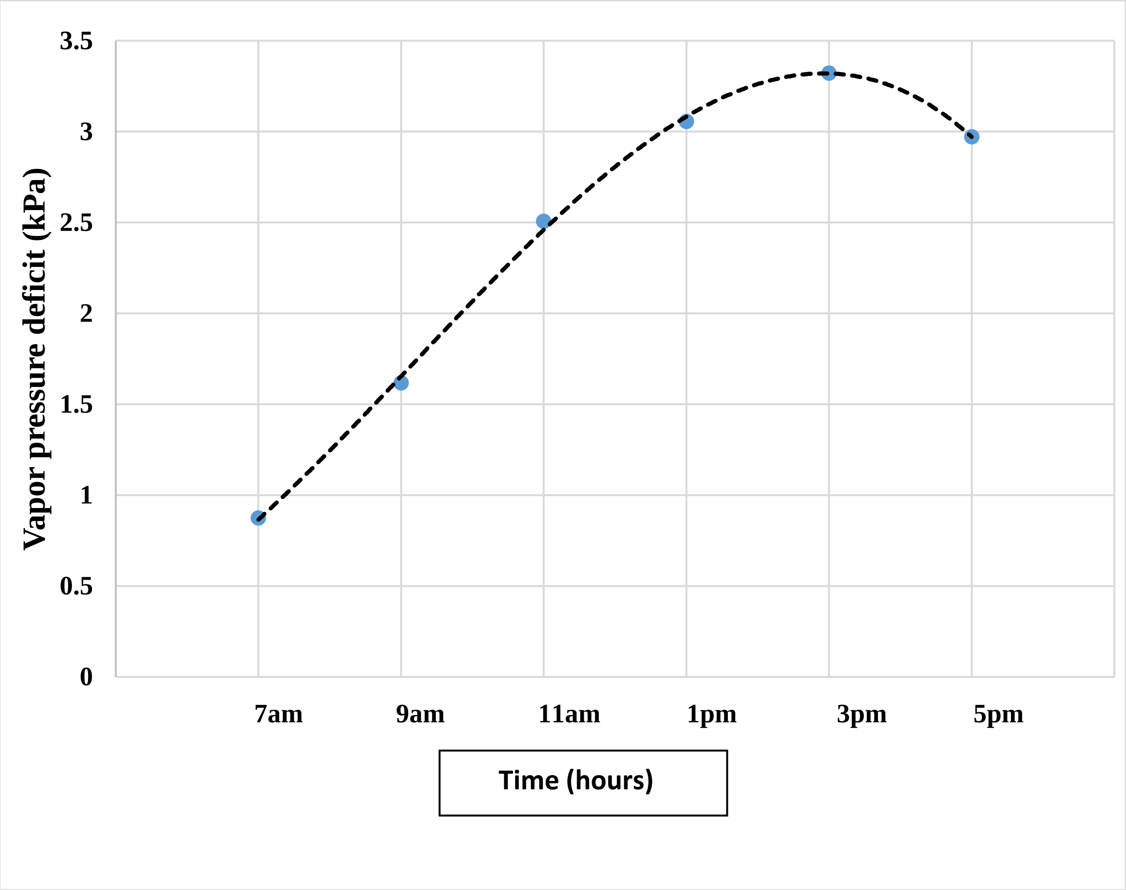


Figure 5.2: Graph showing varying VPD levels on a 2-day experiment.

VPD values increased from 7 am value of 0.87 (kPa) to 2.97 (kPa) at 17:00 hours. Highest VPD value 3.32 (kPa) was recorded at 15:00 hours. The variation in VPD was mainly due to variations in temperature recorded throughout the day.

5.4.2 Transpiration responses of maize hybrids under water deficit conditions.

Table 5.2: Means for maize hybrids and the top 10 best and 10 bottom performing hybrids when evaluated under stressed environments (Soil drying), ranked according to their performance under varying moisture deficit conditions.

Class	Rank	Entry	Hybrid	Day-2	Day-3	Day-4	Day-5	Day-6	Mean Transpiration(days)
Top 10	1	62	**CZH15185**	160.33	131.33^{ab}	107.67	14.00^{a}	4.33	$\mathbf{83.53^{cd}}$
Top 10	2	66	**CZH15572**	197.67	136.00^{ab}	117.67	47.00^{b}	-44.00	$\mathbf{90.87^{bcd}}$
Top 10	3	49	**CZH132119**	199.00	108.00^{b}	115.67	36.00^{b}	-0.33	$\mathbf{91.67^{bcd}}$
Top 10	4	36	**CZH0935**	174.33	137.33^{ab}	108.00	52.67^{b}	-10.67	$\mathbf{92.33^{abcd}}$
Top 10	5	71	**CZH16389**	212.67	133.00^{ab}	110.33	41.00^{b}	-10.00	$\mathbf{97.40^{abcd}}$
Top 10	6	68	**CZH15343**	220.33	121.00^{ab}	120.67	53.00^{b}	0.67	$\mathbf{103.13^{abc}}$
Top 10	7	45	**CZH1261**	178.00	142.00^{ab}	128.67	69.00^{b}	5.33	$\mathbf{104.60^{abc}}$
Top 10	8	61	**CZH15013**	184.67	136.00^{ab}	134.67	67.33^{b}	9.33	$\mathbf{106.40^{abc}}$
Top 10	9	28	**CZH0735**	192.33	141.00^{ab}	132.33	65.00^{b}	7.67	$\mathbf{107.67^{abc}}$
Top 10	10	57	**CZH15002**	194.67	147.33^{ab}	129.67	68.00^{b}	-1.33	$\mathbf{107.67^{abc}}$
Top 10 grand mean				**191.40**	**133.3**	**120.53**	**51.3**	**-3.90**	**98.53**
Bottom 10	11	65	**CZH15467**	248.00	155.00^{ab}	158.00	85.67^{b}	22.00	$\mathbf{133.73^{abc}}$
Bottom 10	12	75	**CZH16048**	225.00	167.67^{ab}	164.67	96.67^{b}	20.00	$\mathbf{134.80^{abc}}$
Bottom 10	13	4	**CZH99015**	222.00	169.00^{ab}	160.67	96.33^{b}	36.67	$\mathbf{136.93^{abc}}$
Bottom 10	14	31	**CZH089**	223.00	177.00^{ab}	166.33	97.33^{b}	26.67	$\mathbf{138.07^{abc}}$
Bottom 10	15	47	**CZH1270**	240.00	256.33^{a}	111.67	71.00^{b}	13.67	$\mathbf{138.53^{abc}}$
Bottom 10	16	2	**SC403**	244.33	166.33^{ab}	147.33	78.67^{b}	56.67	$\mathbf{138.67^{abc}}$
Bottom 10	17	46	**CZH1243**	222.33	199.67^{ab}	156.33	103.33^{b}	26.00	$\mathbf{141.53^{abc}}$
Bottom 10	18	11	**CZH01008**	259.00	159.33^{ab}	156.33	85.67^{b}	55.00	$\mathbf{143.07^{abc}}$
Bottom 10	19	1	**SC513**	240.00	170.00^{ab}	160.67	93.00^{b}	59.00	$\mathbf{144.53^{ab}}$
Bottom 10	20	64	**CZH15212**	266.67	206.00^{ab}	160.67	97.33^{b}	28.33	$\mathbf{151.80^{a}}$
Bottom 10 grand mean				**239.03**	**182.63**	**154.27**	**90.5**	**34.40**	**140.17**

Different letters in columns indicate a statistical difference between hybrids in time interval transpiration at $P<0.05$ (Turkey mean separation).

(Table 5.2) shows that when the pots were well watered, hybrids had increased transpiration which later reduced as the soil drying experiment continued until plants had less water that could be transpired. High transpiration was observed during first days when pots were close to full saturation, the soil provide much water to move through the plant. As the dry down experiment progressed wilting sign in leaves were observed in majority of the hybrids. Trial overall mean transpiration was 122.44 ($\text{g cm}^{-2}\ \text{h}^{-1}$). The highest water conserving hybrid was CZH15185 with an average daily transpiration of 83.53 ($\text{g cm}^{-2}\ \text{h}^{-1}$), whereas the least performing hybrid was CZH15212 from the soil drying experiment with mean daily transpiration of 151.80 ($\text{g cm}^{-2}\ \text{h}^{-1}$) (Table 5.2). In both experiments' hybrids CZH1261, CZH132119 and CZH15103 consistently appeared in the best 10 ranked hybrids that conserve water, whilst hybrids CZH01008, CZH15212 and CZH16048 and commercial check SC403 consistently appeared in the least ranking group for not being able to conserve water (Table 5.1, Table 5.2).

5.4.2.1 Fraction of transpirable soil water (FTSW) threshold value for transpiration decline in four maize genotypes contrasting for drought tolerance in the field.

