Evaluation of genetic gain and diversity in CIMMYT Southern Africa hybrids and open pollinated varieties tested in regional trials from 2000 to 2010

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By

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DECLARATION

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

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Benhildah Pamhidzai Masuka Date

DEDICATION

This piece of work is dedicated to my late father Wilson Koto, my mother Rosa Koto, my husband Abel, my sons Zvikomborero and Nathanael and my daughter Tinaye

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List of abbreviations

AD	Days to mid-anthesis
AFLP	Amplified Fragment Length Polyphormism
ART	Agriculture Research Trust
ASI	Anthesis-silking interval
CIMMYT	International Maize and Wheat Improvement Center
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
DTMA	Drought tolerant maize for Africa programme
DTP	Drought tolerant populations
EA	Ear aspect
EPP	Number of ears per plant
ER	Ear rots
ESA	East and Southern Africa
ET	*Exserohilum (Helminthosporium) tursicum*
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization of the United Nations Statistics
FW	Plot field weight
GART	Golden Valley Agriculture Research Trust
GBS	Genotyping-by-sequencing
GCA	General combining ability
GDP	Gross domestic product
GLS	Grey leaf spot
GMP	Global Maize Programme
GW	Plot grain weight
G x E	Genotype by environment interaction
GYG	Grain yield based on actual grain weight
GY	Grain yield
ha	Hectare
HC	Husk cover

HI	Harvest index
h^2	Heritability
IITA	International Institute of Tropical Agriculture
K	Potassium
KPHA	Number of kernels per hectare
MAF	Minor allele frequency
MAS	Marker assisted selection
MEGA	Molecular evolutionary genetics analysis
MET/s	Multi-environment trial/s
META-R	Multi-environment trial analysis using R-statistical package
MLN	Maize leaf necrosis
MOI	Moisture
MSV	Maize streak virus
mt	Million tonnes
N	Nitrogen
NA	Soil available nitrogen
NDVI	Normalized difference vegetation index
NE	Number of ears
NP	Number of plants
NUE	Nitrogen use efficiency
NUpE	Nitrogen uptake efficiency
NUtE	Nitrogen utilisation efficiency
^{o}C	Degrees celcius
OPVs	Open pollinated varieties
P	Phosphate
PCR	Polymerase Chain Reaction
PH	Plant height
QTLs	Quantitative trait loci
rQTLs	Quantitative trait loci for resistance
S	Selection differential
SADC	Southern Africa Development Community

SAS	Statistical Analysis System
SCA	Specific combining ability
SEN	Senescence
SL	Stem lodging
SNPs	Single nucleotide polymorphism
SSA	Sub-Saharan Africa
SSR	Simple Sequence repeats
t	tonnes
TASSEL	Trait analysis by association, evolution and linkage
TEX	Grain texture
UNESCO	United Nations Education, Scientific and Cultural Organisation
UPGMA	Unweighted pair group method with arithmetic means
USA	United States of America
WCA	West and Central Africa
WUE	Water use efficiency
ΔG	Genetic gain

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Chapter 1

Introduction

1.1 Maize production and demand in sub-Saharan Africa

The world population is growing and demand for food, feed and biofuels is rising, but the rate of growth in global crop production is below what was recommended to cope with the rising demand (Pingali and Pandey, 2001; Ray *et al.*, 2013). Sub-Saharan Africa (SSA) is the most affected by this problem. More than 300 million people in SSA rely on maize for food, feed and a livelihood (Rovere *et al.*, 2010; Prasanna, 2012). SSA has the highest population growth rate, high per capita maize requirement and yet production growth rate is low. Yield in countries including Kenya, Zimbabwe, Zambia, Rwanda, The Democratic Republic of Congo, Somalia and Burundi is declining (Ray *et al.*, 2013). The region has the lowest production levels at less than 2 t ha^{-1} (Bänziger *et al.*, 2000; Pingali and Pandey, 2001; Smale *et al.*, 2011; FAOSTAT, 2012; Kassie *et al.*, 2012) compared to the world average of 4.9 t ha^{-1} as of 2012 (FAOSTAT, 2013). Low maize production means that the small holder farmers face food insecurity and their livelihoods are threatened. Besides, economies for most African countries are agriculture based and the gross domestic product (GDP) is negatively affected if production levels remain low or decline (FAO, 2001). There is an urgent need to improve maize production in SSA for food security and to sustain or improve economies.

Major factors contributing to this low productivity include the use of marginal areas for maize production, low soil fertility, low or no fertiliser use, drought and cultivation of unimproved varieties that are less productive (Bänziger *et al.*, 2000; FAO, 2001; Kassie *et al.*, 2012; Windhausen *et al.*, 2012). A multipronged approach is required to alleviate the effects of the listed factors on maize production. Due to limited capital for acquiring resources such as fertiliser in sufficient quantities and drought being unpredictable and difficult to manage, breeding for tolerance to low nitrogen (N) and drought is a possible intervention to alleviate low maize production (Bänziger *et al.*, 2000; Bänziger and Araus, 2007). Diseases like maize streak virus (MSV), grey leaf spot (GLS) and Northern leaf blight or *Exserohilum (Helminthosporium) tursicum* (ET) also affect maize production and breeding for disease resistance was considered a possible intervention (Bjarnason, 1986; Fajemisin, 2001; Menkir and Ayodele, 2005; Derera *et al.*, 2008; Shepherd *et al.*, 2010; Asea *et al.*, 2011).

1.2 Breeding as a possible intervention

Breeding for drought stress tolerance was initiated at the International Maize and Wheat Improvement Centre (CIMMYT) in 1975 (Bänziger and Araus, 2007) and later (1997) in CIMMYT Southern Africa. The CIMMYT global maize breeding programme in Zimbabwe was established in 1985. Since 1997 CIMMYT East and Southern Africa (ESA) has developed drought tolerant, low N tolerant and disease resistant maize varieties *(*Bänziger *et al.*, 2006; Cairns *et al.*, 2013). Improved drought tolerant maize varieties have been disseminated throughout the SSA region (Bänziger, 2004). These varieties include 60 drought tolerant hybrids and 57 drought tolerant open pollinated varieties (OPVs) (Tsedeke *et al.*, 2013).

In the drought tolerant maize for Africa (DTMA) project the aim was to produce some drought and low N tolerant varieties with resistance to major diseases. The varieties should perform well under stress conditions, producing at least a 1 t ha^{-1} increase in yield by 2016 without a yield penalty under optimal conditions, and to disseminate the varieties to 20 to 30% of the adopting farmers in SSA, with the hope of reaching 30 to 40 million people by 2016 (Rovere *et al.*, 2010).

CIMMYT and International Institute of Tropical Agriculture (IITA) under the DTMA programme set objectives to (i) develop drought tolerant varieties (hybrids and OPVs) that yield at least 1 t ha^{-1} more under drought stress relative to most widely grown hybrids and OPVs under drought stress conditions, (ii) increase maize yield under small holder farming conditions by 20 to 30% under farmer management and (iii) disseminate the developed materials to 30 to 40 million people or five to seven million farming families in SSA by 2016. The main objective of the programme was to alleviate hunger, improve food security and improve income from agriculture and the livelihoods among the resource poor farmers in SSA through the development of drought tolerant varieties. Amid all these developments there is need to assess the genetic gain in yield, drought and low N tolerance and disease resistance in hybrids released[1] by CIMMYT ESA in regional trials from 2000 to 2010.

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[1] For this study release of hybrids is considered as entry into regional trials as this is the point of dissemination

1.3 Evaluation of genetic gain

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). This is not the first era study in maize but it is the first in ESA. Many studies have described long-term trends in grain yield, tolerance to biotic and abiotic stresses and agronomic characters developed over time in selected regions of the world, mainly the United States of America (USA), Canada and South America (Magorokosho, 2006). Ci *et al*. (2011) studied genetic gain in maize yield in China. Recently Badu-Apraku *et al*. (2013) studied genetic gain in early maize varieties in West and Central Africa (WCA). The other studies focused on longer periods, over 60 years in the USA (Duvick, 1997), over three decades in Ontario (Tollenaar, 1989), over three decades in China (Ci *et al*., 2011) and over two decades in WCA (Badu-Apraku *et al*., 2013). This study focused on a shorter period of 11 years (just over a decade). The other studies also had fewer entries, seven in Ontario (Tollenaar, 1989) and 25 entries in China (Ci *et al*., 2011). The more recent study in WCA evaluated 50 entries (Badu-Apraku *et al*., 2013). Seventy hybrids composed of 67 CIMMYT hybrids and three commercial checks were evaluated in a multi-environment trial (MET). The aim of this study was to establish the genetic gain for yield potential and tolerance to drought, low N and disease stresses in CIMMYT hybrids released from 2000 to 2010. Knowledge of the pace of genetic improvement is important in assessing the results and evolution of breeding objectives and strategies (Eyherabide and Damilano, 2001) and for planning.

In earlier studies, the yield increases realised were attributed to both genetic and agronomic improvements. With changing maize crop management over time reflected by the adoption of better cultural practices such as non-tillage agriculture, more rational use of fertilisers and pesticides, and newer and more productive cultivars (Castlebury *et al*., 1983; Eyherabide and Damilano, 2001) yields improved significantly. In separate studies, Russell (1974) and Duvick (1977) established that about 60% of yield increase in maize could be attributed to genetic improvement that includes reduced lodging, ear droppage and barrenness in addition

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to the public and private sector for CIMMYT. The private and public sector will then disseminate selected varieties to farmers.

to increased yield potential. Phenology of the hybrids was evaluated in this study. To focus on genetic gain only, entries were planted side by side in one trial to eliminate variation in agronomic management.

Genetic gain in yield is not only a result of the improved genetic potential for yield, but is influenced by other varietal traits including improved adaptation for improved resource utilisation, improved standability as well as tolerance and resistance to some adverse environmental factors including drought, low N stress and diseases, among other factors. (Tollenaar and Wu, 1999; Duvick, 2005a; 2005b). Drought is quite significant in marginal areas where maize is produced. In SSA drought is frequent, unpredictable, devastating and difficult to manage. The major crop under production in this farming sector in SSA is maize, being the staple crop with a high average requirement of 100 kg capita^{-1} year^{-1} in Southern Africa and 85 kg capita^{-1} year^{-1} in Kenya (Smale and Jayne, 2003). About 40% of the area under maize in SSA is affected by occasional drought while 25% is affected by frequent drought each year (CIMMYT, 2012). The frequency of failed seasons is predicted to increase, making the situation worse. Most of the small holder farmers have no irrigation facilities and are located in the marginal areas where soil fertility is low and drought is frequent. Some of the farmers cannot afford fertilisers in sufficient quantities for crop production.

Diseases devastate potentially good crops and reduce yields by up to 100%. The major maize diseases in Africa include MSV, GLS and ET (Shepherd *et al*., 2010; Tefera *et al*., 2011; Shiferaw *et al*., 2011). Genetically disease tolerant and resistant materials have been developed to curb yield losses. This study assessed the response of the CIMMYT hybrids released from 2000 to 2010 to diseases, focusing mainly on MSV but also ET disease, that developed in the MSV trials from natural infections.

Understanding changes underlying post breeding progress may help to focus research efforts and accelerate future genetic gains (Campos *et al*., 2006). For this reason a study was conducted to evaluate genetic gain and the changes associated with the gain in yield, drought and low N tolerance and disease resistance in CIMMYT hybrids released from 2000 to 2010.

1.4 Evaluation of genetic diversity

Following domestication, crop gene pools have changed to adapt to, and sustain the demands of agricultural systems for thousands of years (Lee, 1998). Breeding results in changes in genetic diversity of populations in defined localities. This is a result of the artificial selection and introductions that are made. Wide adoption of a few varieties of homogenous germplasm reduces crop diversity and reduces stability in crop production (Li *et al.*, 2006). Not much was known earlier about crop content, distribution, architecture, or circuitry (Lee, 1998) but a lot of genetic studies in different crops have been conducted lately including genotyping by sequencing in maize, barley, wheat, rapeseed, lupin, swithgrass and soybean (Liu *et al.*, 2014). Analysing changes over time in genetic diversity of major crops is important for understanding the impact of a plant breeding programme on crop genetic diversity and in setting up baseline indicators for genetic diversity and conservation of genetic resources (Magorokosho, 2006). It is therefore important that the genetic diversity of a breeding programme be assessed constantly in order to prevent loss of crop stability and narrowing of the genetic base.

Characterisation of genetic diversity is highly valuable to breeders. Detailed knowledge of the relationship between maize breeding lines provides a basis for parental selection, genetic analysis and designing of breeding systems (Lu *et al.*, 2009). This study assessed the genetic diversity of the CIMMYT ESA hybrids released from 2000 to 2010. Diversity analysis of germplasm collections can be carried out using data at morphological, geographical, molecular (DNA sequence, gene) and functional levels (Buckler *et al.*, 2006; Prasanna, 2012). For this study hybrids were evaluated at morphological, functional and molecular level for genetic gain and diversity.