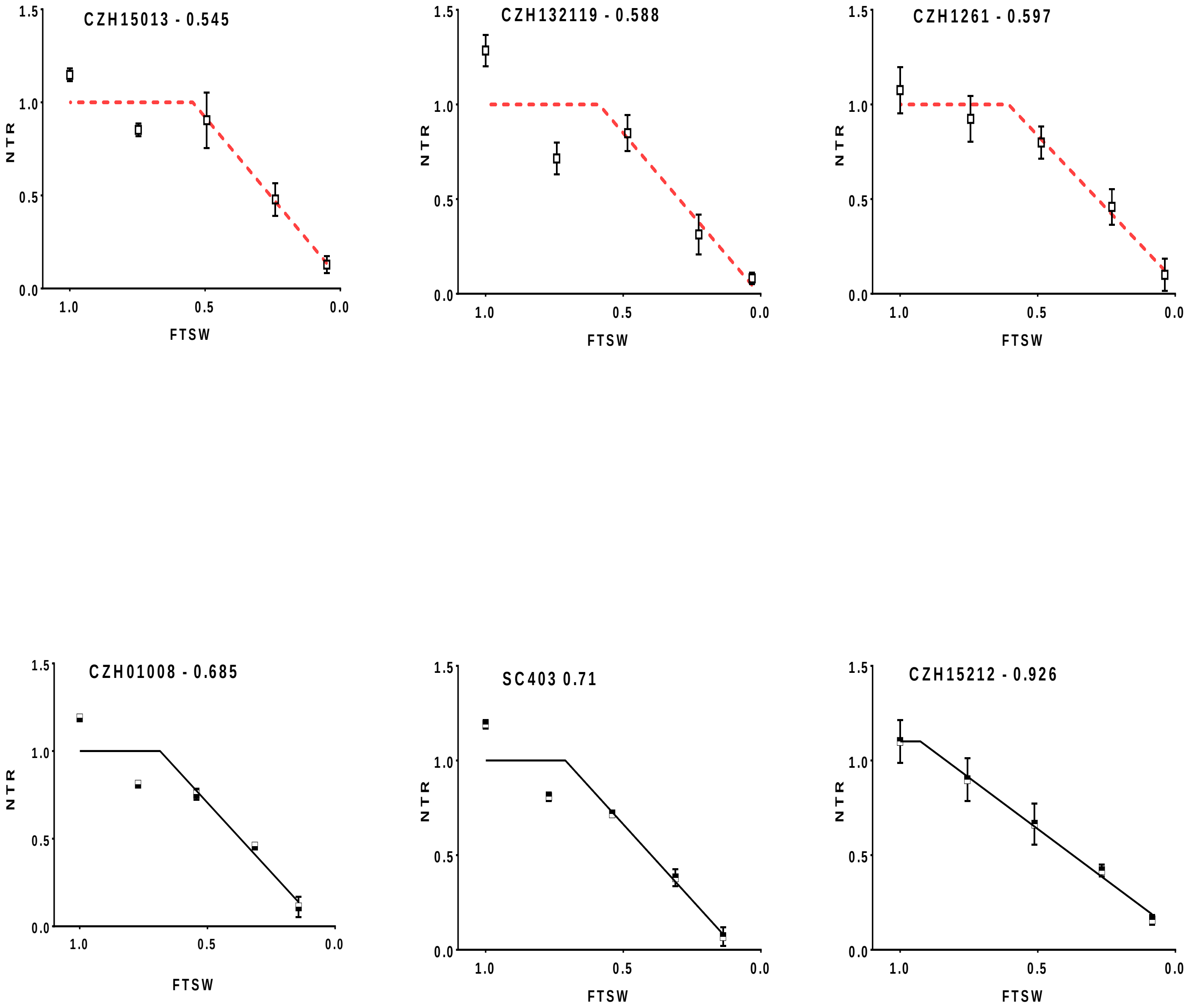


Figure 5.3: Relationship between the normalized transpiration ratio (NTR) and the fraction of transpirable soil water (FTSW) of six maize genotypes contrasting for yield performance under drought stress in the field.

Plants were grown under water deficit for six days with reduction in moisture. The FTSW threshold values were higher for sensitive hybrids CZH1212, CZHSC403 and CHZ01008 compared to tolerant hybrids CZH15013, CZH132119 and CZH1261. Hybrid CZH15013 exhibited the lowest FTSW value of 0.548.

5.4.3 Correlation of roots and leaves biomass

Table 5.3: Mean performance of maize (Zea mays L.) genotypes for biomass partitioning under different water deficit regimes.

Class	Ranking	Entry	Name	Shoot biomass	Root biomass	Root:shoot
Top 10	1	62	**CZH15185**	8.05	5.11	0.63
Top 10	2	66	**CZH15572**	8.35	5.50	0.66
Top 10	3	49	**CZH132119**	6.05	3.67	0.61
Top 10	4	36	**CZH0935**	11.41	6.03	0.53
Top 10	5	71	**CZH16389**	7.34	4.99	0.68
Top 10	6	68	**CZH15343**	7.61	5.33	0.70
Top 10	7	45	**CZH1261**	9.74	4.53	0.47
Top 10	8	61	**CZH15013**	6.53	3.53	0.54
Top 10	9	28	**CZH0735**	7.59	7.78	1.03
Top 10	10	57	**CZH15002**	14.02	4.47	0.32
Top 10 Grand mean				**8.67**	**5.09**	**0.62**
Bottom 10	11	65	**CZH15467**	12.19	6.21	0.51
Bottom 10	12	75	**CZH16048**	10.02	6.11	0.61
Bottom 10	13	4	**CZH99015**	7.40	4.24	0.57
Bottom 10	14	31	**CZH089**	8.99	3.96	0.44
Bottom 10	15	47	**CZH1270**	10.44	8.58	0.82
Bottom 10	16	2	**SC403**	11.11	4.59	0.41
Bottom 10	17	46	**CZH1243**	11.79	7.50	0.64
Bottom 10	18	11	**CZH01008**	13.26	7.38	0.56
Bottom 10	19	1	**SC513**	11.76	6.12	0.52
Bottom 10	20	64	**CZH15212**	16.38	5.63	0.34
Bottom 10 Grand mean				**11.33**	**6.03**	**0.54**

By contrast, no significant discrimination was observed between hybrids ranked as best water conserving and least water conserving under varying water deficit conditions on the basis of root: shoot ratio, (Table 5.3).

$$\boldsymbol{ROOT{:}SHOOT\ RATIO = 0.06518 + 0.0017 * Release}$$

R^2 = 0.0139

$$\boldsymbol{GYD = -67.12530 + 0.03503 * Release}$$

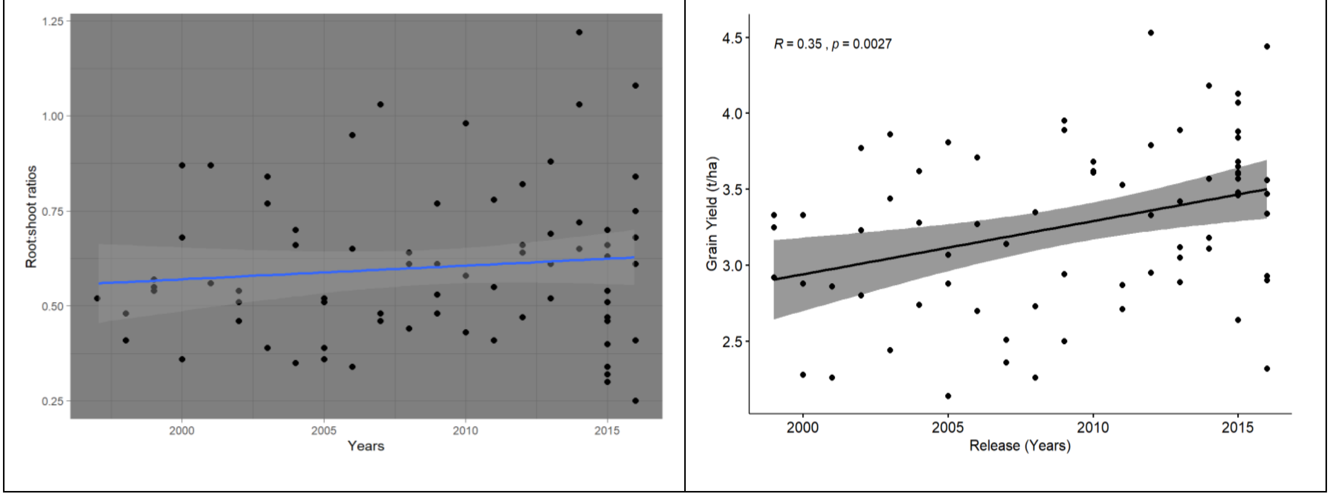


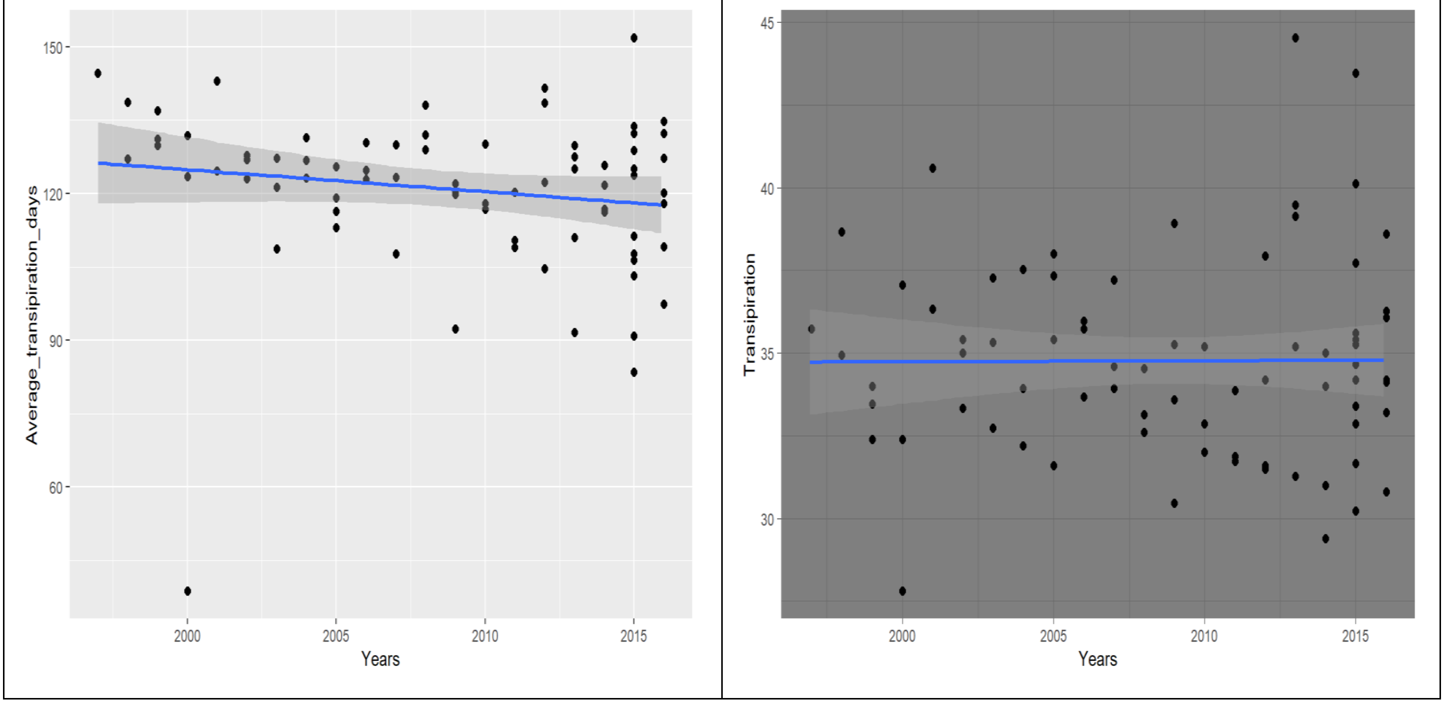
Figure 5.4: Genetic gain for pot experiment root: shoot ratio and field grain yield over 17 years.

Root: shoot ratio generally showed a trend to increase over the 17 years at a rate of 0.002 g plant^{-1} year^{-1} under pot experiment. The study showed significant genetic gain in hybrids developed by the CIMMYT ESA drought tolerance breeding programme over the 17-year period from 1999 to 2016. Genetic gain in grain yield against time of release showed net positive gains of 35.03 kg ha^{-1} year^{-1}.

Table 5.4: Summary of yield and secondary traits of the 10 best and 10 least performing hybrids under drought stress trial in the field.

CLASS	RANKING	ENTRY	HYBRID	GYD		AD		ASI		PH		EH		EPP	SEN1		SEN2		RELEASE
Top 10	**1**	62	**CZH1261**	4.53	a	75.67	efghijklmno	0.0	ab	201.52	defghijklmnopqr	105.60	bcdefghijklmnop	0.89	0.85	ijklm	1.17	bcd	2012
Top 10	**5**	66	**CZH15002**	4.07	abcde	73.00	lmnop	4.3	a	206.30	abcdefghijklmno	90.93	mnopq	0.87	1.97	bcdef	1.50	ab	2015
Top 10	**6**	49	**CZH0935**	3.95	abcdef	75.33	fghijklmno	1.0	ab	201.77	defghijklmnopqr	110.96	abcdefghijklmn	0.86	0.83	ijklm	1.33	bc	2009
Top 10	**9**	36	**CZH15343**	3.88	abcdefgh	81.33	abc	1.0	ab	218.92	abcd	115.73	abcdefgh	0.94	0.67	jklm	1.16	bcd	2015
Top 10	**13**	71	**CZH1243**	3.79	abcdefghij	75.33	fghijklmno	1.3	ab	195.10	ijklmnopqrs	97.22	fghijklmnopq	0.76	0.81	ijklm	1.00	cd	2012
Top 10	**16**	68	**CZH15467**	3.68	abcdefghijk	75.67	efghijklmno	0.0	ab	202.06	defghijklmnopqr	98.40	fghijklmnopq	0.85	1.17	ghijk	1.50	ab	2015
Top 10	**22**	45	**CZH15572**	3.61	abcdefghijklm	75.67	efghijklmno	2.3	ab	213.54	abcdefg	115.22	abcdefghi	0.87	1.81	cdefg	1.33	bc	2015
Top 10	**24**	61	**CZH15013**	3.57	abcdefghijklm	74.00	ijklmnop	0.3	ab	201.04	efghijklmnopqr	86.51	pqr	0.84	1.85	cdefg	1.17	bcd	2015
Top 10	**28**	28	**CZH15212**	3.48	abcdefghijklmno	73.33	klmnop	0.0	ab	194.24	jklmnopqrs	103.98	bcdefghijklmnop	0.74	0.83	ijklm	1.33	bc	2015
Top 10	**33**	57	**SC403**	3.42	bcdefghijklmnopq	75.33	fghijklmno	1.0	ab	213.03	abcdefgh	111.45	abcdefghijkl	0.84	1.51	efghi	1.33	bc	1998
Top 10 grand mean				**3.80**		**75.47**		**1.13**		**204.75**		**103.60**		**0.85**	**1.23**		**1.28**		
Bottom 10	**34**	65	**CZH089**	3.35	bcdefghijklmnopqr	75.67	efghijklmno	2.0	ab	197.11	ghijklmnopqrs	105.35	bcdefghijklmnop	0.79	0.31	lm	1.34	bc	2008
Bottom 10	**35**	75	**CZH16389**	3.34	bcdefghijklmnopqr	78.00	cdefghi	-0.7	b	195.89	hijklmnopqrs	103.88	bcdefghijklmnop	0.79	1.36	fghij	1.17	bcd	2016
Bottom 10	**41**	4	**CZH99015**	3.25	cdefghijklmnopqrs	75.33	fghijklmno	0.3	ab	190.42	opqrst	96.39	ghijklmnopq	0.79	1.36	fghij	1.51	ab	1999
Bottom 10	**49**	31	**SC513**	3.02	defghijklmnopqrs	76.00	defghijklmn	1.7	ab	207.77	abcdefghijklmno	115.22	abcdefghi	0.77	1.83	cdefg	1.18	bcd	1997
Bottom 10	**50**	47	**CZH1270**	2.95	efghijklmnopqrs	79.33	bcdef	0.3	ab	199.27	efghijklmnopqrs	112.73	abcdefghijk	0.85	1.16	ghijk	1.00	cd	2012
Bottom 10	**55**	2	**CZH132119**	2.89	fghijklmnopqrs	76.00	defghijklmn	0.3	ab	206.42	abcdefghijklmno	90.50	nopq	0.71	0.67	jklm	1.17	bcd	2013
Bottom 10	**66**	46	**CZH15185**	2.64	klmnopqrs	75.67	efghijklmno	3.0	ab	216.41	abcde	110.32	abcdefghijklmn	0.84	1.81	cdefg	1.33	bc	2015
Bottom 10	**67**	11	**CZH0735**	2.51	lmnopqrs	72.67	mnopq	1.0	ab	186.88	qrst	101.19	defghijklmnopq	0.77	0.82	ijklm	1.49	ab	2007
Bottom 10	**71**	1	**CZH16048**	2.32	pqrs	78.00	cdefghi	0.0	ab	199.72	efghijklmnopqr	107.43	bcdefghijklmno	0.77	2.36	bcd	1.16	bcd	2016
Bottom 10	**74**	64	**CZH01008**	2.26	rs	84.00	a	0.0	ab	184.70	rst	103.87	bcdefghijklmnop	0.69	1.15	ghijk	1.49	ab	2001
Bottom 10 grand mean				**2.85**		**77.07**		**0.80**		**198.46**		**104.69**		**0.78**	**1.28**		**1.28**		
			Mean	**3.26**		**76.30**		**0.8**		**201.30**		**103.20**		**0.8**	**1.29**		**1.24**		
			Mse	**0.78**		**20.33**		**2.9**		**282.00**		**359.38**		**0.01**	**1.09**		**0.11**		
			LSD	**1.14**		**4.19**		**1.9**		**17.56**		**20.53**		**0.137**	**0.96**		**0.49**		
			h2	**0.54**		**0.68**		**0.6**		**0.66**		**0.57**		**0.257**	**0.07**		**0.00**		
			p	******		*******		*******		*******		*******		**ns**	*******		**ns**		
			Min	**2.14**		**68.7**		**-0.67**		**162.8**		**69.27**		**0.657**	**0.16**		**0.83**		
			Max	**4.53**		**84**		**4.33**		**223.6**		**130.1**		**0.937**	**3.18**		**1.84**		

Yield data was used to assess how the best and least ranked hybrids in pot experiment conferred to drought tolerance in the field. Table 5.4 shows results from field data shows that hybrids CZH0935 (4th), CZH15343 (6th), CZH1261 (7th) and CZH15002 (10th) were consistently found in the best 10 ranked as they conserved water in pot soil drying experiment and also yielded best in the field CZH1261 (1st), CZH15002 (5th), CZH0935 (6th) and CZH15343 (9th). These four hybrids yielded 4.53 t ha^{-1}, 4.07 t ha^{-1}, 3.95 t ha^{-1} and 3.88 t ha^{-1} which was above the trial mean of 3.26 t ha^{-1}. These four hybrids showed better adaptation in stressed environment. Overall, hybrids produced in recent years exhibited trait to conserve water under stress in pot experiment than old hybrids however, newer hybrids proved to be more yielding as compared to old ones in the field. Older hybrids showed better water conservative trait under stress through their ability to roll their leaves. However, leaf rolling has a negative effect of yield as it reflects hydraulic conductance and it reduces the quantity of light intercepted by the canopy. Hybrids CZH16048 (71st) and CZH01008 (74th) were ranked in the bottom 10 ranking for either grain yield in field attaining 2.32 t ha^{-1} and 2.26 t ha^{-1} respectively and this was below the trial mean. In addition, CZH15185 and CZH0735 were ranked 1st and 9th as hybrid that best conserve water in water deficit environment under soil drying experiment but they were ranked 66th and 67th on grain yield table ranking. These two hybrids had mean average yield of 2.64 t ha^{-1} and 2.51 t ha^{-1} which was far below the trial mean of 3.26 t ha^{-1}.



a) Changes in transpiration for overall hybrids in soil drying conditions.
$y = -0.0931x + 310.73$. $R^2 = 0.0017$

b) Changes in transpiration in overall hybrids under varying VPD regimes.
$y = 0.0669x - 99.562$. $R^2 = 0.0141$

Figure 5.5: Transpiration plotted against year for the best contrasting hybrids from local trials between 1999 and 2016 under soil drying and different VPD levels.

There is significant decrease (a) in genetic gain for water conservation under soil drying conditions from year 1999 to 2016. Transpiration rate under VPD shows a slight significant increase from year 1999 to 2016. Under soil drying experiment, the rate of transpiration progress measured was 93.1 (g cm^{-2} h^{-1} y^{-1}) (Fig 5.4a) and 66.9 (g cm^{-2} h^{-1} y^{-1}) (Fig 5.4b) under varying VPD conditions respectively.

5.5 Discussion

5.5.1 Measuring the sensitivity of transpiration of different maize hybrids to different VPD levels

(Kramer, 1983) observed that Vapor pressure difference between leaves and the surrounding atmosphere is the driving gradient of water loss from plants. Results from (Fig 5.1, Table 5.1 and Table 5.2) helped reflect how much maize experiment under varying VPD regimes can be able to identify hybrids which could be grown in water limited environments across many agro-ecological regions. Furthermore, the difference in transpiration observed between non-water stress (Table 5.1) and water stress (Table 5.2) was in accordance with the finding of many experiments that managed to classify hybrids having ability to conserve water when exposed to low and high VPD and also different water deficit conditions.

Limited transpiration mechanism at high VPD has already been highlighted in previous research work amongst many species to be the key trait that helps research develop hybrids tolerant to drought stress: maize (Zea mays L.) (Gholipoor *et al.*, 2013), soybean (*Glycine max* (L.) Merr.) (Sadok and Sinclair, 2009) and chickpea (C*icer arietinum* L.; (Zaman-Allah *et al.,* 2011).

When soil moisture is adequate, this allows plants to perform all their physiological processes efficiently which include cooling the plant under high temperatures and also allowing stomas to remain open so as to take up carbon dioxide. Limited soil moisture reduces stomatal opening thus causing a reduction in carbon dioxide taken up by the plant, this leads to increased leaf temperatures, leaf rolling - limiting photosynthesis to be performed efficiently. Limited transpiration is attributed to water conservation traits namely early and partial stomatal closure under high Vapor pressure deficit. Reduction in stomatal opening controlled water loss in the measured hybrids. Limited transpiration trait as a crop water management strategy under drought conditions is now well defined across crop genotypes and species, including maize, (Choudhary *et al.,* 2013).

5.5.2 Measuring the sensitivity of transpiration of different maize hybrids under varying water deficit conditions

In the soil drying experiment Fraction of transpirable soil water ranged from 1 to 0.06 in the soil drying experiment amongst 75 tested hybrids. Transpiration decreased as moisture levels in the pots reduced over days. This left maize plants with little to water to use and forced leaves to roll so as to conserve the water. This observation is supported by previous studies of (Slatyer,

1957) who explained that the transpiration of *Gossypium barbadense* L. decreases with decreases in soil water potential.

Closure of the stomata improves water use efficiency under water deficit conditions. Stomatal close closure has also been observed to lead to decrease in CO_2 assimilation thus lowering photosynthetic efficiency. This could explain the results that were observed in this study which showed that hybrids that were able to conserve water under pot experiment obtained low yield in field conditions. This can be further explained by the fact that under well-watered conditions maize hybrids lose a lot of water as they transpire more, increasing photosynthetic capacity and in reserve water deficit conditions encourages plant to perform leaf rolling thus reducing surface area for light interception thus reducing photosynthesis in the field.