1.5 Objectives

The objectives of this study were:

1. To assess genetic gain in yield under optimal conditions in the best performing CIMMYT hybrids released from 2000 to 2010

2. To assess genetic gain in drought tolerance in the best performing CIMMYT hybrids released from 2000 to 2010

3. To assess genetic gain in low N tolerance in the best performing CIMMYT hybrids released from 2000 to 2010
4. To assess genetic gain in MSV tolerance in the best performing CIMMYT hybrids released from 2000 to 2010
5. To identify traits associated with genetic gain in CIMMYT hybrids released from 2000-2010
6. To evaluate genetic diversity in CIMMYT hybrids released from 2000 to 2010

1.6 Hypotheses

The hypotheses for this study were:

1. There was a net positive genetic gain in grain yield in CIMMYT hybrids released from 2000 to 2010
2. New CIMMYT hybrids released from 2000 to 2010 are more drought tolerant than earlier ones
3. New CIMMYT hybrids released from 2000 to 2010 are more low N tolerant than earlier ones
4. New CIMMYT hybrids released from 2000 to 2010 are more disease resistant than earlier ones
5. There has been a change in secondary traits in CIMMYT hybrids released from 2000 to 2010
6. Parental lines of CIMMYT hybrids released from 2000 to 2010 are genetically diverse

Entries were evaluated from 2011 to 2013 in MET using an alpha lattice design with three replications. A total of 67 CIMMYT hybrids and three commercial checks (Appendix I) were evaluated in the MET. The CIMMYT hybrids were evaluated for genetic gain under optimal, low N, disease stress and drought environments. Fifty-four parental lines from CIMMYT Southern Africa were fingerprinted using genotyping by sequencing at Cornell University.

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Chapter 2

Literature review: Maize improvement for yield and multiple stress tolerance

2.1 Introduction

While maize provides 30% of food calories to more than 4.5 billion people of 94 developing countries, and a livelihood to millions of poor farmers (Shiferaw *et al.*, 2011) food production in SSA is still low and does not match the population growth rate (Ray *et al.*, 2012; Ray *et al.*, 2013; Hall and Richards, 2013; Cairns *et al.*, 2013). In Africa alone 29 million ha out of 194 million ha of arable land is used for maize production. About 480 million of the agriculture population in about 46 countries in SSA are cultivating maize for food and feed (CIMMYT, 2012a; b).

More than 300 million people in SSA depend on agriculture for their food, feed and income. Maize is the staple food for the region but its production at 69.45 mt (Figure 2.1) in 2012 (FAOSTAT, 2013) is not sufficient to meet the per capita calorie requirement. The per capita maize requirement is at an average of 100 kg year^{-1} for Africa, 94 kg year^{-1} in Kenya (Smale and Jayne, 2003) and 174 kg year^{-1} in Lesotho (IITA, 2013). Production per unit area in Africa, excluding South Africa, at 1.1 t ha^{-1}(Kassie *et al.*, 2012) is low compared to other regions such as the USA that produces well above 7 t ha^{-1}as shown in Figures 2.1 (FAOSTAT, 2012; 2013). As of 2009, Africa produced 57.07 mt, net imported 12.86 mt and consumed 67.07 mt (Figure 2.1) that converts to 42 kg^{-1} capita^{-1} year^{-1} (FAOSTAT, 2012) and is way below the average of 100 kg^{-1} capita^{-1} year^{-1}. Africa often needs maize imports to supplement local production (Pingali and Pandey, 2001; Smale *et al.*, 2011; Kassie *et al.*, 2012).

The demand for maize is rising due to population growth. On the other hand, economies of some countries in SSA are growing and the demand for meat products in such countries is rising, pushing up the demand for feed and indirectly for maize (Pingali and Pandey, 2001; Betrán *et al.*, 2003a; Shiferaw *et al.*, 2011). The demand for feed rose by 4.3% from 2001/02 to 2005/06. Demand for maize globally is predicted to surpass that for wheat and rice by 2020, yet the production in SSA remains low. Global demand for maize is expected to increase to 837 mt by 2020 with an estimated 504 mt demand in the developing world.

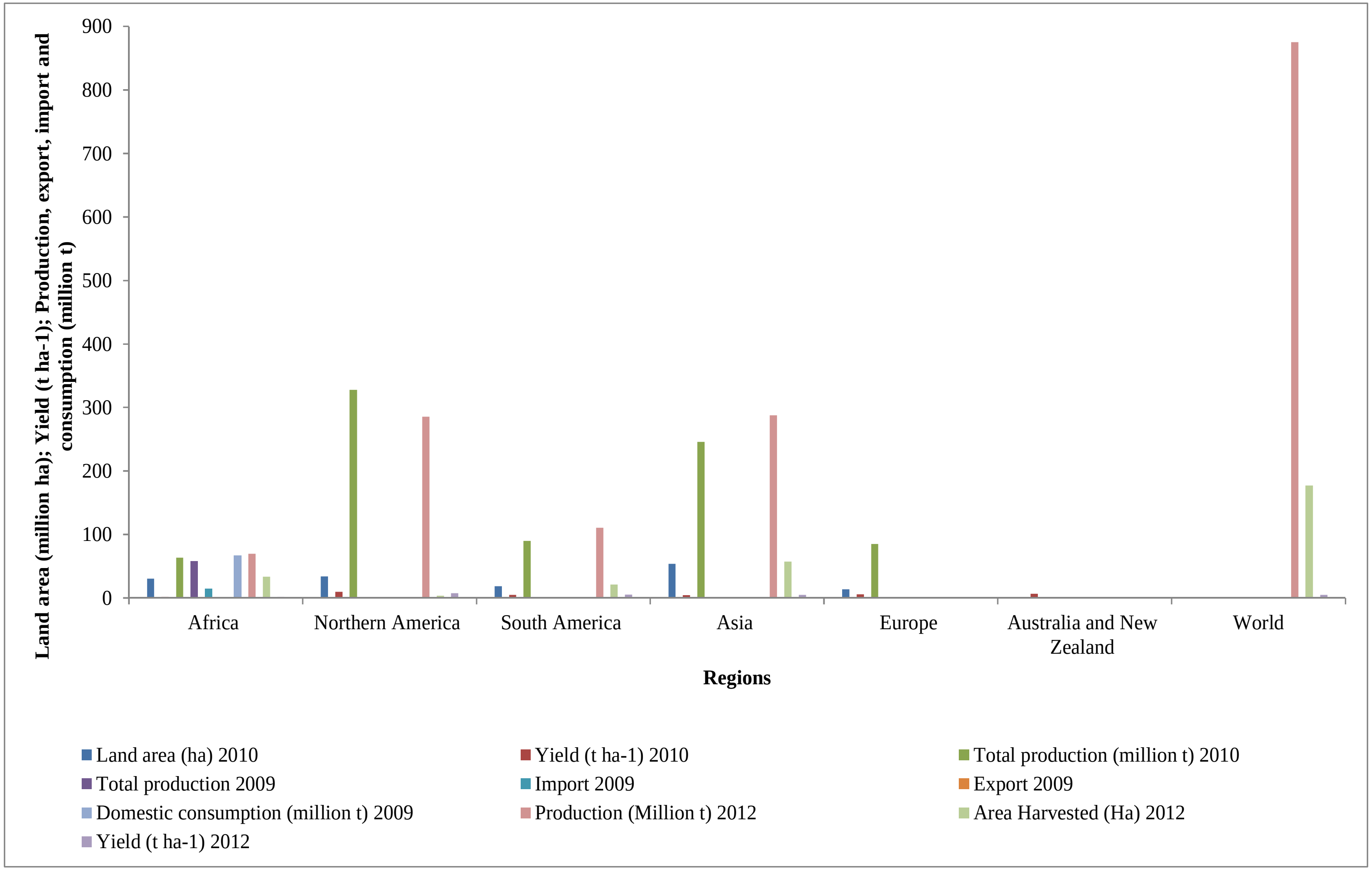

Figure 2.1 World maize production and yield by region (2010 and 2012) and production, consumption, import and export in Africa for 2009 (FAOSTAT, 2012; 2013)

In SSA alone annual maize demand was predicted to increase to 52 mt by 2020 (Pingali and Pandey, 2001) but it has already surpassed the prediction as shown by FAOSTAT (2012) where maize consumption in SSA in 2009 was 67.07 mt. There are maize production shortfalls in SSA that are worsened by increasing input prices, low soil fertility, drought and biotic stresses.

The use of marginal areas for maize cultivation depresses yields in SSA. Marginal areas are characterised by frequent and at times severe drought, degraded soils, diseases, insect pests, weeds, low soil fertility and low fertiliser use (Waddington and Heissey, 1997; Bänziger *et al.*, 2000; Sánchez, 2010; Cairns *et al.*, 2013; FAO, 2013a). Maize production in the small holder sector is mainly rainfed and suffers frequent and sometimes severe drought (Cairns *et al.*, 2013) affecting more than 200 million people in SSA (FAO, 2013a). Maize is more susceptible to drought stress compared to all the other cereals except rice (Campos *et al.*, 2006; Bänziger and Araus, 2007). This is a major drawback in maize production in developing countries among subsistence farmers. Climate change is predicted to worsen the situation (FAO, 2013b). The predicted increase in temperature in SSA is expected to reduce maize yields further (Lobell *et al.*, 2008; Cairns *et al.*, 2013). Most of the tropical maize is also grown under low N conditions due to low N status in the tropical soils, low N use efficiency (NUE) under drought, limited availability of fertilisers and the low purchasing power of farmers, among other causes. Fertiliser use in SSA is low. Fertiliser rates have been reported by different authorities to average less than 10 kg ha^{-1} (Morris *et al.*, 2007) ranging from less than 7 kg ha^{-1} (Pingali and Pandey, 2001; Monneveux *et al.*, 2005) to about 10 to 15 kg ha^{-1} (Phillip *et al.*, 2009). Total crop failure is common in SSA, especially under drought and at times compounded by low or non-use of fertilisers and improved seed. The result is food insecurity and poverty (Holden and Shiferaw, 2004; CIMMYT 2013b). Due to low productivity per unit area, expanding area of production instead of intensification is the more affordable option because of limited access and affordability of fertilisers and other inputs. Land is finite and labour is limited, limiting the capacity to expand area of production. Maize production in SSA, therefore, remains low.

As a result of low production, SSA suffers acute malnourishment with more than 260 million people (30% of the population) affected (Sánchez, 2010). There is need to increase maize production per unit area (Shiferaw *et al.*, 2011) to mitigate maize production shortfalls, hunger and poverty in SSA. Over the past decades new varieties with high yield potential have been developed and they produce well under optimal conditions. Yields are reduced by varying degrees of up to 100% under various biotic and abiotic stresses. This raised the need to develop some compound stress tolerant varieties for the small holder farmers producing maize in the marginal areas under rainfed conditions. Breeding for drought and low N tolerance in addition to breeding for yield and quality was initiated by CIMMYT as a possible intervention (Edmeades *et al.*, 1997; Bänziger *et al.*, 2002). Sinebo (2005) recommended that breeding for the developing countries should be targeted to benefit resource poor farmers in marginalised areas, producing varieties that are both high yielding and highly stable under different environmental stresses.

Breeding for drought tolerance in CIMMYT started in 1975 (Bänziger and Diallo, 2001). The CIMMYT maize breeding programme was established in SSA in 1985 with the establishment of the Southern Africa maize research station in Harare, Zimbabwe and later with another station in East Africa in Nairobi, Kenya. The main objective was the development of stress tolerant and high yielding maize materials adapted to mid-altitude environments of ESA (Hassan *et al.*, 2001). Breeding for drought and low N stress tolerance started later, in 1997 (Bänziger and Diallo, 2001; Bänziger *et al.*, 2006). This programme targeted high yield potential hybrids that still perform well in marginal areas. Breeding for compound stress tolerance can potentially alleviate the maize deficit in SSA where maize production among the resource poor farmers is sometimes in marginalised areas where season failure is high (Figure 2.3) (Rovere *et al.*, 2010; Kassie *et al.*, 2012).

The CIMMYT maize breeding programme in ESA focused on producing drought tolerant varieties with compound stress tolerance. The aims of the breeding programme were to develop varieties that give at least 1 t ha^{-1} yield increase under drought and 30 to 40% yield increase under optimal conditions, and to disseminate the materials to 30 to 40 million people in SSA (Rovere *et al.*, 2010). This intervention for SSA can potentially increase productivity per unit area or yield despite the stresses experienced. All breeding programmes aim to increase yield and assess the genetic yield gain over a specified period of time.