Results from Fig 5.3 shows different patterns of Fraction of transpirable soil water (reduction of transpiration) among tested hybrids under during the progressive soil drying trial. Hybrid CZH15013 exhibited the lowest FTSW value of 0.548 enabling it to perform normal physiological functions even when water levels in the soil are around 50%.

(Vadez *et al.,* 2012) asserts that high leaf–air VPD imposes high leaf–air gradients and drives water out of the leaves at a faster rate, leading to more rapid depletion of soil moisture, especially when water is available. Therefore, plants cannot continue to transpire without wilting if the soil is very dry since water in the xylem that moves out through the leaves will not being replaced by the soil water.

5.5.3 Understanding the morpho-physiological traits that confer drought tolerance under different water stress levels and varying Vapor pressure deficit.

The results obtained in this study helped to have an understanding of CIMMYT maize hybrids under genetic gain being assessed for the past 17 years, with terminal drought tolerance capacity through the water limitation trait under high temperature and moisture stress. These results indicate that yield itself in field experiments is not a sufficient criterion for identifying hybrids with tolerance to drought, as pot experiments also aid in identifying hybrids with limited transpiration rate at varying VPD. Gradual decrease in transpiration from 3pm (after peak) to 5pm transpiration in relation VPD could be related to reduced light intensity on the experiment and also causing temperature reductions. In addition, water stress conditions results in loss of turgor, which translates to reduced cell division, controlled leaf expansion with early stomatal closure lowering intercepted radiation and reduced biomass production (Blum and Blum, 2011).

In relation to genetic gains, hybrids released later showed good water conservative use under optimum environment (VPD) as compared to old hybrids. However, with the same comparison made under soil drying conditions, hybrids released in later years exhibited good water conservative use as compared to those released earlier. Furthermore, genetic gain as evaluated in era studies will help breeding program to select genes that are in earlier bred hybrids that allow them to conserve water under stressed conditions.

5.6 Conclusion

Drought tolerant hybrids had lower TR, particularly at high VPD and varying Soil drying conditions as compared to susceptible ones. Hybrids CZH1261, CZH132119 and CZH15013 were observed to conserve water efficiently whilst hybrids CZH01008, CZH15212 and CZH15467 exhibited the least ability to conserve water (Table 5.1 and 5.2) under VPD and soil drying experiment. It is also hypothesized that hybrids showing water conservative traits in stressed environments have the ability to use water efficiently and use it during terminal drought stress later in the season. This could aid to improve grain yield as hybrids will have some water to aid in their physiological activities during kernel formation and maturation. Variations in both water deficit environment and varying VPD in relation to transpiration rates were observed. This suggests that these two experimental environments can be used to select best hybrids with the water conservation trait that is need to help adapt maize hybrids in the face of climate change.

5.7 Recommendations

Soil drying and Vapor deficit environments can also be used to screen poorly adapted hybrids that have minimum ability to conserve water. Best ranked hybrids to conserve water should be put for further analysis so that hybrids exhibiting the efficient water use under increased temperature and high humidity and also water deficit environment. Based on transpiration stability across VPD and Soil drying experiment, hybrids recommended from the study includes CZH1261, CZH132119 and CZH15013 as they exhibited the conserve water conservation trait. The results from genetic gain analysis also showed that hybrids bred earlier conserve water efficiently than hybrids released in later years.

5.8 Acknowledgements

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CHAPTER 6

6.1 Introduction

Maize is the principal staple in most countries within Eastern and Southern Africa, hence its availability and affordability is central to ensuring food security in the region. Maize, which is grown mainly under rain-fed conditions, is susceptible to increasing intensity and frequency of drought due to climate change. This in turn increases chances of crop failure, negatively affecting livelihoods and food security status of millions of smallholder farmers and their families. Maize's susceptibility to water deficit is more pronounced at the reproductive stage and causes the greatest reduction in grain yield when stress coincides with the irreversible reproductive processes. Since 1997 CIMMYT ESA has focused on producing drought tolerant varieties but incorporated into the selection screening for high yielders under optimal conditions and low N stress tolerance and MSV, GLS and ET disease resistance including.

6.2 Research summary

72 hybrids developed between 1999 and 2016 at CIMMYT in Zimbabwe, were used in this study. The hybrids were coded CZH (CIMMYT Zimbabwe hybrids) which are early to intermediate maturing hybrids. The hybrids together with 3 local checks (SC513, SC403 and PAN413) were grown in Chiredzi Research Station (21° 0' 58"S, 31° 34' 17E") during the 2018-19 growing season under heat and managed drought stress. The experimental design was a 15*15 a-lattice (Patterson and Biometrika, 1976) with 75 hybrids that will be replicated three times.

The same CIMMMYT hybrids were also grown in a pot experiment. These hybrids were grown to observe how they manage transpiration under varied Vapor pressure deficit regimes and also varying water deficit conditions. Another study was conducted at CIMMYT research station (17°43′37.21″ S, 31°01′00.60″ E, and altitude 1489 m above sea level) in Harare, Zimbabwe in a pot experiment. 72 CIMMYT hybrids and 3 local checks from (SC513, SC403 and PAN413) contrasting in tolerance to drought stress were selected for the study from CIMMYT. Plants were grown in pots during October to November 2018 under outdoors conditions.

6.3 Conclusions

Under **Drought stress** genetic gain in grain yield against time of release showed net positive gains of 35.03 kg ha^{-1} year^{-1}. The new hybrids yielded better than the old hybrids. There was no change in number of days to mid-anthesis over the 17 years ($P \leq 0.57$). Newer and older hybrids flowered almost within the same days. ASI showed a trend to increase at a rate of 0.03 d year $^{-1}$ but the change was not significant ($P \leq 0.15$). There was a significant change in plant height ($P \leq 0.04$ as it showed a trend of increasing over the 16-year period at an estimated rate of 0.46 cm year^{-1}. There was no significant change in ear height over the 17 years at $P \leq 0.31$ but showed a trend to increase at a rate of 0.25 cm year^{-1}. From 1999 to 2016 the number of ears per plant increased at a rate of 0.003 year^{-1} at $P \leq 0.04$.

Under **Heat stress** a genetic gain in yield of 39.36 kg ha^{-1} year^{-1} was recorded. Days to mid-anthesis did not significantly change over the 17 years but showed a tendency to increase at a rate of 0.001 days' year^{-1}. New hybrids flowered within the same range as the old hybrids but yielded better than the old hybrids. A net decrease of, on average, 0.02 days' year^{-1} was recorded for ASI under heat stress. New hybrids had relatively shorter ASI compared to the old hybrids. Plant height showed a trend to increase at an average rate of 0.31 cm year^{-1} but there was no significant change. Ear height did not significantly change over the 17 years but showed a tendency to increase at a rate of 0.14 cm year^{-1}. New hybrids had a relatively higher number of ears per plant that increased at a rate of 0.002 which is not significant at $P \leq 0.05$. New hybrids that yielded better than old hybrids had shorter ASI or improved synchronization at flowering and increased number of ears per plant.

In the pot experiment gradual increase in transpiration from varying **Vapor Pressure Deficit** regimes were observed. 9am interval recorded the least mean averages on both the best ranked and least ranked in transpiration, with 1pm and 3pm recording peak transpiration values and 5pm mean averages for both ranked hybrids started decreasing gradually. Transpiration at 9am, 11am, 1pm and 5pm showed no significant differences but 3pm time interval showed very significant difference in comparison with transpiration from 9am.

In the **Soil drying experiment**, hybrids had increased transpiration when pots were all saturated which later reduced as the soil drying experiment continued until plants had less water that could be transpired. As the dry down experiment progressed wilting sign in leaves were observed in majority of the hybrids. Overall, recently released hybrids shower better ability to conserve water under water deficit conditions as compared to older hybrids.

6.4 Recommendations

Based on transpiration stability across **Vapor Pressure Deficit** and **Soil drying experiment**, hybrids recommended from the study includes CZH1261, CZH132119 and CZH15013 as they exhibited the conserve water conservation trait. Hybrid CZH15013 exhibited the lowest FTSW value of 0.548.

Drought stress results indicated that hybrids with strong drought tolerance are CZH1261, CZH16340, CZH142020, CZH15603 and CZH15002, these can be further grown in different environments to determine their tolerance stability.

Results from **Heat stress** trial indicated that hybrids with strong heat tolerance are CZH15575, CZH0527, CZH15017, CZH16389 and CZH15572, these should be further grown in different sites to determine their tolerance.

6.5 Areas for further research

There is need to do further trials to confirm the performance of the genetic gain hybrids so as to identify drought and heat tolerant hybrids and secondary traits contributing to gains in grain yield. Genetic gain hybrids tested should also be grown in further experiments as grain yield and other secondary traits showed they can still be improved as no signs of reaching plateaus were observed.

Appendices

Appendix 1: Mean separation for drought stress trial: grain yield and yield attributing traits of maize

RANK	HYBRID	GYD	AD	ASI	PH	EH	EPP	NP	SEN1	SEN2
1	CZH1261	4.53 a	75.67 efghijklmno	0.00 ab	201.52 defghijklmnopqr	105.60 bcdefghijklmnop	0.89 abc	29.92 abcdefg	0.85 ijklm	1.17 bcd
2	CZH16340	4.44 ab	75.33 fghijklmno	0.33 ab	223.56 a	130.14 a	0.93 ab	27.03 abcdefghijklm	1.49 efghi	1.17 bcd
3	CZH142020	4.18 abc	77.33 cdefghijk	2.33 ab	213.36 abcdefgh	115.58 abcdefgh	0.78 cdefghijklmn	28.75 abcdefghi	0.83 ijklm	1.16 bcd
4	CZH15603	4.13 abcd	77.00 defghijkl	0.00 ab	198.02 fghijklmnopqrs	111.01 abcdefghijklmn	0.86 abcdefg	28.19 abcdefghijk	1.86 cdefg	0.83 d
5	CZH15002	4.07 abcde	73.00 lmnop	4.33 a	206.30 abcdefghijklmno	90.93 mnopq	0.87 abcdefg	27.69 abcdefghijkl	1.97 bcdef	1.50 ab
6	CZH0935	3.95 abcdef	75.33 fghijklmno	1.00 ab	201.77 defghijklmnopqr	110.96 abcdefghijklmn	0.86 abcdefgh	25.43 defghijklm	0.83 ijklm	1.33 bc
7	CZH132163	3.89 abcdefg	76.00 defghijklmn	1.00 ab	201.28 efghijklmnopqr	95.56 hijklmnopq	0.87 abcdef	28.90 abcdefghi	2.17 bcde	1.01 cd
8	CZH0928	3.89 abcdefgh	73.67 jklmnop	0.67 ab	201.05 efghijklmnopqr	100.18 fghijklmnopq	0.83 abcdefghijk	28.47 abcdefghij	0.34 lm	1.33 bc
9	CZH15343	3.88 abcdefgh	81.33 abc	1.00 ab	218.92 abcd	115.73 abcdefgh	0.94 a	28.20 abcdefghijk	0.67 jklm	1.16 bcd
10	CZH03002	3.86 abcdefghi	78.33 cdefgh	-0.33 b	215.25 abcdef	123.81 abc	0.80 abcdefghijklm	27.48 abcdefghijkl	0.79 ijklm	1.32 bc
11	CZH15017	3.84 abcdefghij	74.33 hijklmnop	0.33 ab	197.65 ghijklmnopqrs	87.08 opqr	0.79 cdefghijklm	30.68 abcd	2.69 ab	1.00 cd
12	CZH0526	3.81 abcdefghij	76.00 defghijklmn	0.33 ab	210.92 abcdefghijk	101.66 defghijklmnopq	0.74 fghijklmn	27.69 abcdefghijkl	0.50 klm	1.16 bcd
13	CZH1243	3.79 abcdefghij	75.33 fghijklmno	1.33 ab	195.10 ijklmnopqrs	97.22 fghijklmnopq	0.76 cdefghijklmn	29.14 abcdefgh	0.81 ijklm	1.00 cd
14	CZH02008	3.77 abcdefghijk	70.67 pq	2.33 ab	202.91 cdefghijklmnopq	97.85 fghijklmnopq	0.81 abcdefghijklm	28.71 abcdefghij	0.99 hijkl	1.00 cd
15	CZH0623	3.71 abcdefghijk	77.67 cdefghij	0.33 ab	200.30 efghijklmnopqr	98.91 fghijklmnopq	0.87 abcdef	25.89 bcdefghijklm	1.00 hijkl	1.33 bc
16	CZH15467	3.68 abcdefghijk	75.67 efghijklmno	0.00 ab	202.06 defghijklmnopqr	98.40 fghijklmnopq	0.85 abcdefghij	29.57 abcdefgh	1.17 ghijk	1.50 ab
17	CZH1032	3.68 abcdefghijk	76.67 defghijklm	0.67 ab	182.07 st	94.35 jklmnopq	0.89 abcd	31.99 a	1.01 hijkl	1.00 cd
18	CZH15188	3.65 abcdefghijk	74.67 hijklmnop	0.67 ab	205.83 bcdefghijklmno	105.40 bcdefghijklmnopq	0.84 abcdefghijk	28.76 abcdefghi	2.18 bcde	1.33 bc
19	CZH04007	3.62 abcdefghijkl	78.00 cdefghi	0.00 ab	198.83 efghijklmnopqrs	97.74 fghijklmnopq	0.80 bcdefghijklm	26.78 abcdefghijklm	1.67 defgh	1.33 bc
20	CZH1033	3.62 abcdefghijklm	76.33 defghijklm	-0.67 b	191.69 nopqrst	98.26 fghijklmnopq	0.86 abcdefgh	30.28 abcdef	1.70 defgh	1.50 ab
21	CZH1036	3.61 abcdefghijklm	76.00 defghijklmn	0.00 ab	193.44 klmnopqrs	101.13 efghijklmnopq	0.86 abcdefgh	27.31 abcdefghijkl	1.80 cdefg	1.00 cd
22	CZH15572	3.61 abcdefghijklm	75.67 efghijklmno	2.33 ab	213.54 abcdefg	115.22 abcdefghi	0.87 abcdef	29.94 abcdefg	1.81 cdefg	1.33 bc
23	CZH15183	3.60 abcdefghijklm	77.33 cdefghijk	1.00 ab	221.49 ab	116.43 abcdefg	0.75 efghijklmn	27.55 abcdefghijkl	1.69 defgh	1.16 bcd
24	CZH15013	3.57 abcdefghijklm	74.00 ijklmnop	0.33 ab	201.04 efghijklmnopqr	86.51 pqr	0.84 abcdefghijk	28.30 abcdefghijk	1.85 cdefg	1.17 bcd
25	CZH142060	3.57 abcdefghijklmn	79.33 bcdef	1.33 ab	206.66 abcdefghijklmno	124.06 ab	0.78 cdefghijklmn	29.50 abcdefgh	2.17 bcde	1.83 a
26	CZH16333	3.56 abcdefghijklmn	78.00 cdefghi	-0.33 b	209.66 abcdefghijkl	121.21 abcde	0.80 abcdefghijklm	26.17 bcdefghijklm	2.50 abc	1.17 bcd
27	CZH1123	3.53 abcdefghijklmn	71.67 opq	1.67 ab	194.78 ijklmnopqrs	69.27 r	0.84 abcdefghijk	26.54 abcdefghijklm	0.85 ijklm	1.50 ab
28	CZH15212	3.48 abcdefghijklmno	73.33 klmnop	0.00 ab	194.24 jklmnopqrs	103.98 bcdefghijklmnopq	0.74 fghijklmn	24.81 fghijklm	0.83 ijklm	1.33 bc
29	CZH16334	3.47 abcdefghijklmno	79.00 cdefg	0.67 ab	209.32 abcdefghijklm	114.75 abcdefghij	0.82 abcdefghijklm	25.84 bcdefghijklm	1.49 efghi	0.84 d
30	CZH15575	3.46 abcdefghijklmnop	76.33 defghijklm	1.00 ab	202.67 defghijklmnopq	104.31 bcdefghijklmnopq	0.85 abcdefghij	29.33 abcdefgh	1.50 efghi	1.17 bcd
31	CZH03006	3.44 abcdefghijklmnop	76.33 defghijklm	1.00 ab	202.16 defghijklmnopq	112.26 abcdefghijk	0.82 abcdefghijkl	30.29 abcdef	1.68 defgh	1.00 cd
32	CZH132169	3.42 abcdefghijklmnopq	76.00 defghijklmn	1.33 ab	203.77 cdefghijklmnopq	91.09 lmnopq	0.75 cdefghijklmn	30.03 abcdefg	3.18 a	1.17 bcd
33	SC403	3.42 bcdefghijklmnopq	75.33 fghijklmno	1.00 ab	213.03 abcdefgh	111.45 abcdefghijkl	0.84 abcdefghijk	27.55 abcdefghijkl	1.51 efghi	1.33 bc
34	CZH089	3.35 bcdefghijklmnopqr	75.67 efghijklmno	2.00 ab	197.11 ghijklmnopqrs	105.35 bcdefghijklmnopq	0.79 cdefghijklmn	28.31 abcdefghijk	0.31 lm	1.34 bc
35	CZH16389	3.34 bcdefghijklmnopqr	78.00 cdefghi	-0.67 b	195.89 hijklmnopqrs	103.88 bcdefghijklmnopq	0.79 cdefghijklm	25.12 efghijklm	1.36 fghij	1.17 bcd
36	CZH99014	3.33 bcdefghijklmnopqr	76.33 defghijklm	0.00 ab	194.36 jklmnopqrs	96.91 fghijklmnopq	0.82 abcdefghijk	28.25 abcdefghijk	0.16 m	1.50 ab
37	CZH1258	3.33 bcdefghijklmnopqr	76.00 defghijklmn	1.00 ab	191.01 nopqrst	94.70 ijklmnopq	0.84 abcdefghijk	29.36 abcdefgh	1.18 ghijk	1.16 bcd
38	CZH00020	3.33 bcdefghijklmnopqr	78.00 cdefghi	0.00 ab	212.17 abcdefghi	121.60 abcd	0.83 abcdefghijk	26.56 abcdefghijklm	0.64 jklm	1.16 bcd
39	CZH04005	3.28 cdefghijklmnopqrs	76.33 defghijklm	0.67 ab	198.30 fghijklmnopqrs	109.51 bcdefghijklmn	0.87 abcdef	28.54 abcdefghij	1.32 fghij	1.33 bc
40	CZH0629	3.27 cdefghijklmnopqrs	76.67 defghijklm	0.33 ab	205.07 bcdefghijklmnop	117.32 abcdef	0.89 abcde	28.76 abcdefghi	1.35 fghij	1.33 bc
41	CZH99015	3.25 cdefghijklmnopqrs	75.33 fghijklmno	0.33 ab	190.42 opqrst	96.39 ghijklmnopq	0.79 cdefghijklmn	27.94 abcdefghijk	1.36 fghij	1.51 ab
42	CZH02004	3.23 cdefghijklmnopqrs	75.33 fghijklmno	0.67 ab	188.20 pqrst	99.62 fghijklmnopq	0.79 cdefghijklmn	29.03 abcdefgh	1.49 efghi	1.34 bc
43	CZH142055	3.18 cdefghijklmnopqrs	76.67 defghijklm	1.00 ab	209.57 abcdefghijklm	104.84 bcdefghijklmnopq	0.82 abcdefghijkl	24.24 hijklmn	1.18 ghijk	1.00 cd
44	CZH0728	3.14 cdefghijklmnopqrs	83.33 ab	2.00 ab	205.51 bcdefghijklmnop	124.02 abc	0.79 cdefghijklmn	27.15 abcdefghijklm	1.16 ghijk	1.17 bcd
45	CZH132117	3.12 cdefghijklmnopqrs	76.33 defghijklm	-0.33 b	206.68 abcdefghijklmno	97.52 fghijklmnopq	0.75 defghijklmn	24.27 hijklmn	1.83 cdefg	1.16 bcd
46	CZH142056	3.11 cdefghijklmnopqrs	75.33 fghijklmno	3.00 ab	203.17 cdefghijklmnopq	106.30 bcdefghijklmnop	0.80 cdefghijklm	27.53 abcdefghijkl	1.48 efghi	1.51 ab
47	CZH0527	3.07 defghijklmnopqrs	75.00 ghijklmno	3.00 ab	198.23 fghijklmnopqrs	100.44 fghijklmnopq	0.73 hijklmn	31.18 abc	0.64 jklm	1.00 cd
48	CZH132118	3.05 defghijklmnopqrs	76.00 defghijklmn	0.33 ab	206.55 abcdefghijklmno	100.58 fghijklmnopq	0.75 fghijklmn	29.22 abcdefgh	1.34 fghij	1.17 bcd
49	SC513	3.02 defghijklmnopqrs	76.00 defghijklmn	1.67 ab	207.77 abcdefghijklmno	115.22 abcdefghi	0.77 cdefghijklmn	27.80 abcdefghijkl	1.83 cdefg	1.18 bcd
50	CZH1270	2.95 efghijklmnopqrs	79.33 bcdef	0.33 ab	199.27 efghijklmnopqrs	112.73 abcdefghijk	0.85 abcdefghi	24.71 ghijklm	1.16 ghijk	1.00 cd
51	CZH0932	2.94 fghijklmnopqrs	76.33 defghijklm	0.33 ab	192.16 mnopqrst	103.53 cdefghijklmnopq	0.75 cdefghijklmn	25.95 bcdefghijklm	0.66 jklm	1.18 bcd
52	CZH16335	2.93 fghijklmnopqrs	79.67 bcde	1.00 ab	207.26 abcdefghijklmno	114.26 abcdefghij	0.74 fghijklmn	27.05 abcdefghijklm	0.65 jklm	1.16 bcd
53	CZH99019	2.92 fghijklmnopqrs	79.33 bcdef	0.00 ab	206.27 abcdefghijklmno	114.75 abcdefghij	0.66 n	26.12 bcdefghijklm	1.66 defgh	1.17 bcd
54	CZH16374	2.90 fghijklmnopqrs	78.33 cdefgh	0.33 ab	200.27 efghijklmnopqr	110.54 abcdefghijklmn	0.80 cdefghijklm	21.73 mn	0.84 ijklm	1.17 bcd
55	CZH132119	2.89 fghijklmnopqrs	76.00 defghijklmn	0.33 ab	206.42 abcdefghijklmno	90.50 nopq	0.71 jklmn	28.98 abcdefgh	0.67 jklm	1.17 bcd
56	CZH0528	2.88 fghijklmnopqrs	74.00 ijklmnop	0.00 ab	192.66 lmnopqrs	92.29 klmnopq	0.73 ghijklmn	29.58 abcdefgh	0.70 jklm	1.33 bc
57	CZH00011	2.88 fghijklmnopqrs	81.33 abc	0.00 ab	190.87 nopqrst	91.67 lmnopq	0.78 cdefghijklmn	30.45 abcde	1.17 ghijk	1.16 bcd
58	CZH1122	2.87 fghijklmnopqrs	73.00 lmnop	1.67 ab	188.09 pqrst	92.86 klmnopq	0.76 cdefghijklmn	25.71 cdefghijklm	0.83 ijklm	1.00 cd
59	CZH01006	2.86 fghijklmnopqrs	76.00 defghijklmn	0.33 ab	208.04 abcdefghijklmn	95.50 hijklmnopq	0.71 klmn	29.47 abcdefgh	1.48 efghi	1.17 bcd
60	PAN413	2.80 ghijklmnopqrs	80.00 abcd	1.00 ab	194.78 ijklmnopqrs	97.76 fghijklmnopq	0.78 cdefghijklmn	28.50 abcdefghij	1.50 efghi	1.16 bcd
61	CZH02006	2.80 ghijklmnopqrs	75.67 efghijklmno	1.00 ab	196.26 ghijklmnopqrs	91.40 lmnopq	0.83 abcdefghijk	24.23 hijklmn	1.00 hijkl	1.16 bcd
62	CZH04001	2.74 hijklmnopqrs	81.33 abc	1.00 ab	207.79 abcdefghijklmno	113.84 abcdefghij	0.82 abcdefghijklm	23.19 jklmn	2.04 bcdef	1.33 bc
63	CZH0836	2.73 ijklmnopqrs	76.67 defghijklm	3.00 ab	220.38 abc	107.54 bcdefghijklmno	0.76 cdefghijklmn	31.25 ab	1.37 fghij	1.33 bc
64	CZH1134	2.71 jklmnopqrs	73.33 klmnop	1.33 ab	209.65 abcdefghijklm	92.44 klmnopq	0.72 ijklmn	29.62 abcdefgh	0.33 lm	1.17 bcd
65	CZH0615	2.70 jklmnopqrs	76.00 defghijklmn	0.67 ab	197.78 ghijklmnopqrs	88.75 opqr	0.84 abcdefghijk	26.06 bcdefghijklm	0.49 klm	1.33 bc
66	CZH15185	2.64 klmnopqrs	75.67 efghijklmno	3.00 ab	216.41 abcde	110.32 abcdefghijklmn	0.84 abcdefghijk	22.88 klmn	1.81 cdefg	1.33 bc
67	CZH0735	2.51 lmnopqrs	72.67 mnopq	1.00 ab	186.88 qrst	101.19 defghijklmnopq	0.77 cdefghijklmn	25.36 defghijklm	0.82 ijklm	1.49 ab
68	CZH0946	2.50 mnopqrs	72.00 nopq	-0.33 b	175.00 tu	84.89 qr	0.77 cdefghijklmn	27.53 abcdefghijkl	1.66 defgh	1.84 a
69	CZH03005	2.44 nopqrs	78.00 cdefghi	1.00 ab	211.45 abcdefghij	111.19 abcdefghijklm	0.80 abcdefghijklm	25.38 defghijklm	2.15 bcde	1.33 bc
70	CZH0733	2.36 opqrs	76.00 defghijklmn	0.67 ab	196.51 ghijklmnopqrs	99.38 fghijklmnopq	0.68 mn	24.80 fghijklm	0.85 ijklm	1.33 bc
71	CZH16048	2.32 pqrs	78.00 cdefghi	0.00 ab	199.72 efghijklmnopqr	107.43 bcdefghijklmno	0.77 cdefghijklmn	19.15 n	2.36 bcd	1.16 bcd
72	CZH00013	2.28 qrs	74.00 ijklmnop	-0.33 b	199.31 efghijklmnopqrs	97.52 fghijklmnopq	0.81 abcdefghijklm	26.15 bcdefghijklm	0.84 ijklm	1.17 bcd
73	CZH088	2.26 rs	68.67 q	1.67 ab	162.83 u	85.83 pqr	0.81 abcdefghijklm	28.17 abcdefghijk	0.30 lm	1.34 bc
74	CZH01008	2.26 rs	84.00 a	0.00 ab	184.70 rst	103.87 bcdefghijklmnopq	0.69 lmn	23.42 ijklmn	1.15 ghijk	1.49 ab
75	CZH0524	2.14 s	74.67 hijklmnop	-0.67 b	203.00 cdefghijklmnopq	88.43 opqr	0.75 cdefghijklmn	22.28 lmn	1.31 fghij	1.34 bc