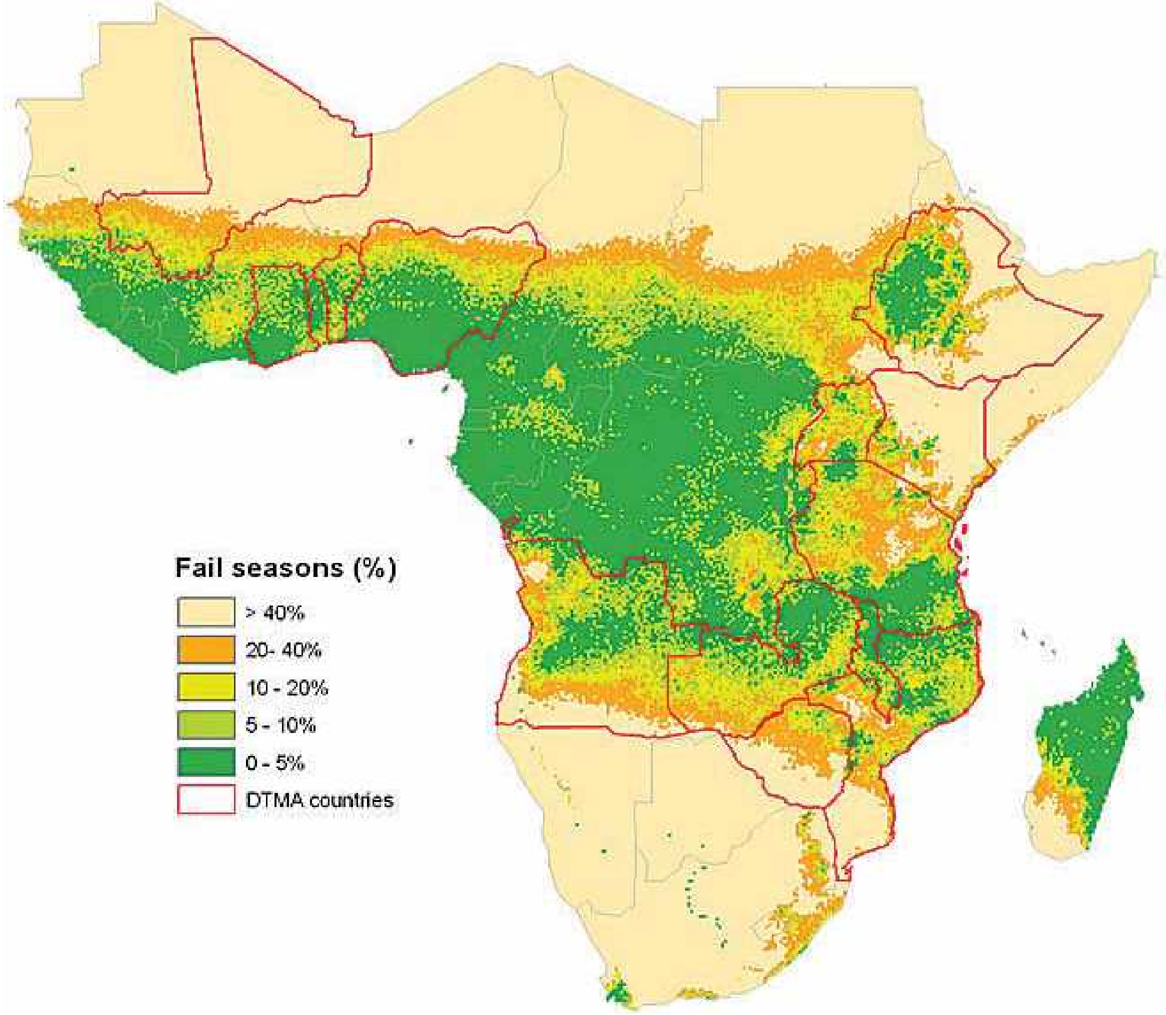

Figure 2.2 Probability/percentage failed seasons in Africa (adapted from Rovere *et al*., 2010)

Genetic gain is the amount of increase in performance that is achieved through genetic improvement programmes. Breeding therefore aims at combining favourable genetic traits with high positive responsiveness to the environment in one or a few varieties. Genetic gain results from genetic changes that improve grain production and stress tolerance, causing higher grain yield production in new varieties (Tollenaar, 1989; Duvick, 2005a).

Genetic diversity is important, as it facilitates breeding by providing the necessary variation from which the breeder can select from and recombine. A breeding programme therefore needs a wide base of genetic diversity. Through artificial selection in a breeding programme, if diversity is not checked and monitored, the genetic base may eventually narrow down (Whitt *et al*., 2002). This can result in loss of diversity for future breeding programmes which will, in turn, narrow down the chances of success in breeding in response to new problems that may arise with time. It is therefore necessary for each breeding programme to maintain a wide genetic base to realise genetic gain.

2.2 Drought in sub-Saharan Africa

Drought is unpredictable and is a major factor in genotype by environment interaction (G x E) (Bankoungou, 1996; Bänziger and Araus, 2007; Wang *et al*., 2011) that can cause significant yield losses (Bruce *et al*., 2002). Drought is a limiting environmental stress in most parts of the world (Bruce *et al*., 2002) with 60% of SSA vulnerable (FAO, 2013a). For SSA drought is described as complex and chronic (UNESCO, 1999). In ESA drought is the most important challenge to livelihoods (Kassie *et al*., 2012). Rainfall amount, intensity and distribution vary greatly in space and time, from year to year and within the season, yet farming in the SSA region is largely rainfed (Bankoungou, 1996).

Maize is susceptible to drought stress because it has

i) separate male and female flower parts

ii) a lesser depth of water extraction

iii) greater transpiration rate due to the large leaves

iv) a slower growth rate and a longer grain filling period and

v) no tillering capacity (Bänziger and Araus, 2007).

Symptoms of susceptibility to post anthesis drought stress include premature leaf and stem senescence, charcoal rot, Fusarium stalk rot, lodging and reduced seed size (Borell *et al.*, 2000). Studies have shown that the sensitive period extends from a week before to two weeks after mid-silking, with yield losses of around 45% to 60% respectively. Losses of up to 70% and 40% to 54% can be experienced when the crop is stressed at silking and 10 to 31 days after silking respectively (Campos *et al.*, 2006). Delayed senescence or stay green during grain filling has been noted as a mechanism of surviving drought stress that increases grain yield (Tollenaar and Wu, 1999; Borrell *et al.*, 2000). Improved water use efficiency (WUE) and NUE under low water stress, and the remobilisation of assimilates promote grain development, increasing grain yield (Edmeades *et al.*, 1997). Live plants have stronger stems, hence lodging is reduced in stay green varieties, and grain filling continues when it ceases in senescing varieties (Borrell *et al.*, 2000).

In the face of climate change, maize yields are predicted to decrease due to reduced rainfall reliability and increase in temperature (Lobell *et al.*, 2008; Cairns *et al.*, 2012). Climate change can further reduce maize productivity in the region. In a study by Lobell *et al.* (2008) maize is considered important for food security in SSA, but is predicted to be the most affected by climate change in Southern Africa. In a separate study by Lobell *et al.* (2011) each degree day increase at above $30^{o}C$ reduces final maize yield by 1% under optimal conditions and 1.7% under drought. Drought tolerant materials may be climate ready if they are heat stress tolerant such that they will be able to tolerate the predicted rainfall decrease and rise in temperature. By testing drought tolerant maize varieties for heat and drought stress tolerance, varieties that are potentially climate ready, may be identified.

Maize production is reduced under drought due to reduced leaf expansion that reduces photosynthetic area, early or hastened leaf senescence, stomatal closure and photo oxidation that damages photosynthetic mechanisms (Bruce *et al.*, 2002). Poor yields under drought conditions have detrimental effects on the income of most of the small holder farmers who depend on agriculture for food and a livelihood. The impact of drought is detrimental in Southern Africa considering that the economies are mainly agriculture based (Kassie *et al.*, 2012). Agriculture contributes significantly to the economy and small holder farmers contribute about 70% of agriculture output (UNESCO, 1999). Even though maize is the primary crop in production in Southern Africa, some small holder farmers are not investing much in improved varieties and fertiliser with the fear of losing the crop due to drought. Small holder farmers are resorting to expanding areas of production (Kassie *et al*., 2012) at times into marginal areas instead of intensifying production by using improved varieties and more fertilisers.

Irrigation is not affordable to most of the small holder farmers (FAO, 2001a) and that calls for alternative interventions. Breeding initially focused on increasing average yield. Yield stability across environments, including biotic and abiotic stresses, was not considered (Bänzinger and Diallo, 2001) but because of the effects of drought there is now need to focus on developing drought tolerant varieties to enhance production under drought stress (Bruce *et al*., 2002). While major breeding efforts focus on increasing productivity under favourable conditions where breeding progress for grain yield is high (Bänziger *et al*., 2006) CIMMYT has been developing drought tolerant materials that can cushion farmers against drought and maximise yield under optimal conditions (Bänzinger and Diallo, 2001; Weber *et al*., 2012; Cairns *et al*., 2013). Breeding for drought and low N tolerance to alleviate poor maize production in most regions of the developing world is a major focus in CIMMYT (Monneveux *et al*., 2006). Maize production in these regions is in marginal areas. Badu-Apraku *et al*. (2013) stated that maize, compared to other crops, has the potential of yield improvement under improved management practices and can fit well in different farming systems. Once improved and adapted to stress conditions, maize production should improve under drought stress.

According to Edmeades *et al*. (1999) and Bolaños and Edmeades (1996) breeding for drought tolerance was initiated in 1975 with screening for drought tolerance within the white dented Tuxpeno crema 1 population in cycle 11. The selected population that was to be further screened for drought tolerance was named Tuxpeno sequia. Sequia means drought. The Tuxpeno sequia population underwent eight cycles of recurrent full sib selection for drought tolerance. Families that showed drought tolerance within the population were selected. Screening was done in three environments; well watered, drought stressed from two weeks before flowering throughout the grain filling period and water stressed from three weeks after emergence (Bolaños and Edmeades, 1993).

Two drought tolerant populations (DTPs) later named DTP1 and DTP2 were also developed by CIMMYT through recurrent selection that involved inter crossing sources from different regions of the world in the form of landraces and lines. Selection was done under well watered and drought stress conditions across the world. DTP1 was developed and later on DTP2. DTP2 contained a large proportion of DTP1 (Edmeades *et al.*, 1999). DTP2 was developed by introgressing 25 drought sources into DTP1. DTP1 and DTP2 were later combined and separated into yellow and white grained populations to cater for varying preferences of different regions of the world. The DTP products had no yield penalty under optimal conditions because selection was done in METs, even though more focus was given to stress environments. DTP2 was prolific under optimal conditions.

In the study by Edmeades *et al.* (1999) Tuxpeno sequia and other sources of drought tolerance including the Pool 26 sequia, La Posta Sequia, DTP1 and DTP2 populations were evaluated in METs that included water stressed, well watered and moderate moisture stress environments. Early maturing varieties yielded better under drought stress. Selection during the screening focused on reduced barrenness, short anthesis-silking interval (ASI) and yield under stress conditions. Drought tolerance was associated with reduced ASI for better grain set, reduced stem biomass, increased number of ears per plant (reduced barrenness), increased harvest index and reduced senescence. Increased yield in DTP1 was a result of increased number of ears per plant or reduced barrenness, reduced ASI, increased harvest index, reduced stem and tassel size that improved biomass partitioning towards the ear and reduced number of kernels per ear that indirectly reduced abortion. No changes were recorded for senescence, leaf rolling and chlorophyl concentration. The selection that was done under drought resulted in some gains under low N. Selection also focused on early maturity and small plants with small tassels. A conventional system that relies on selection in METs is used in CIMMYT to develop varieties that are stable and perform well across all environments without a yield penalty under optimal conditions (Edmeades *et al.*, 1999).

More drought tolerant populations, DTP1-Y with yellow grain colour and DTP1-W with white grain, were developed from DTP1. Two populations, DTP2YC9 (yellow) and DTP2WC9 (white) were developed later. DTP2YC9 and DTP2WC9 are widely adapted to low N and drought stress and are used to develop drought and low N tolerant populations and hybrids that are screened in METs. The drought tolerant and low N tolerant source populations are freely available to breeders (Monneveux *et al.*, 2006).

In separate studies by Bolaños and Edmeades (1996) and Monneveux *et al.* (2008) heritability for grain yield under drought stress was low, but high for secondary traits. Even though heritability is low under drought, significant yield increases have been recorded (Bänziger and Araus, 2007). Bolaños and Edmeades (1996) and Monneveux *et al.* (2008) proposed that secondary traits can therefore be used for selection under stress conditions. Using secondary traits in selection for drought stress eliminates effects of other soil stresses (Monneveux *et al.*, 2008). Genetic variance compared to environmental variance for yield decreases rapidly under drought stress between plots. The experiment showed that individual kernel weight is not affected by drought but the number of kernels per ear and the number of ears per plant are affected. Days to mid-anthesis were not affected by drought stress but days to mid-silking were delayed.