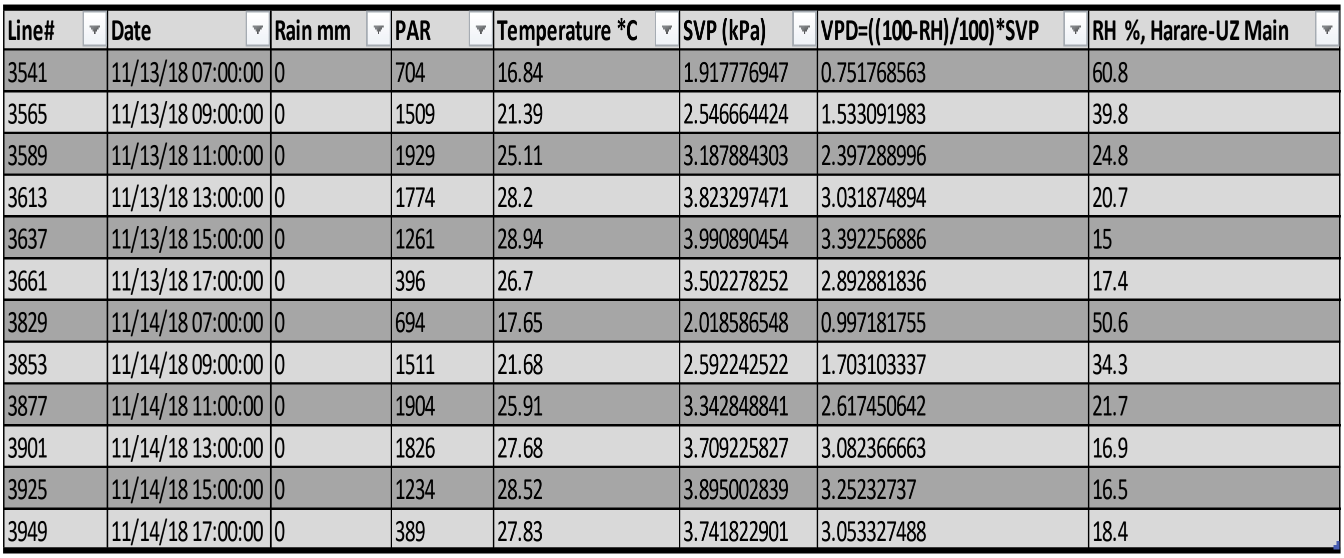
Appendix 2: Mean separation for heat stress trial: grain yield and yield attributing traits on maize