Grain yield was linearly related to single kernel weight and number of kernels per ear. However, the heritability of grain yield, single kernel weight and number of kernels per ear decreased as stress increased. Grain yield was curvilinear related to ears per plant and ASI, yield decreased with increasing ASI up to zero. The heritability and variability of number of ears per plant and ASI increased as stress increased and yield declined. The heritability for days to mid-anthesis remained constant and anthesis, plant height, tassel branch number and leaf angle had the highest heritabilities across all moisture levels. Days to mid-anthesis and days to mid-silking negatively correlated with grain yield as moisture stress increased. Varieties that can produce ears under water stress at flowering are therefore important, as they overcome barrenness induced by stress. N uptake was restricted under drought, resulting in remobilisation of N from older leaves to meet the requirements of the ear. This resulted in senescence of older leaves and a low N content in the leaves (Bolaños and Edmeades, 1996).

Selection for drought tolerance aims at varieties that can capture water from deeper levels, and with early N uptake stored in leaves for remobilisation. This has been possible through selection based on yield as a primary trait and secondary traits genetically correlated with grain yield under drought. The secondary traits used should be stable across environments, measurable and not associated with yield penalties under optimal conditions (Bolaños and Edmeades, 1996; Bruce *et al*., 2002). Some of the secondary traits considered included short ASI, stay green (low senescence), reduced barrenness and leaf rolling (Bänziger *et al*., 2000; Bruce *et al*., 2002). Selection for drought tolerant materials is done under random and managed drought. Good materials are selected in METs that include optimal sites, low N, random stress, disease stresses and lately heat and heat-and-drought stress. This is to ensure compound stress tolerance in the drought tolerant varieties and insurance that there will not be a yield penalty under optimum conditions since a variety is finally selected based on satisfactory performance across the different environments (Bruce *et al*., 2002; Weber *et al*., 2012; Cairns *et al*., 2013). Selection under heat and combined heat and drought stress aims to produce climate ready material in the face of climate change. Improvement in yield stability of varieties under different environments is important in yield improvement (Bruce *et al*., 2002).

Under normal conditions in the farmers' fields in SSA, crops do not face a single stress factor but usually combined stress conditions. Varieties that have gone through screening under different stress environments should be adapted to the random drought stress typical to the farmers' fields. An additional abiotic stress environment was included in the METs. A random stress site that is totally rain fed (like typical farmers' fields) in a low rainfall areas where yield potential is low was included. The random drought stress site usually suffers one or more prolonged and severe natural mid-season droughts.

Improved drought tolerant varieties have been released in 13 countries in SSA. By July 2012 57 drought tolerant OPVs and 48 drought tolerant hybrids had been released in different countries in SSA through collaborators and are currently under cultivation (CIMMYT, 2012b). The most significant production of drought tolerant varieties has been recorded in Kenya, Malawi, Zambia and Zimbabwe (Kassie *et al*., 2012). In Malawi in 2013, four drought tolerant hybrids and four drought tolerant OPVs were released onto the market for the 2012/13 season (Kaonga, 2013 personal communication). More than 2 million farmers across Africa are already benefitting from the CIMMYT drought tolerant products (CIMMYT, 2012b).

2.3 Low soil fertility and low fertiliser use

Low soil fertility and low or no fertiliser use contributes towards low crop production in SSA (FAO, 2001b; Monneveux *et al*., 2005). Marginal areas are used for maize production by some resource poor farmers while high value crops take up the more productive lands. The marginal areas are characterised by inherently poor soils (FAO, 2001a; M'mboyi *et al*., 2010). Due to reduced or non-fertiliser use, the soil is mined as farmers harvest each year without fully replacing nutrients harvested in the crop. This is compounded by mono cropping, mainly of maize (FAO, 2001b). Yields in SSA have remained low due to low soil fertility and other challenges.

Low soil fertility is a result of a number of factors (FAO, 2001b). Low or no fertiliser use contributes significantly to low soil fertility, especially in inherently low fertility soils. There was a reduction in fertiliser use in maize production in ESA (Weber *et al*., 2012) due to the limited availability of the commodity, cost of the commodity which is twice in SSA compared to the world market price, distance or access to markets, access to information, contact with agriculture extension officers, access to credit, membership to cooperatives and extreme poverty (Heisey and Mwangi, 1996; Bänziger *et al*., 2006; Kassie *et al*., 2012). Without subsidies, the N fertiliser to maize ratio is relatively high (it ranged from 2.2 to 10.7 in SSA in the 1990s). The structural adjustment programmes introduced in Africa with the aim of improving economies resulted in the liberalisation of fertiliser markets among other resources such as seed (Heisey and Mwangi, 1996; Smale and Jayne, 2003). Subsidies on farming inputs were withdrawn, pushing up the fertiliser prices. Fertiliser use among resource poor farmers that had shown high adoption was less than 10 kg ha^{-1} (FAO, 2001a). Fertiliser use in Africa and other regions of the world for 2010 is shown in Figure 2.4 (FAOSTAT, 2013).

Low soil fertility and fertiliser use makes maize production unsustainable. Yields in the small holder farming sector are low due to low and depleted soil fertility (Gladwin, 2013). Problems of soil fertility are compounded by low rainfall and increased incidence of drought. N becomes less available under water stressed conditions (Monneveux *et al*., 2005).

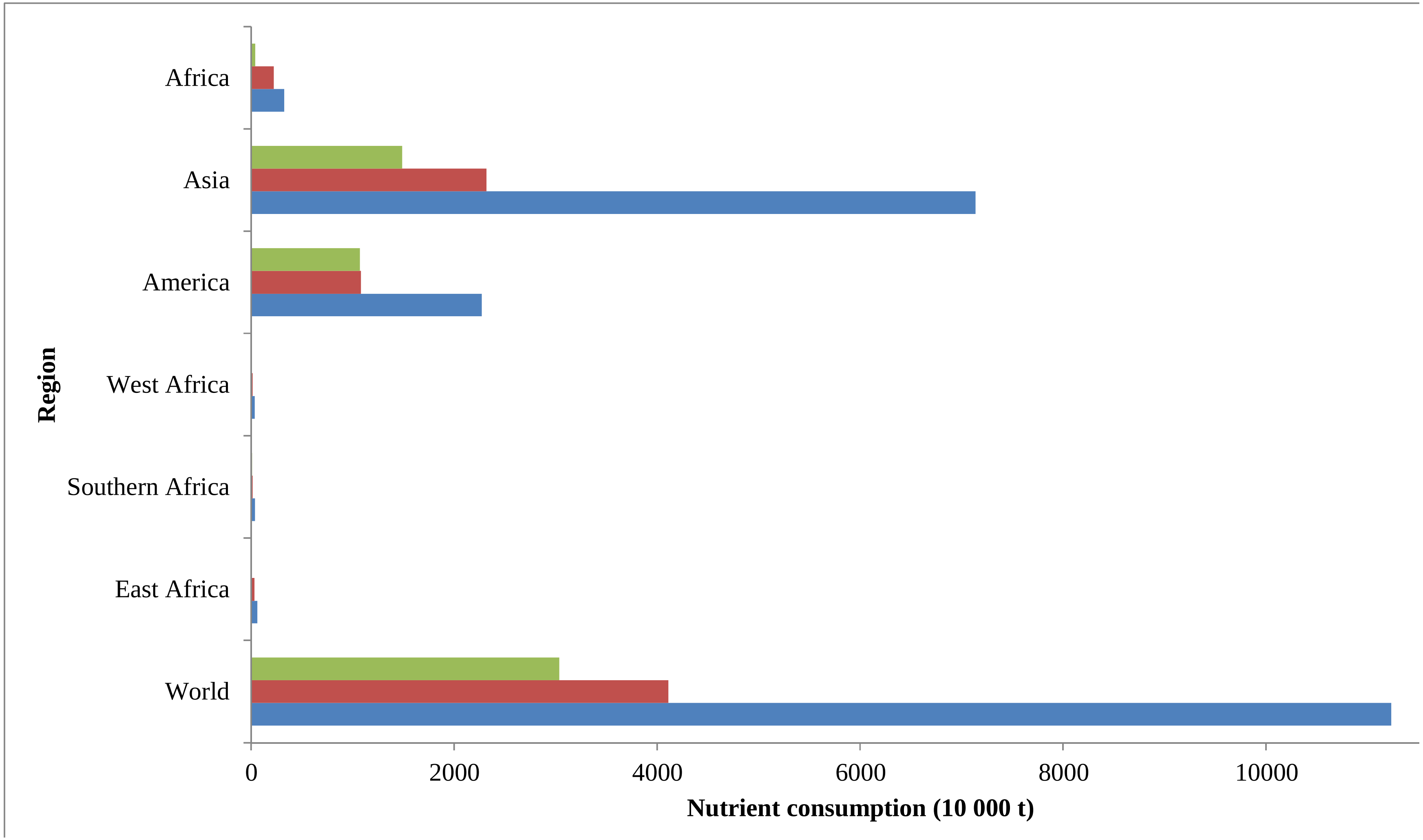

Figure 2.3 Fertiliser use in Africa in kg ha^{-1} (2010) compared to the rest of the world (FAOSTAT, 2013)

All field stresses have resulted in low food production against an increasing population (Bänziger and Diallo, 2001). As possible interventions, FAO (2001b) recommended the use of livestock manure, crop residues, green manure, cover crops and agroforestry to enhance soil fertility and augment the average mineral fertiliser application of less than 10 kg ha^{-1}. The rate is low compared to the world average of 90 kg ha^{-1} and 130 kg ha^{-1} in Asia. Even though these recommendations can potentially alleviate problems of low fertility and low food production in SSA, there are challenges in obtaining organic manure.

Farmers who have livestock have varying herd sizes and some are not large enough to provide all the manure that is needed. In some cases animal manure is used as fuel. Crop residues are at times used as fodder and/or fuel (FAO, 2001b). The manure is often not sufficient to cover the cropped area if used. Some households do not have livestock at all and as such, will not have a ready source of manure and for such farmers organic fertilisers are not a ready alternative. At times farmers with the manure may have labour shortage and as manure digging, transportation and application are laborious, the manure will not be used, even though available.

Agroforestry as an alternative to improve soil fertility is promising (Franzel *et al.*, 2001; Chihota *et al.*, 2007). However, agroforestry faces challenges ranging from limited or small land holdings that cannot be spared for agroforestry as they may not be sufficient for food production, limited access to, or short supply of seed of agroforestry species, lack of knowhow, land tenure systems. With communal land ownership livestock roam in the fields for off season feeding and destroying established agroforestry species. Another challenge is limited water availability (low rainfall) impeding establishment of species (Chihota *et al.*, 2007; Kabwe *et al.*, 2009; Chitakira and Torquebiau, 2010; Parwada *et al.*, 2010). As researchers continue to work on the solution to challenges in agroforestry adoption, there is need to work on other interventions.

N is a major determinant of grain yield. This is expressed in an equation

$GY = [NA \times NUpE \times NUE] \times HI$ where

GY is grain yield,

NA is N availability (soil),

NUpE is N uptake efficiency

NUE is N use efficiency and

HI is harvest index.

In this relationship, grain yield is determined by the amount of N available in the soil, the amount of N that the crop can extract from the soil, the efficiency of the crop to produce grain from the N extracted and the harvest index or biomass partitioning. In the tropics recovery of N (N uptake) by the plants is 35 to 50% and is lower under waterlogged conditions. In maize, NUE is around 30 to 70 kg grain kg^{-1} N applied at low levels. NUE decreases with increased N supply (Bänziger *et al*., 2000). Based on this concept, varieties with better N uptake and/or NUE will perform better than those without this under conditions where soil N is limited.

N stress affects a number of plant processes including reduction of photosynthesis, reduced leaf growth that results in reduced radiation interception, early and at times rapid senescence of older leaves as N is relocated to developing parts of the plant (Bruce *et al*., 2002) and delayed anthesis and silking. Effectively low N stress reduces grain yield. N stress at 12 to 14 leaf stage will reduce the number of rows of the ear while stress at 16 to 18 leaf stage will reduce the number of kernels per row. This, combined with kernel and ear abortion due to delayed silking, significantly affects grain yield (Bänziger *et al*., 2000). It is therefore important that N stress be avoided through the use of fertilisers and varieties that are more responsive to N application (Heisey and Mwangi, 1996). Where the use of fertiliser is low or limited like in SSA, N stress tolerant varieties should be available to at least improve maize production and enhance food security. There is need to screen and develop low N tolerant varieties. Development of low N tolerant varieties for the tropics started in CIMMYT in 1986 with a recurrent breeding programme (Bänziger and Lafitte, 1997).

There is low correlation between grain yield under optimal conditions and grain yield under N stress. For this reason screening for low N tolerance should be done under low N stress, but selection should include optimal and drought environments (Bänziger and Lafitte, 1997;

Bänziger *et al.*, 2000). Heritability of grain under low N stress is low, making it difficult to select directly for grain yield. This makes secondary traits more ideal when selecting under stress conditions. Secondary traits that correlate well with grain yield under N stress are used to select for tolerant varieties (Betrán *et al.*, 2003b; Monneveux *et al.*, 2005; Badu-Apraku *et al.*, 2012). Such traits include ASI, plant aspect, ear aspect, number of ears per plant or barrenness, stay green or senescence, plant height and in some cases percentage root and stem lodging (Bänziger *et al.*, 2000; 2006). These traits were observed to be stable and reliable in a study by Badu-Apraku *et al.* (2012) and the findings agreed with recommendations by Lafitte *et al.* (1997). Grain yield as the primary selection trait and secondary traits that have high correlation with yield are therefore all used to select for low N tolerance in the CIMMYT breeding programme.

In CIMMYT low N tolerant varieties are screened for in N depleted fields that cause an average of 70% reduction in yield. Land is depleted of N by continuous cropping of wheat in the main rainy season and irrigated wheat off season, removing all the stover and not applying any N fertilisers. The land is depleted until yields under low N are about 30% of yields of the same entries under optimal conditions (Bänziger *et al.*, 2006; Weber *et al.*, 2012). It is recommended to select varieties in METs so that varieties that perform well across all environments are selected. There is a possibility that if selection for low N tolerance is done under low N stress only, there may be a yield penalty when the variety is produced under optimal conditions (Weber *et al.*, 2012; Cairns *et al.*, 2013).

From an evaluation by CIMMYT of a wide range of landraces only about 3%, compared to favourably elite adapted germplasm, was N tolerant. This justified the screening of elite germplasm under low N and drought rather than screening landraces. Screening for low N is much simpler than screening for drought tolerance due to the different effects of drought on maize at different developmental stages. Low N and drought stress affect performance of maize varieties in similar ways such that some common secondary traits are used in selection. Some materials screened under drought stress were also found to be low N tolerant. Low N and drought tolerant materials in the CIMMYT breeding pipeline are screened under different stresses for integration into other germplasm (Bänziger *et al.*, 2000). In another study by Betrán *et al.* (2003b), severe drought stress and low N reduced plant heights of maize plants by up to 48%. The general combining ability (GCA) of all secondary traits except stalk lodging for the lines, OPVs and hybrids tested, was significant. The specific combining ability (SCA) for days to mid-anthesis, days to mid-silking, ASI, ear height, tassel size and erect leaves was significant. There was a negative correlation between grain yield and days to mid-anthesis and mid-silking. Plant height, ear height and the number of ears per plant positively correlated with grain yield.

2.4 Selection for combined stress tolerance

The farmers' fields in the marginal areas do not have specific stresses like low N only, or drought only, but usually have a combination of biotic and abiotic stresses. Selection for a single stress becomes a partial solution, but a holistic approach is necessary. The CIMMYT breeding programme, in addition to screening for drought tolerance and low N, grain yield, plant architecture, stalk and root quality, ear aspect and combining ability under optimal conditions, screens for disease resistance (Betrán *et al*., 2003b), random stress, heat stress and combined heat and drought stress tolerance.

2.4.1 Disease resistance

According to Asea *et al*. (2011), foliar diseases reduce maize production globally but they can be managed through quantitative and partial host resistance. The main foliar diseases affecting maize production in SSA include MSV, GLS and ET (Asea *et al*., 2011). If these diseases are not controlled, they can cause significant yield losses. Chemical control of the diseases or vectors raises production costs and may not be an affordable option among resource poor farmers. Use of resistant varieties is the best control measure recommended by most researchers (Menkir and Ayodele, 2005; Shepherd *et al*., 2010; Asea *et al*., 2011). Various studies have shown that selection for resistance to diseases is effective in disease management. Marker assisted selection (MAS) can be used for stacking quantitative trait loci (QTLs) for resistance in favourable varieties (Asea *et al*., 2011).

2.4.2 Maize streak virus disease

MSV, introduced to Africa through Ghana from Portugal is widespread in Africa, affecting maize production and a wide range of other *Gramminae* species that provide alternative hosts off season (Bosque-Perez *et al*., 1998; Magenya *et al*., 2008; Shepherd *et al*., 2010). In SSA MSV was first reported by Fuller in 1901 (Damsteegt, 1983; Shepherd *et al*., 2010) and is considered economically as the most important foliar disease in maize production in SSA (Ramusi and Flett, 2012). MSV disease causes severe losses in maize production in Africa. Losses of up to 100% in early infections have been recorded (Shepherd *et al*., 2010; Tefera *et al*., 2011; Asea *et al*., 2011). Most small scale farmers cannot control this disease chemically and once a crop without resistance is infected, the losses can be devastating.

MSV disease is caused by MSV A virus strain (Shepherd *et al*., 2010) that is transmitted by the *Cicadulina* hoppers including *C. storey* (Damsteegt, 1983; Shepherd *et al*., 2010). The vector and pathogen overwinter on alternative *Gramminae* species such as winter wheat and grass (CIMMYT, 2004; Ramusi and Flett, 2012). The MSV has a high evolution rate (van der Walt *et al*., 2008) and its mutants are virulent. Mutants infect maize, causing development of MSV symptoms (Lazarowitz and Pinder, 1989).

Following an infection, chloroplasts fail to develop in tissues surrounding vascular bundles (Rose, 1978). White to yellow, sometimes red circular chlorotic spots develop that coalesce to form white streaks. This reduces photosynthesis in the plants and increases respiration. Leaf length and plant height are reduced. Severe symptoms include stunting, undersized and misshapen cobs, barrenness, reduced seed set and even premature death. If infections occur early, losses of up to a 100% can be recorded (Damsteegt, 1983; Mawere *et al*., 2006; Shepherd *et al*., 2010). MSV does not only affect the quantity of grain yield but also its quality. Resistant varieties have better yield and quality of both grain and stover (Lukuyu *et al*., 2002). Low stover quality and quantity affect maize silage yield and quality, while reduced grain yield reduces food security.

For control of MSV, maize is planted early to escape increased viral load. Barriers of bare ground can be generated to keep the hoppers out of the fields. Hoppers can be controlled by spraying to control spreading of the disease. Younger or newer crops should be planted up wind. Development of resistant maize varieties is recommended as the most effective and economical method of control. Resistant varieties are poor sources of secondary infection, maintaining low viral loads throughout the season (Bosque-Perez *et al*., 1998; Magenya *et al*., 2008; Shepherd *et al*., 2010). Good soil fertility management reduces the impact of MSV (Magenya *et al*., 2008). Mawere *et al*. (2006) recommended host plant resistance as the best alternative, citing that it is cost effective, a long term solution, economically viable and most appropriate alternative. The technology is more affordable to small holder farmers of SSA who may not be able to afford chemicals to control the vector. Breeding for MSV tolerance reduces the losses caused by the disease and helps to maintain yields. Tolerant maize varieties yield well despite being infected.

CIMMYT, IITA and other private seed companies have been breeding for resistance to MSV since the 1980s (Bosque-Perez *et al*., 1998; Pingali and Pandey, 2001). Resistance to MSV is controlled by a major gene with two to a few modifying genes (Mawere *et al*., 2006). MSV resistant varieties were developed by selection of full sibs under disease pressure during inbreeding (Pixley *et al*., 2006). MAS was used in the development of some tolerant varieties (Asea *et al*., 2009; Shepherd *et al*., 2010; Asea *et al*., 2011). For introgressing different genes from different chromosomes into better developed varieties and lines, MAS was used (Shepherd *et al*., 2010). Since the sources of MSV resistance were temperate materials that were not adapted to the tropics, MAS was effective in selecting progeny in which only the desirable resistance genes were successfully introgressed into adapted tropical materials without the undesirable background of donor materials.

By 1995 CIMMYT was disseminating MSV tolerant varieties (Magorokosho and Pixley, 1997). In CIMMYT, MSV resistant varieties were tested regionally in METs such that by the time they are released they would be suitable for cultivation in different areas of SSA and compound stress tolerant with no yield penalty under optimal conditions. To date all varieties developed in CIMMYT ESA are tested in METs that include optimal, abiotic and biotic stresses and under artificial MSV infestation in Harare.

2.4.3 Gray leaf spot

GLS is a major biotic constraint in maize production in SSA that causes large yield losses, affecting the viability of maize production (Verma, 2002; Menkir and Ayodele, 2005; Derera *et al*., 2008). The disease spread northwards from South Africa to the rest of SSA (Meisel *et al*., 2009). Even though GLS is widespread in South America and SSA, the impact is worse in SSA where maize is the staple food crop for most of the population. Reduced yield negatively affects both the producers due to loss of income and consumers in the form of product supply shortfalls and the associated shifting of price (Ward and Nowell, 1998).

GLS is caused by *Cercospora zea-maydis* (CIMMYT, 2004; Asea *et al*., 2009). *C. zea-maydis* is carried over and spread in plant residues. Once infected with GLS, photosynthetic tissue is damaged, especially the leaves. Infection is promoted by extended leaf wetness and cloud cover. Initially small regular, elongated brown gray necrotic spots develop. Lesions normally start developing on lower leaves and develop upwards. Lesions will enlarge to 3 to 4 cm in length. Other symptoms include poor grain filling and leaf senescence after flowering (CIMMYT, 2004; CIMMYT, 2013a). *C. zea-maydis* overwinters in maize residues (Derera *et al*., 2008). Yield reductions are caused by reduced photosynthesis and increased stem and root lodging. Yield losses due to GLS may be more than 30% to 70% (Asea *et al*., 2009; CIMMYT, 2013a). GLS is a threat to most small scale maize farmers in SSA who cannot afford fungicides. There is need to develop host plant resistance against GLS to reduce yield losses due to the disease in SSA and South America (Verma, 2002; CIMMYT, 2004; Menkir and Ayodele, 2005; Mawere *et al*., 2006; Pixley *et al*., 2006; Derera *et al*., 2008). In SSA CIMMYT, IITA and some private seed houses are developing varieties with GLS resistance.

Resistance to GLS is quantitative, controlled by both additive and non-additive genes (Derera *et al*., 2008). Resistance to GLS can be managed through MAS. Phenotyping combined with MAS was used to identify GLS resistant lines. Selected lines were used in breeding for GLS tolerance. Genes for resistance to GLS, MSV and ET are pyramided in desirable germplasm using MAS (Asea *et al*., 2009; 2011). Through the programme for breeding maize for biotic and abiotic stresses, all varieties developed by CIMMYT ESA are screened for GLS in Harare, Zimbabwe under natural inoculation, in Cedara, South Africa and Kakamega, Kenya in addition to other environments in the METs.

2.4.4 Northern corn leaf blight

ET is a fungal disease caused by *Exserohilum (Helminthosporium) tursicum* (Levy and Pataky, 1992; Abebe and Singburaudom, 2006; Zhu *et al*., 2011). The disease is a problem worldwide (Asea *et al*., 2011; Technow *et al*., 2013) causing yield losses of more than 50% in maize production (Ogliari *et al*., 2005; Carson, 2006; Zhu *et al*., 2011; Chandrashekara *et al*., 2012). Farmers in SSA need an economically effective way of controlling the disease, bearing their economic status in mind. Use of fungicides is costly and unaffordable to most of these farmers.

Disease development following infection is promoted by low temperatures and high humidity (Ogliari *et al.*, 2005; Abebe and Singburaudom, 2006; Carson, 2006; Chandrashekara *et al.*, 2012). *E. tursicum* overwinters in crop residues (Carson, 2006). The pathogen infects the crop from the previous season's crop debris. The disease spreads rapidly under humid conditions (Levy and Pataky, 1992). Initially small water soaked spots develop on the leaves. Spots later elongate into spindle shaped necrotic lesions. Symptoms start developing on lower leaves and spread upwards. Under serious infestations of the disease a maize crop can suffer complete senescence. Early infections are more devastating compared to later infections (CIMMYT, 2004). The degree of yield loss varies with level of resistance of a variety (Levy and Pataky, 1992). The presence of host plant resistance significantly reduces the effect of ET on yield.