HYBRID	GYD	AD	ASI	PH	EH	EPP	NP
CZH15575	6.54 a	70.61 pqrstuvwx	0.67 a	224.60 bcdefg	110.04 cdefghijklmnopq	0.88 abcdefg	16.55 bcdefg
CZH0527	6.41 ab	73.01 fghijklmn	1.67 a	221.73 bcdefghijkl	123.60 abcdef	0.86 abcdefghi	16.93 b
CZH15017	6.32 abc	72.38 ghijklmnopq	-0.33 a	214.04 cdefghijklmnopqrs	112.58 bcdefghijklmn	0.88 abcdefg	16.42 bcdefg
CZH16389	6.04 abcd	72.49 ghijklmnop	-1.33 a	207.23 ghijklmnopqrstuvw	112.13 bcdefghijklmn	0.86 abcdefghi	15.18 bcdefghij
CZH15572	5.90 abcde	70.97 nopqrstuv	0.67 a	219.18 bcdefghijklmn	103.86 ghijklmnopqr	0.90 abcde	16.30 bcdefghi
CZH15188	5.86 abcdef	72.45 ghijklmnop	-0.67 a	213.62 cdefghijklmnopqrs	112.99 abcdefghijklm	0.87 abcdefg	15.89 bcdefghi
CZH0935	5.85 abcdef	70.76 opqrstuvw	0.00 a	210.00 efghijklmnopqrstuv	111.56 bcdefghijklmno	0.88 abcdef	16.79 bcd
CZH15603	5.83 abcdefg	72.50 ghijklmnop	-0.67 a	220.77 bcdefghijklm	121.31 abcdefgh	0.86 abcdefghi	15.86 bcdefghi
CZH15185	5.81 abcdefgh	74.29 cdefgh	1.33 a	233.14 ab	126.49 abcd	0.85 abcdefghij	15.66 bcdefghi
CZH0928	5.73 abcdefghi	70.03 stuvwxy	0.00 a	200.64 opqrstuvw	91.03 qr	0.92 ab	16.54 bcdefg
CZH1243	5.71 abcdefghi	72.84 fghijklmno	-1.67 a	201.59 nopqrstuvw	92.50 opqr	0.88 abcdefg	16.10 bcdefghi
CZH132119	5.67 abcdefghi	68.75 wxyz	-0.33 a	217.93 bcdefghijklmno	93.96 mnopqr	0.88 abcdef	14.62 efghij
CZH0615	5.64 abcdefghi	72.15 hijklmnopqrs	-0.33 a	209.48 efghijklmnopqrstuv	102.16 ijklmnopqr	0.93 a	15.28 bcdefghij
CZH02008	5.60 abcdefghi	66.65 zAB	2.33 a	213.64 cdefghijklmnopqrs	102.17 hijklmnopqr	0.83 bcdefghij	15.85 bcdefghi
CZH132118	5.59 abcdefghi	71.26 klmnopqrstuv	1.00 a	217.32 bcdefghijklmnop	98.66 jklmnopqr	0.87 abcdefg	15.87 bcdefghi
CZH16340	5.58 abcdefghi	71.49 klmnopqrstu	-1.00 a	222.48 bcdefghijk	112.50 bcdefghijklmn	0.88 abcdefg	14.19 ij
CZH0733	5.57 abcdefghij	71.66 jklmnopqrstu	0.67 a	201.98 nopqrstuvw	105.15 efghijklmnopqr	0.84 abcdefghij	16.82 bcd
CZH16334	5.38 abcdefghijk	72.49 ghijklmnop	0.33 a	221.89 bcdefghijkl	128.44 abc	0.82 bcdefghij	16.69 bcdef
CZH15013	5.37 abcdefghijkl	68.30 yzA	0.67 a	211.97 defghijklmnopqrstu	91.13 pqr	0.84 abcdefghij	16.12 bcdefghi
CZH142055	5.35 abcdefghijklm	72.15 hijklmnopqrs	0.33 a	223.82 bcdefghij	122.72 abcdefg	0.88 abcdef	14.25 hij
CZH99014	5.34 abcdefghijklm	73.10 efghijklmn	0.33 a	205.56 klmnopqrstuvw	99.23 ijklmnopqr	0.88 abcdef	16.58 bcdef
CZH03006	5.32 abcdefghijklmn	73.16 efghijklm	0.33 a	211.08 efghijklmnopqrstuv	110.23 cdefghijklmnop	0.86 abcdefghi	16.39 bcdefg
CZH16374	5.31 abcdefghijklmn	72.02 ijklmnopqrst	0.67 a	207.66 ghijklmnopqrstuv	99.90 ijklmnopqr	0.89 abcde	15.18 bcdefghij
CZH15467	5.29 bcdefghijklmno	71.83 jklmnopqrst	-0.33 a	205.40 klmnopqrstuvw	98.72 jklmnopqr	0.88 abcdef	16.72 bcdef
CZH142056	5.27 bcdefghijklmno	71.19 lmnopqrstuv	0.33 a	204.07 lmnopqrstuvw	100.58 ijklmnopqr	0.84 abcdefghij	15.36 bcdefghi
CZH03002	5.24 bcdefghijklmno	74.30 cdefgh	0.00 a	206.63 hijklmnopqrstuvw	99.21 ijklmnopqr	0.84 abcdefghij	15.24 bcdefghij
CZH142060	5.23 bcdefghijklmno	74.11 defghi	0.33 a	221.37 bcdefghijkl	128.20 abc	0.85 abcdefghij	15.91 bcdefghi
CZH16335	5.21 bcdefghijklmno	76.05 abcd	1.00 a	215.60 bcdefghijklmnopqr	115.57 abcdefghijk	0.88 abcdefg	16.56 bcdef
CZH1036	5.20 bcdefghijklmno	70.60 pqrstuvwx	1.00 a	200.43 opqrstuvw	98.32 jklmnopqr	0.87 abcdefg	15.94 bcdefghi
CZH0836	5.18 cdefghijklmno	73.41 efghijk	1.67 a	220.43 bcdefghijklm	112.88 abcdefghijklm	0.80 efghij	16.56 bcdef
CZH04007	5.17 cdefghijklmno	73.26 efghijkl	0.67 a	211.77 efghijklmnopqrstu	112.30 bcdefghijklmn	0.84 abcdefghij	16.89 bc
CZH142020	5.16 cdefghijklmno	74.15 defghi	1.00 a	231.52 abc	132.17 a	0.80 cdefghij	15.20 bcdefghij
CZH089	5.16 cdefghijklmno	71.70 jklmnopqrst	2.00 a	194.14 uvwx	99.94 ijklmnopqr	0.90 abcd	16.46 bcdefg
SC513	5.15 cdefghijklmno	73.13 efghijklmn	0.33 a	225.21 bcdefg	118.42 abcdefghi	0.87 abcdefgh	16.02 bcdefghi
CZH132117	5.14 cdefghijklmno	72.25 hijklmnopqr	1.33 a	217.13 bcdefghijklmnop	96.65 klmnopqr	0.87 abcdefgh	15.08 bcdefghij
CZH0623	5.11 cdefghijklmno	74.27 cdefgh	0.00 a	203.44 mnopqrstuvw	110.24 cdefghijklmnop	0.81 cdefghij	15.46 bcdefghi
CZH01008	5.06 defghijklmno	76.11 abcd	0.00 a	221.75 bcdefghijkl	122.87 abcdefg	0.75 j	19.83 a
CZH99019	5.04 defghijklmno	74.73 bcdef	0.67 a	227.20 abcde	114.39 abcdefghijkl	0.86 abcdefghi	16.84 bc
CZH0629	4.93 defghijklmnop	71.67 jklmnopqrstu	-1.33 a	198.13 rstuvw	93.33 nopqr	0.88 abcdef	16.74 bcde
CZH1033	4.91 defghijklmnop	72.34 ghijklmnopq	-0.33 a	193.00 vwxy	96.77 klmnopqr	0.87 abcdefgh	15.66 bcdefghi
CZH132169	4.90 defghijklmnop	71.92 jklmnopqrst	1.00 a	194.66 tuvwx	86.95 r	0.83 bcdefghij	15.74 bcdefghi
CZH0528	4.90 defghijklmnop	71.52 klmnopqrstu	0.00 a	212.17 defghijklmnopqrstu	108.60 defghijklmnopq	0.87 abcdefgh	15.56 bcdefghi
CZH132163	4.87 defghijklmnop	72.46 ghijklmnop	0.67 a	205.84 jklmnopqrstuvw	102.60 hijklmnopqr	0.86 abcdefghi	15.97 bcdefghi
CZH02004	4.87 defghijklmnop	71.09 mnopqrstuv	-0.67 a	207.97 ghijklmnopqrstuv	112.78 abcdefghijklm	0.86 abcdefghi	16.09 bcdefghi
CZH1122	4.86 defghijklmnop	68.53 xyz	1.00 a	198.95 qrstuvw	100.22 ijklmnopqr	0.90 abc	16.55 bcdefg
CZH0735	4.85 defghijklmnop	66.34 AB	1.33 a	196.87 stuvwx	102.38 hijklmnopqr	0.92 ab	16.19 bcdefghi
CZH1032	4.85 defghijklmnop	73.42 efghijk	0.00 a	200.98 opqrstuvw	98.24 jklmnopqr	0.90 abcd	15.96 bcdefghi
CZH1123	4.84 defghijklmnop	68.31 yzA	1.00 a	199.72 pqrstuvw	101.39 ijklmnopqr	0.85 abcdefghi	15.86 bcdefghi
CZH0932	4.84 defghijklmnop	73.33 efghijkl	-1.00 a	189.52 wxy	113.51 abcdefghijkl	0.86 abcdefghi	14.43 ghij
CZH15002	4.77 efghijklmnop	72.53 ghijklmnop	2.33 a	223.93 bcdefghi	100.87 ijklmnopqr	0.92 ab	14.94 bcdefghij
CZH16333	4.76 efghijklmnop	73.16 efghijklm	1.33 a	208.98 fghijklmnopqrstuv	106.76 efghijklmnopq	0.87 abcdefg	16.33 bcdefgh
CZH15212	4.75 efghijklmnop	72.68 fghijklmnop	-1.67 a	206.43 hijklmnopqrstuvw	104.17 ghijklmnopqr	0.81 cdefghij	16.15 bcdefghi
CZH1270	4.74 efghijklmnop	74.49 bcdefg	-1.33 a	211.39 efghijklmnopqrstu	102.20 hijklmnopqr	0.88 abcdefg	16.35 bcdefgh
CZH0728	4.73 efghijklmnop	75.21 abcde	0.67 a	229.43 abcd	124.23 abcde	0.86 abcdefghi	16.97 b
CZH0526	4.69 efghijklmnop	72.15 hijklmnopqrs	1.00 a	205.12 klmnopqrstuvw	102.04 ijklmnopqr	0.86 abcdefghi	16.22 bcdefghi
CZH15343	4.69 fghijklmnop	76.90 a	1.33 a	243.71 a	130.85 ab	0.88 abcdefg	16.20 bcdefghi
CZH04001	4.64 fghijklmnop	76.32 abc	1.00 a	226.19 abcdef	110.96 cdefghijklmno	0.88 abcdefg	16.24 bcdefghi
CZH15183	4.62 fghijklmnop	72.61 fghijklmnop	0.67 a	208.67 fghijklmnopqrstuv	86.18 r	0.80 cdefghij	15.40 bcdefghi
CZH0946	4.61 fhijklmnop	66.26 AB	-0.33 a	179.98 xy	92.77 opqr	0.81 cdefghij	15.99 bcdefghi
CZH1261	4.61 fhijklmnop	70.24 qrstuvwxy	-0.67 a	214.84 cdefghijklmnopqrs	112.50 bcdefghijklmn	0.84 abcdefghij	16.35 bcdefgh
CZH03005	4.58 ghijklmnop	71.62 klmnopqrstu	2.00 a	222.40 bcdefghijk	114.84 abcdefghijkl	0.88 abcdefg	16.12 bcdefghi
CZH04005	4.53 ijklmnop	71.68 jklmnopqrstu	-0.67 a	206.05 ijklmnopqrstuvw	117.38 abcdefghij	0.78 ghij	14.79 cdefghij
CZH0524	4.33 jklmnop	69.53 uvwxy	0.00 a	226.09 abcdef	102.54 hijklmnopqr	0.85 abcdefghij	15.94 bcdefghi
CZH02006	4.19 klmnop	70.11 rstuvwxy	-0.33 a	205.11 klmnopqrstuvw	101.02 ijklmnopqr	0.85 abcdefghij	13.21 j
CZH00020	4.18 klmnop	76.59 ab	1.67 a	213.74 cdefghijklmnopqrs	115.89 abcdefghijk	0.80 defghij	16.92 b
CZH99015	4.18 klmnop	71.50 klmnopqrstu	2.33 a	198.57 rstuvw	95.54 lmnopqr	0.85 abcdefghij	15.14 bcdefghij
CZH16048	4.17 klmnop	73.30 efghijkl	1.33 a	212.54 defghijklmnopqrst	107.35 defghijklmnopq	0.78 ghij	14.61 fghij
CZH00011	4.15 lmnop	69.27 vwxy	-0.33 a	208.26 fghijklmnopqrstuv	100.60 ijklmnopqr	0.79 fghij	15.47 bcdefghi
CZH00013	4.15 mnop	70.81 opqrstuvw	0.33 a	211.86 defghijklmnopqrstu	96.53 klmnopqr	0.76 ij	15.86 bcdefghi
CZH01006	4.09 nop	71.54 klmnopqrstu	0.33 a	217.00 bcdefghijklmnopq	104.35 fghijklmnopqr	0.84 abcdefghij	14.72 defghij
SC403	4.06 op	71.50 klmnopqrstu	0.33 a	224.14 bcdefgh	98.87 jklmnopqr	0.78 fghij	17.06 b
CZH1134	3.81 p	71.83 jklmnopqrst	1.67 a	209.02 fghijklmnopqrstuv	97.18 klmnopqr	0.77 hij	15.88 bcdefghi
PAN413	3.81 p	73.83 efghij	1.33 a	198.38 rstuvw	103.53 ghijklmnopqr	0.87 abcdefgh	15.12 bcdefghij
CZH1258	3.80 p	69.89 tuvwxy	1.67 a	224.12 bcdefghi	113.08 abcdefghijkl	0.85 abcdefghij	15.22 bcdefghij
CZH088	3.76 p	65.26 B	1.67 a	175.86 y	92.55 opqr	0.85 abcdefghij	15.90 bcdefghi