Building up of host plant resistance in varieties cultivated by farmers is an effective intervention that will reduce losses due to ET (Freymark *et al.*, 1994; Abebe and Singburaudom, 2006; Fullerton, 2012). This will also cut down on production costs since spraying with fungicides against ET will be reduced. Resistance breeding is done by CIMMYT and the private sector. The genes for resistance to ET have an additive effect. Resistance can be controlled by one major gene that will confer resistance and is specific to an ET race, or can be polygenic. In commercial maize, mostly polygenic resistance is utilised (Freymark *et al.*, 1994; Carson, 2006). Several studies were conducted to identify potential sources of resistance genes, map maize genes and identify Quantitative trait loci for resistance (rQTLs) (Freymark *et al.*, 1994; Chandrashekara *et al.*, 2012). Conventional and MAS breeding were employed in developing lines and varieties with resistance to ET (Carson, 2006; Asea *et al.*, 2011). Total resistance was not realised, but graded resistance was obtained. Lines were classified as resistant if they had a score of 1 to 2 on a scale of 1 to 5. For scores between 2.5 and 3.5 the lines were considered moderately tolerant (Chandrashekara *et al.*, 2012). Quantitative resistance was associated with a reduced number of lesions and increased latent period (Freymark *et al.*, 1994; Carson, 2006). Reduced lesion length was also initially used, but had low heritability (Carson, 2006). Selection was finally based on increased latent period and number of lesions.

In CIMMYT ESA, breeding nurseries and trials are exposed to ET and selection in the nurseries are done based on performance of the lines or parental material in the nurseries as well as performance of progeny in hybrid trials. Through the breeding pipeline to the final stages of variety development, varieties are selected in METs that include ET and other diseases, abiotic stresses and optimal conditions. The final products are supposed to be stable across all environments without a yield penalty under optimal conditions.

2.5 The CIMMYT breeding pipeline

Entries that perform well in these METs are advanced to the next testing level until they reach regional trial testing where they are tested throughout SSA. Best performers in the regional trials are disseminated to farmers through collaborators. The breeding pipeline is shown in Figure 2.5.

2.6 Genetic gain in maize

Every breeding programme needs to be assessed for progress. In maize breeding this is done through evaluation of the products from different years to estimate genetic gain for the various traits of interest. As described by Hallauer *et al.* (2010) genetic gain is a product of the heritability and selection differential. It is calculated using the formula

$$\Delta G = h^2 S$$

Where ΔG is the genetic gain

h^2 is the heritability and

S is the selection differential

Heritability is calculated using the equation

$$h^2 = \sigma^2_g / (\sigma^2_{e/re} + \sigma^2_{ge/e} + \sigma^2_g)$$

where σ^2_g is genetic variance

$\sigma^2_{e/re}$ is environmental variance in replications in the different environments and

$\sigma^2_{ge/e}$ is variance due to gene by environment interaction in the different environments.

Selection differential is the difference between the mean of the selected progeny and the population mean, that is

$$S = X_s - X$$

Where X_s is the mean of the selected progeny and

X is the population mean.

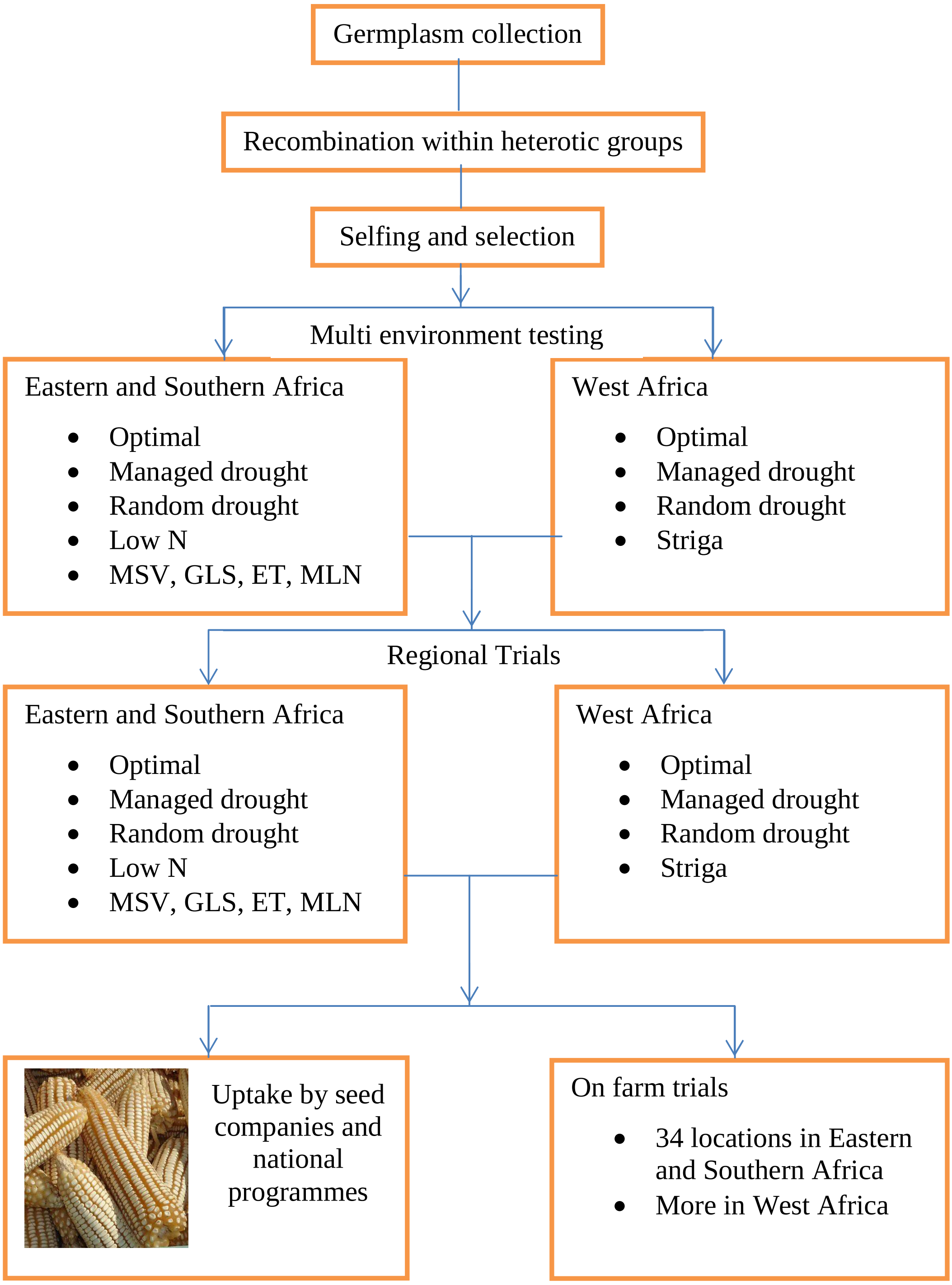

Figure 2.5 Overview of the maize drought breeding programme in SSA

Genetic gain results from changes in the allele constitution that improves a variety (Smith *et al.*, 2006). The genetic changes result in physiological changes that contribute to improvement in performance of the developed varieties. Breeding in maize focuses on improving light interception by altering leaf angle, flowering dates, photosynthesis, functional and visual stay green, kernel number, kernel set and dry matter partitioning (Lee and Tollenaar, 2007). Hammer *et al.* (2009) however, based on simulations and empirical data reported that since the 1990s change in leaf angle has not contributed significantly towards grain yield.

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, 2005b). Barrero Farfan *et al.* (2013) agree that changes in secondary traits contribute to grain yield improvement. Studies have shown that ability of the new varieties to withstand high plant density stress contributes to increase in grain yield (Sangoi, 2001; Duvick, 2005b). Increase in yield will therefore be a product of the grain yield per plant and the number of plants per unit area (Duvick, 2005a). For maize production, hybrids make use of heterosis to increase yield compared to lines. It is therefore critical to maintain a wide genetic base that can be used for line, population and hybrid development.

2.6.1 Genetic diversity

Maize is both phenotypically and genetically diverse (Xu and Crouch, 2008). The molecular diversity of maize is estimated at two to five times higher than that of other domesticated grass crops (Tenaillon *et al.*, 2001). Diversity in maize landraces exceeds variability in any other crop species. Tropical maize is more diverse compared to temperate genotypes. Sources of diverse germplasm in maize include wild relatives such as teosinte, about 300 landraces in the world, OPVs, synthetics, inbreds, hybrids, pools and various stocks that include near-isogenic lines, mutants and permanent populations (Xu and Crouch, 2008). With wide diversity there is high likelihood of successfully breeding new varieties with additional desirable traits that may be in response to crop production problems such as new diseases.

Domestication of cultivated plants generally reduces diversity relative to their wild ancestors and this can have an impact on the future improvement of the crop (Vigourox *et al*., 2008). According to Coors and Pandey (1999) and Tollenaar and Wu (1999), maize improvement throughout the world has been associated with narrowing of the germplasm base as newer lines and varieties are derived from inter-crosses of elite materials. This raises concerns that bottlenecks may restrict breeding flexibility and slow response to new opportunities, pests, pathogens and agronomic practices. It is important to monitor the diversity of domesticated maize so that breeding and selection will not narrow down the diversity resulting in lack of heterosis and loss of diversity when breeding for arising problems. Conserving and studying maize variability provides resources for agricultural improvement, such as breeding, to reduce hunger and poverty, and to provide a solid knowledge base (Taba *et al*., 2013). Where the genetic base is narrow, introductions can widen the diversity (Louette and Smale, 1996). A wide genetic diversity assures successful breeding and genetic gains for grain yield and other traits.

2.6.2 Genetic gain studies

Various studies in different parts of the world have shown varying degrees of genetic gain. Increase in grain yield per unit area over the years has been attributed to both genetic gain and improvement in cultural management. In the USA improvements in grain yield of 65 to 75 kg ha^{-1} year^{-1} over 70 years from 1934 to 2004 was reported to have been due to genetic gain and changes in cultural management respectively, contributing 50 to 60% and 40 to 50% to the total increase (Duvick, 2005a). Eyherabide and Damilano (2001) reported a genetic gain in Argentina of 2.9% per year. Tollenaar and Wu (1999) studied yield improvement in Ontario and reported a genetic gain of 1.5% per year over five decades since the 1940s. Kamara *et al*. (2011) studied genetic gain over 30 years from 1974 to 2004 in grain and fodder yield of cow peas in WCA. Results showed that genetic gain for yield was higher in the determinate compared to indeterminate varieties. For fodder, genetic gain was higher in the indeterminate varieties. The study indicated that physiological differences can contribute to overall genetic gain, and gain also varies with the trait of interest. Little or no genetic improvement had been recorded prior to the hybrid era that was in the 1940s.

Breeding has contributed significantly to gains in yield and other traits, but the total gain in yield is a result of genetic gain, gains due to improved agronomic practices and environmental influence (Duvick, 2005b). With improvement in agronomic practices without improvement in genetic makeup, particularly the development of hybrids, gain in yield and other traits may reach a limit. It is therefore important in yield gain studies to separate gain due to genetic improvement and gain due to improved agronomic or cultural practices as described by Duvick (2005b).

2.7 Conclusions

The CIMMYT breeding programme has developed varieties targeted for ESA since the establishment of the Southern Africa regional station in 1985. The SSA region faces frequent droughts and most of the farmers are small holders who cannot afford sufficient fertilisers. From 1997 CIMMYT ESA focused on development of drought and low N tolerant varieties aiming to improve productivity among resource poor farmers in the region. Several varieties were released and some of the best performing varieties were released to the farmers through public and private sector collaborators. As of September 2013, 117 drought tolerant varieties comprised of 60 hybrids and 47 OPVs from the CIMMYT ESA and IITA for WCA had been released to farmers in different countries in the region. Adoption of the varieties has been a result of superior performance of the drought tolerant varieties in regional and on-farm variety trials. Genetic gain in the IITA early maize hybrids programme was evaluated (Badu-Apraku *et al*., 2013) but the CIMMYT programme has not been evaluated for genetic gain.

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Chapter 3

Genetic gain in maize breeding in Eastern and Southern Africa under optimal conditions

Abstract

More than 300 million people in sub-Saharan Africa depend on agriculture for their food, feed and livelihood. Maize is the staple crop for most countries in the region, but its production on more than 39 million ha at an average of 1.1 t ha^{-1} is low and insufficient to meet the annual requirements for food. Low maize production is a compound effect of various biotic and abiotic stresses including frequent droughts, heat stress, climate change, low nitrogen stress, diseases and pests. As a possible intervention that is more affordable to resource poor small holder farmers in the region, CIMMYT has been developing a range of compound stress tolerant hybrids. Some of the developed varieties include 57 open pollinated varieties and 60 hybrids that performed well in regional trials from 2000 to 2010, were disseminated to farmers through the public and private sector. This study evaluated genetic gain in the CIMMYT drought tolerant maize breeding programme. The best performing hybrids from each year were evaluated from the 2011/12 season to the 2012/13 season under optimum conditions at 14 sites in a multi-environment trial across East and Southern Africa. The study showed an estimated net genetic gain in yield of 109.4 kg ha^{-1} (1.4%) year^{-1} over the 11 year period from 2000 to 2010. This was accompanied by some changes in secondary traits including an average decrease in anthesis-silking interval of 0.08 d year^{-1} and a trend of reduced in barrenness corresponding to an increase of 0.004 ears plant^{-1} year^{-1}.