Appendix 3: CIMMYT hybrids tested by year of first testing or release

Year of first testing or release	Number of entries
1999	3
2000	3
2001	2
2002	3
2003	3
2004	3
2005	4
2006	3
2007	3
2008	3
2009	4
2010	3
2011	3
2012	4
2013	5
2014	4
2015	12
2016	7

Appendix 4: Pot experiment weather data summary



Line#	Date	Rain mm	PAR	Temperature *C	SVP (kPa)	VPD=((100-RH)/100)*SVP	RH %, Harare-UZ Main
3541	11/13/18 07:00:00	0	704	16.84	1.917776947	0.751768563	60.8
3565	11/13/18 09:00:00	0	1509	21.39	2.546664424	1.533091983	39.8
3589	11/13/18 11:00:00	0	1929	25.11	3.187884303	2.397288996	24.8
3613	11/13/18 13:00:00	0	1774	28.2	3.823297471	3.031874894	20.7
3637	11/13/18 15:00:00	0	1261	28.94	3.990890454	3.392256886	15
3661	11/13/18 17:00:00	0	396	26.7	3.502278252	2.892881836	17.4
3829	11/14/18 07:00:00	0	694	17.65	2.018586548	0.997181755	50.6
3853	11/14/18 09:00:00	0	1511	21.68	2.592242522	1.703103337	34.3
3877	11/14/18 11:00:00	0	1904	25.91	3.342848841	2.617450642	21.7
3901	11/14/18 13:00:00	0	1826	27.68	3.709225827	3.082366663	16.9
3925	11/14/18 15:00:00	0	1234	28.52	3.895002839	3.25232737	16.5
3949	11/14/18 17:00:00	0	389	27.83	3.741822901	3.053327488	18.4

Appendix 5: Experimental sites summary

Site	TrialName	Location	Country	Environment	PlantingDate	Season	GPS coordinates	Collaborator
9	EEGG17	Chiredzi	Zimbabwe	Drought stress	Off		21° 0' 58"S, 31° 34' 17E"	Dr. Z. Mainassara
11	EEGG17	Chiredzi	Zimbabwe	Heat stress	Off		21° 0' 58"S, 31° 34' 17E"	Dr Z. Mainassara

Appendix 6: Individual site analysis of variance of grain yield for drought stress.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Replication	1	39.356	39.356	0.63	55.535	0.0000	***
Hybrid	74	89.069	1.204	440.15	1.6984	0.0033	**
Block(Replication)	1	6.272	6.272	1482.60	8.8505	0.0034	**
Error	148	104.883	0.709				
Total	224	239.58					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 7: Individual site analysis of variance of anthesis date for drought stress.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Replication	1	52.81	52.807	0.06	7.3297	0.0076	**
Hybrid	74	1504.51	20.331	39.82	2.822	0.0000	***
Block(Replication)	1	24.26	24.263	134.15	3.3677	0.0685	.
Error	148	1066.26	7.204				
Total	224	2647.84					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 8: Individual site analysis of variance of anthesis-silking interval for drought stress.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Replication	1	0.667	0.6667	0.34	0.4523	0.5023	
Hybrid	74	212.062	2.8657	238.90	1.9443	0.0003	***
Block(Replication)	1	10.525	10.5251	804.72	7.1409	0.0084	**
Error	148	218.142	1.4739				
Total	224	441.396					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 9: Individual site analysis of variance of plant height for drought stress.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Replication	1	8944.4	8944.4	0.00	63.031	0.0000	***
Hybrid	74	24414.9	329.9	1.92	2.325	0.0000	***
Block(Replication)	1	694.5	694.5	6.45	4.894	0.0285	*
Error	148	21001.9	141.9				
Total	224	55055.7					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 10: Individual site analysis of variance of ear height for drought stress.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Replication	1	3933.2	3933.2	0.00	24.033	0.0000	***
Hybrid	74	27444.2	370.9	1.90	2.2661	0.0000	***
Block(Replication)	1	1.5	1.5	6.39	0.0089	0.9248	
Error	148	24221	163.7				
Total	224	55599.9					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 11: Individual site analysis of variance of ears per plant for drought stress.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Replication	1	0.08812	0.08812	80.69	12.35	0.0006	***
Hybrid	74	0.71269	0.00963	56723.27	1.3498	0.0629	.
Block(Replication)	1	0.00219	0.00219	191067.80	0.3063	0.5808	
Error	148	1.05603	0.00714				
Total	224	1.85903					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 12: Individual site analysis of variance of sen1 for drought stress.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Replication	1	7.935	7.935	1.33	16.929	0.0001	***
Hybrid	74	33.182	0.4484	931.68	0.9566	0.5780	
Block(Replication)	1	2.692	2.6922	3138.30	5.7436	0.0178	*
Error	148	69.373	0.4687				
Total	224	113.182					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 13: Individual site analysis of variance of sen2 for drought stress.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Replication	1	0.0417	0.04167	7.129278	0.436	0.5101	
Hybrid	74	6.7067	0.09063	5011.882	0.9484	0.5945	
Block(Replication)	1	0.1488	0.1488	16882.13	1.5572	0.2141	
Error	148	14.1429	0.09556				
Total	224	21.0401					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 14: Individual site analysis of variance of grain yield for heat stress.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Replication	1	6.814	6.8144	0.67	8.9663	0.0032	**
Hybrid	74	99.574	1.3456	470.35	1.7705	0.0017	**
Block(Replication)	1	5.329	5.3286	1584.32	7.0113	0.009	**
Error	148	112.48	0.76				
Total	224	224.197					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 15: Individual site analysis of variance of anthesis date for heat stress.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Replication	1	9.63	9.6267	0.10	4.8623	0.0290	*
Hybrid	74	1216.22	16.4354	69.43	8.3014	0.0000	***
Block(Replication)	1	0.02	0.0235	233.86	0.0119	0.9134	
Error	148	293.02	1.9798				
Total	224	1518.89					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 16: Individual site analysis of variance of anthesis-silking interval for heat stress.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Replication	1	3.84	3.84	0.33	2.3074	0.1309	
Hybrid	74	205.76	2.7805	230.36	1.6708	0.0043	**
Block(Replication)	1	1.852	1.8522	775.95	1.1129	0.2932	
Error	148	246.308	1.6642				
Total	224	457.76					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 17: Individual site analysis of variance of plant height for heat stress.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Replication	1	5407	5407.2	0.00	39.6865	0.0000	***
Hybrid	74	32511	439.3	1.81	3.2245	0.0000	***
Block(Replication)	1	215	214.5	6.09	1.5744	0.2115	
Error	148	20165	136.2				
Total	224	58298					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 18: Individual site analysis of variance of ear height for heat stress.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Replication	1	514.1	514.12	0.00	3.3951	0.0674	.
Hybrid	74	26844.4	362.76	2.10	2.3956	0.0000	***
Block(Replication)	1	453.7	453.67	7.07	2.9959	0.0856	.
Error	148	22411.5	151.43				
Total	224	50223.7					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 19: Individual site analysis of variance of ears per plant for heat stress.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Replication	1	0.00356	0.003558	165.13	0.9051	0.343	
Hybrid	74	0.32299	0.004365	116089.00	1.1103	0.2933	
Block(Replication)	1	0.00001	0.000013	391036.60	0.0032	0.9552	
Error	148	0.5818	0.003931				
Total	224	0.90836					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 20: Combined analysis of variance for grain yield of 75 hybrids across two environments.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Hybrid	74	118.01	1.59	259.60	1.7389	0.0007	***
Environment	1	348.63	348.63	0.14	380.136	0.0000	***
Hybrid*Environment	74	70.63	0.95	1038.54	1.0407	0.3994	
Error	300	275.13	0.92				
Total	449	812.4					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 21: Combined analysis of variance for anthesis date of 75 hybrids across two environments.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Hybrid	74	2365.9	31.97	33.61	6.6331	0.0000	***
Environment	1	2108.17	2108.17	0.02	437.379	0.0000	***
Hybrid*Environment	74	354.83	4.8	134.46	0.9948	0.4965	
Error	300	1446	4.82				
Total	449	6274.9					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 22: Combined analysis of variance for anthesis-silking interval of 75 hybrids across two environments.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Hybrid	74	307.19	4.1512	230.99	2.5873	0.0000	***
Environment	1	13.87	13.8689	0.12	8.644	0.0035	**
Hybrid*Environment	74	110.63	1.495	924.09	0.9318	0.6347	
Error	300	481.33	1.6044				
Total	449	913.02					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 23: Combined analysis of variance for plant height of 75 hybrids across two environments.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Hybrid	74	46354	626.4	1.69	3.3303	0.0000	***
Environment	1	11224	11224	0.00	59.6735	0.0000	***
Hybrid:environment	74	10572	142.9	6.77	0.7596	0.9218	
Error	300	56427	188.1				
Total	449	124577					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 24: Combined analysis of variance for ear height of 75 hybrids across two environments.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Hybrid	74	40155	542.63	1.97	3.1588	0.0000	***
Environment	1	1287	1287.27	0.00	7.4936	0.0066	**
Hybrid*Environment	74	14134	191	7.88	1.1119	0.2677	
Error	300	51535	171.78				
Total	449	107111					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 25: Combined analysis of variance for ears per plant of 75 hybrids across two environments.

Source of variation	Df	Sum Sq	Mean Sq	Variance ratio	F value	Pr(>F)	Sig.
Hybrid	74	0.62872	0.0085	68835.69	1.4719	0.0132	*
Environment	1	0.29644	0.29644	36.72	51.3547	0.0000	***
Hybrid*Environment	74	0.40696	0.0055	275379.50	0.9527	0.5891	
Error	300	1.7317	0.00577				
Total	449	3.06382					

† DF = degrees of freedom; SS = sum of squares; MS = mean square

Appendix 26: Weather data – Chiredzi 2018-2019 season