3.1 Introduction

3.1.1 Drought tolerant maize in sub-Saharan Africa

Over 300 million people in SSA depend on agriculture for their food, feed and income. Maize is the staple food for the region, but its production at 63.5 mt in 2010 (FAOSTAT, 2012) is insufficient to meet the per capita calorie requirement. The per capita maize requirement is at an average of 100 kg year^{-1} for SSA, 94 kg year^{-1} in Kenya, 148 kg year^{-1} in Malawi, 140 kg year^{-1} in Zambia, 122 kg year^{-1} in Zimbabwe (Smale and Jayne, 2003) and 174 kg year^{-1} in Lesotho (IITA, 2013). Production per unit area in Africa, excluding South Africa, at 1.1 t ha^{-1} (Kassie *et al.*, 2012) is low compared to other regions of the world such as the United States (US) that produces about 9 t ha^{-1} (FAOSTAT, 2012). In 2009, Africa net imported 12.86 mt to supplement produced maize in an effort to meet maize food requirement but only managed to achieve an average of about 42 kg capita^{-1} year^{-1} (FAOSTAT, 2012) that is way below the average of 100 kg capita^{-1} year^{-1}. With the current production Africa often needs maize imports to supplement local production (Pingali and Pandey, 2001; Smale *et al.*, 2011; Kassie *et al.*, 2012) but improving production is a more sustainable solution.

The SSA region faces a great challenge in trying to improve maize production levels to cope with increased demand for food and to improve nutrition and livelihoods. Challenges faced in the region include various stresses limiting maize production that include drought and low N. Displacement of maize production from productive areas to marginal areas as high value crops are introduced or expanded in production systems. Low or no fertiliser use and soil pulverisation have reduced production and average yields in the tropics (Bänziger *et al.*, 2000; FAO, 2001). With more than 48% of the population living in the rural areas (World Bank, 2013) depending on subsistence agriculture for food and livelihoods, crop failures can have disastrous consequences. The predicted impact of climate change further raises the need to improve yields and adaptation of maize production to harsh environments (Lobell *et al.*, 2008). Bänziger *et al.* (2000) expressed the need to develop varieties that combine tolerance to multiple stresses.

With this vision, CIMMYT and IITA jointly implemented maize breeding for drought and low N stress tolerance aimed at developing compound stress tolerant varieties that can increase yield by 1 t ha^{-1} under drought stress by 2016. The materials were supposed to increase yield under small holder farming by 20-30% when farmers use the drought stress varieties and to reach 30-40 million people in SSA (Rovere *et al.*, 2010). Initial breeding for drought tolerance in CIMMYT started in the 1970s (Bänziger and Aurus, 2007). The maize breeding programme expanded to ESA with the establishment of the CIMMYT maize station in Harare, Zimbabwe in 1985 and later in Nairobi, Kenya. Breeding for stress tolerance in ESA started in 1997 (Edmeades *et al.*, 1997). CIMMYT has since developed new varieties for better performance under optimum and stress conditions by breeding and selecting in METs that included optimal, low N, drought and disease stress environments (Weber *et al.*, 2012). The varieties have been released in regional trials conducted annually. Sixty hybrids and 57 OPVs developed by CIMMYT ESA have been disseminated throughout SSA (Table 3.1) through collaboration with the National Research programmes and private seed companies in the region (CIMMYT, 2013). There is need to evaluate the breeding programme for genetic gain for yield to review progress. From the evaluation results, objectives of the programme can be maintained or modified, or new objectives will be set if necessary.

Table 3.1 CIMMYT drought tolerant maize varieties released in SSA from 2007 to 2013 (CIMMYT, 2013)

Country	Hybrid	OPV	Total
Angola	2	5	7
Benin	0	6	6
Ethiopia	2	4	6
Ghana	5	7	12
Kenya	1	1	2
Malawi	5	3	8
Mali	4	3	7
Mozambique	3	3	6
Nigeria	6	13	19
Tanzania	10	2	12
Uganda	6	1	7
Zambia	9	6	15
Zimbabwe	7	3	10
Total	60	57	117

3.1.2 Genetic gain in a breeding programme

Globally, breeding has contributed significantly to maize yield improvements. Improvement in maize yield in the farmers' fields over the years has been a combination of breeding and advanced agronomic practices (Duvick, 2005a; Tollenaar and Lee, 2011). Figure 3.1 shows yield increases over time from 1961 to 2010 for different regions of the world, including Africa. Grain yield increase was higher in the US, Europe and Asia. Increases were low in Africa and the yield levels are still low. Maize grain yield in North America is above 8 t ha^{-1}, on average 4.2 t ha^{-1} in South America, around 6 t ha^{-1} in Europe compared to less than 2 t ha^{-1} in SSA (FAOSTAT, 2012).

Genetic gain in maize breeding has been evaluated in some regions of the world. In China gains in yield over 30 years averaged 94 kg ha^{-1} year^{-1} and 53% could be attributed to improved germplasm (Ci *et al.,* 2011). Maize grain yields in the US started to rise in the late 1930s with the introduction of hybrids and improved cultural methods. On-farm yield gains averaged 115 kg ha^{-1} year^{-1} during the years 1934-2004 with genetic improvements contributing 50-60% of the on-farm gains while changes in cultural practices (management) are responsible for the remainder (Duvick, 2005b).

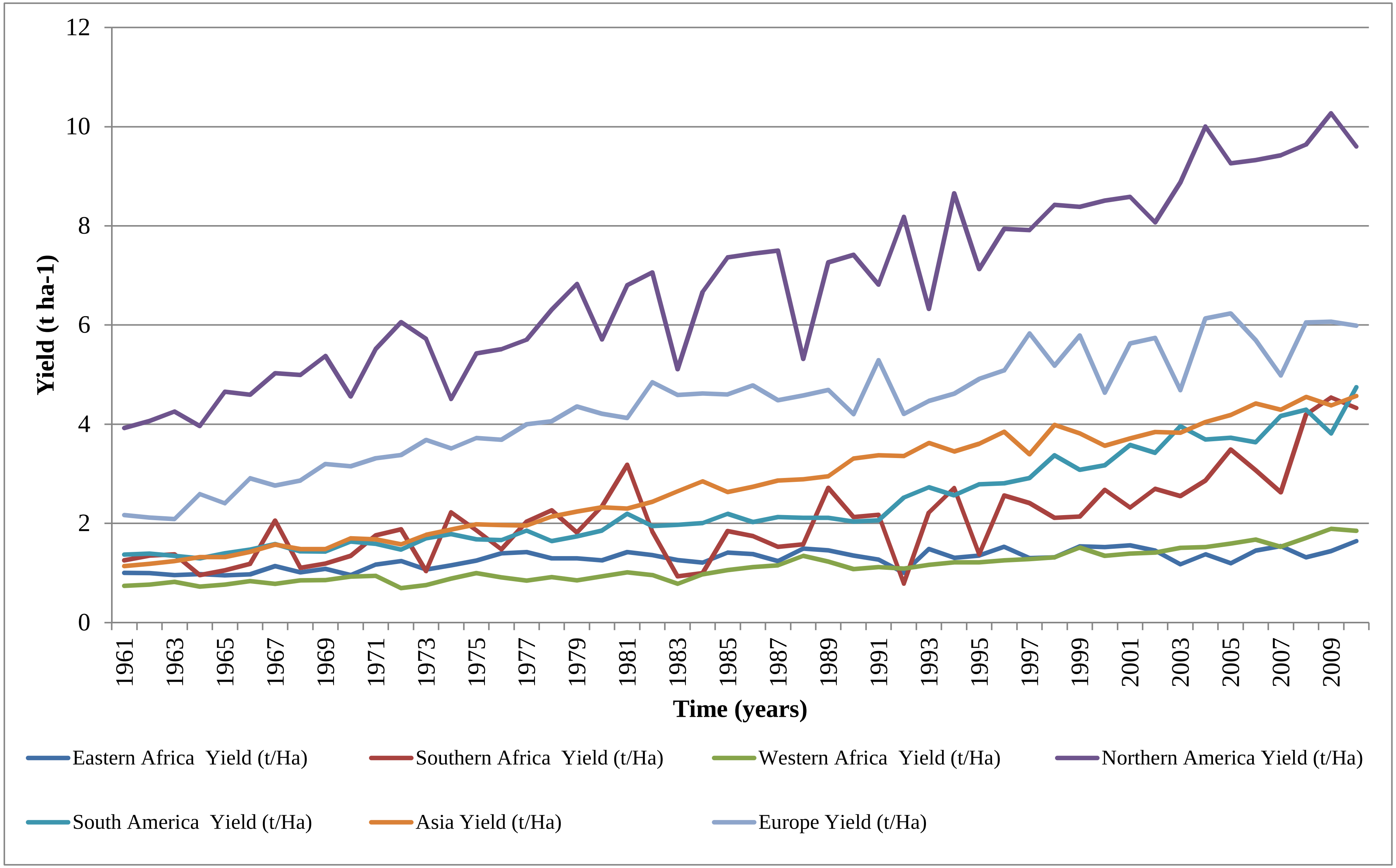


Figure 3.1 Trends in yield (t ha^{-1}) for Africa, America and Europe (FAOSTAT, 2013)

A study by Wang *et al.* (2011) evaluating single cross hybrids in China from 1964 to 2001 showed an across site average genetic gain estimate of 60 kg ha^{-1} year^{-1}. Li *et al.* (2011) compared genetic gain in Chinese hybrids to US hybrids covering the period from 1970 to 2001. The US hybrids showed a gain of 81 kg ha^{-1} year^{-1} that was higher than the 62 kg ha^{-1} year^{-1} recorded for China. In Western Africa Badu-Apraku *et al.* (2013) recorded genetic gain of 40 kg ha^{-1} year^{-1} under optimal conditions and 13.5 kg ha^{-1} year^{-1} under drought stress in early maturing maize hybrids.

Every breeding programme needs evaluation to review progress and based on the findings, to modify, maintain or set new breeding objectives and strategies to improve breeding efficiency (Ci *et al.,* 2011). Studying genetic gains in breeding programmes provides an understanding of unexploited genetic potential. This study evaluated the progress of the CIMMYT breeding programme in ESA.

The aim of this study was to evaluate the genetic gain for yield and other secondary traits in CIMMYT varieties developed and released in regional trials from 2000 to 2010 under optimum conditions throughout the ESA region. The objectives of the study were to

i) evaluate genetic gain in yield in CIMMYT drought tolerant hybrids released from 2000 to 2010
ii) evaluate changes in secondary traits of CIMMYT drought tolerant hybrids released from 2000 to 2010
iii) dissect genetic gain in CIMMYT drought tolerant hybrids released from 2000 to 2010

3.2 Materials and methods

3.2.1 Germplasm

A total of 67 best performing hybrids were selected from hybrids that were developed by the CIMMYT maize breeding programme in East (Kenya) and Southern (Zimbabwe) Africa, selected for disease, drought, low N stress tolerance and high yield under optimal conditions as described in the CIMMYT breeding pipeline (Figure 2.5). More focus was initially on drought and disease stress tolerance with low N screening constituting a small percentage. Hybrids were selected based on superior performance in regional trials within ESA between 2000 and 2010. Information on the pedigree, breeding programme, and first year of entry in the regional trials of all hybrids is presented in Appendix I. Hybrids were grouped based on their first year of entry into regional trials. The number of hybrids per year ranged from two (in 2002 and 2003) to 11 in 2004 (Appendix II). Selection of hybrids evaluated in the study was done independently in East and Southern Africa. The entries in this study were therefore a combination of the best selections form the Eastern and Southern Africa programme selected independently and then combined in the experiment. Considering the East Africa programme started later than the Southern Africa programme, entries from East Africa were mostly from the most recent years. The proportion of later hybrids was therefore higher than that of earlier hybrids. Three popular commercial hybrids SC513, SC635 and SC727 released in 1997, 2003 and 2010, were used as checks.

3.2.2 Trial sites

A total of 14 trials were planted between 2011/12 and 2012/13 seasons throughout ESA (Appendix III), some of which are shown in Figure 3.2. In Zimbabwe, trials were conducted at the CIMMYT maize experimental station in Harare (-17^{o}50'S, +31^{o}2'E, 1483 masl), the Agricultural Research Trust Farm (ART) in Harare, (-17^{o}42'9.1", +31^{o}3'48.4", 1556 masl) and in Chiredzi (21° 0' 58"S, 31° 34' 17E"); in Zambia at Golden Valley Agricultural Research Trust (GART) (-14°10'6.72"S, +28° 22' 12.48"E, 1170 masl) and Nanga (Irrigation Research Centre) in Mazabuka (-15° 49' 21.35"S, +27° 46' 35.48"E) and in Malawi at Chitedze Research Station in Lilongwe (-13° 58' 51", +33° 38' 13") at 3742 masl. In Harare three sites Harare2012a to c were planted during the 2011/12 season and one site Harare 2013 was palanted in the 2012/13 season. Harare, Chiredzi, ART and Nanga have red clayey loam soils with high water holding capacity. Chitedze has sandy clay loam soils.

3.2.3 Trial layout

Experiments were planted in an alpha-lattice design, replicated three times at ART Farm, GART, Nanga, Chitedze and one site at the CIMMYT station in Harare and replicated twice in three sites planted during the first season at the CIMMYT station in Harare. Experiments were planted in two row plots with a final plant density of 53 000 plants ha^{-1} at CIMMYT station in Harare, Chitedze, Nanga and GART. At CIMMYT Harare, Chitedze and Nanga the crop was spaced at 0.75 m between the rows and 0.25 m in the row with one plant per station (53 000 plants ha^{-1}), at 0.75 m between the rows and 0.5 m within the row with two plants per station in GART (53 000 plants ha^{-1}) and at 0.9 m x 0.5 m within the row with two plants per station at ART Farm (44 000 plants ha^{-1}). Plant population ha^{-1} varied at some sites managed by collaborators as they used their management practices in terms of plant spacing.

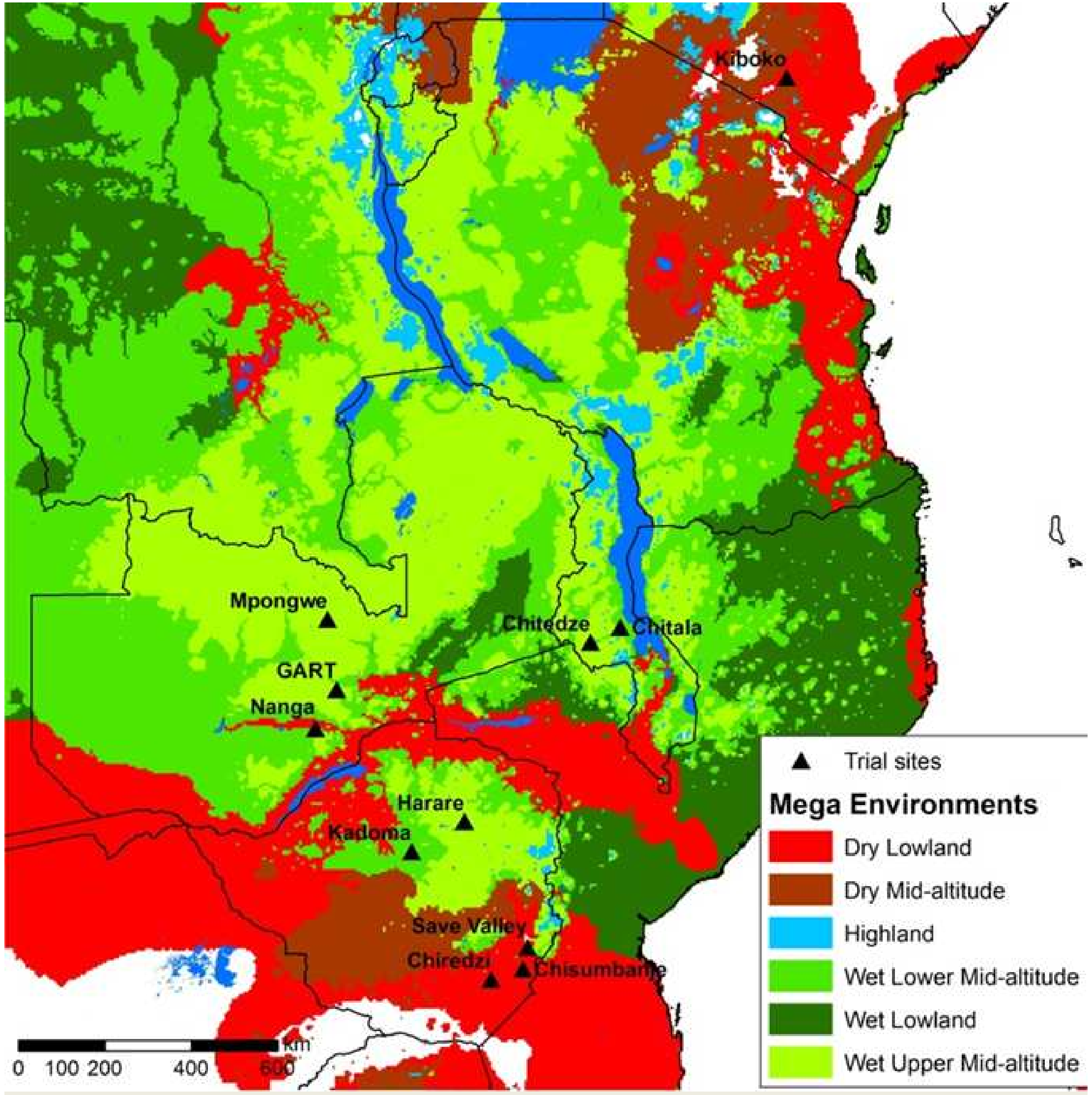


Figure 3.2 Location of trial sites in ESA for the 2011/12 and 2012/13 season

3.2.4 Trial management

Trials at the CIMMYT station in Harare were planted on the 17th of December 2011 and harvested on the 12th of June 2012. All plots in Harare received 28 kg N ha^{-1}, 56 kg P ha^{-1} and 28 kg K ha^{-1} at sowing as Compound D (7 N 14 P_2O_5 7 K_2O 6.5 S) at planting. A second application of N (69 kg N ha^{-1}) was applied approximately 5 weeks after sowing (V6 stage, (Iowa State University, 2009)) and a final application of N (69 kg N ha^{-1}) at flowering. At ART farm the field was limed to a pH of 5.5. The trial was planted on the 4th of November 2011 and harvested on the 30th of April 2012. All plots received 35 kg N ha^{-1}, 70 kg P ha^{-1} and 35 kg K ha^{-1} as Compound D (7 N 14 P_2O_5 7 K_2O 6.5 S) at planting. The crop was side dressed with 69 kg N ha^{-1} as Ammonium Nitrate at four leaf stage and 69 kg N ha^{-1} again at eight leaf stage. In Zambia at GART farm the experiment was planted on the 23rd of December 2011 and harvested on the 17th of July 2011. At Nanga in Zambia the experiment was planted on the 1st of December and harvested 23rd of May 2011. The crop was dressed with 20 kg N ha^{-1}, 40 kg P ha^{-1} and 20 kg K ha^{-1} as a 10 N 20 P_2O_5 10 K_2O compound fertiliser at planting and top dressed with 92 kg N ha^{-1}. Trials in Chitedze were planted on the 1st of January 2012 and harvested on the 19th of June 2012. Basal dressing was applied at emergence at a rate of 46 kg N ha^{-1}, 42 kg P ha^{-1} and 8 kg S ha^{-1} as a compound fertiliser (23 N 21 P_2O_5 0 K_2O 4 S) . The crop was top dressed with 74 kg N ha^{-1} as Urea 46% N at three weeks after emergence.

At all sites recommended plant diseases, weed, and insect control measures were used. In Harare trials received a total of 50 mm per irrigation cycle applied at planting and emergence. Trials were rainfed for the rest of the season. Trials in Chitedze and GART were rainfed. At ART farm trials were irrigated at planting, a week after planting for emergence and at flowering with 50 mm per irrigation cycle.

3.2.5 Data recorded

The listed traits were recorded as described in Appendix IV. Days to mid (50%) anthesis and to mid (50%) silking were recorded (Table 3.2). There was no artificial inoculation of diseases but where diseases occurred at a significant level, maize streak virus (MSV), grey leaf spot (GLS), northern leaf blight (ET) and common rust were scored on a scale of 1 to 5. Ear leaf chlorophyl content measured using the SPAD meter (SPAD) and senescence (SEN) were recorded at 2, 4 and 6 weeks after mid silking. Plant height from the base of the plant to the first branch of the tassel, ear height, plant stand (NP), stem and root lodging (SL and RL) and husk cover (HC) as the number of ears per plot with open tips then converted to a percentage of the total number of ears per plot were recorded within 2 to 3 weeks before harvesting. At harvesting the total number of ears harvested from each plot (NE), ear rots (ER) incidence from each plot as the estimated proportion of rotten ears per plot , ear aspect (EA) on a 1-5 scale with 1 as the best, ear texture (TEX) on a 1-5 scale (Appendix V), field weight (FW) as the total weight of unshelled ears for each plot, grain weight (GW) for each plot and grain moisture (MOI) were recorded at harvesting. Traits recorded are listed in Table 3.2.

Table 3.2 Traits recorded under optimal condition

Secondary traits (phenology)	**Secondary traits (stress tolerance)**	**Direct yield assessment**	**Secondary traits recorded at harvesting**
Days to mid-anthesis Days to mid-silking Plant height Ear height Tassel size Lodging	Chlorophyl content (SPAD) Disease scores Number of ears per plant / barrenness Grain yield/ grain moisture content ratio	Field weight and / or Grain weight Grain moisture	Plant stand Ear aspect Grain texture Ear rots Husk cover Number of ears per plant

3.2.6 Statistical analysis

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). Grain yield was standardised by adjusting the yield of all entries to a standard 12.5 % moisture content before analysing for variance. Further analysis for correlations and heritability was done using META-SAS codes in CIMMYT's DEMO-META analytical software. Regression analysis for genetic gain was processed in SAS 9.3 (SAS, 2012). using the PROC mixed model. Heritability (h^2) was used to test variability of hybrid consistency of the experiment acrsoss sites. Heritability was calculated using the formulae

$$MSE_{(t)} / [MSE_{(t)} + (MSE_{(res)} / r)]$$

Where:

$MSE_{(t)}$ is the mean square error of the treatment

$MSE_{(res)}$ is the residual mean square error

r is the number of replications in the experiment

The following models were used in SAS 9.3. for the evaluation of

i) analysis of variance

y = loc rep(loc) block(rep*loc) release loc*release loc*entry(release) and

ii) genetic gain

y = loc release loc*release entry(release) loc*entry(release)

where:

loc is location or site

entry is the treatments equivalent to the different germplasm entries or hybrids

release is the year of first testing in regional trials

block is the incomplete block within replications in each site or location and

rep is the replication within a location.

A regression was plotted to determine the genetic gain for yield and changes that occurred in different traits over the 11 years from 2000 to 2010. This was done in SAS 9.3. using the mixed model. Yield and secondary traits were plotted against time and the regression coefficient was generated.

Genotypic correlations among the sites were calculated using META SAS. META is a suite of SAS programmes for analysing multi-environment breeding trials data (Vargas *et al.*, 2013). In META SAS distance matrices are calculated and these are used to generate the phenotypic and genetic correlations using the PROC Corr and the dendrograms using the PROC Cluster and PROC Tree. Genetic correlations among locations were calculated using the formulae

$$\rho_g = \frac{\overline{\sigma_{g(jj')}}}{\overline{\sigma g_{(j)}\sigma_{g(j')}}}$$

where $\overline{\sigma_{g(jj')}}$ is the arithmetic mean of all pairwise genotypic covariances between environments j and j′, and

$\overline{\sigma_{g(j)}\ \sigma_{g(j')}}$ is the arithmetic average of all pairwise geometric means among the genotypic variance components of the environments (Cooper, 1996) .

Genetic correlations among traits were calculated using the formulae

$$r(x,y) = \sigma^2 xy/\sqrt{(\sigma^2 x.\sigma^2 y)}$$

where r(x,y) is the correlation between trait x and y

σ^2xy is the covariance between x and y

σ^2x is the variance of x

and σ^2y is the variance of y (Singh and Caudhary, 1977).

The analysis was done using DEMO-META (CIMMYT, 2014) that is a META SAS programme. The analysis used grain yield as the main response variable (MRV) with days to mid-anthesis as the covariate (Vargas *et al*., 2013). The generated genotypic correlations were used for cluster analysis. A dendrogram was generated from the genetic correlations data.

3.3 Results

3.3.1 Analysis of variance of hybrid performance under optimal trials

Grain yield standardised to 12.5% moisture content significantly varied among hybrids at $P \leq 0.05$ at all sites except Harare2012c (Table 3.3). Grain yield varied at $P \leq 0.01$ in Chiredzi, Serere and GART and $P \leq 0.001$ at Bulindi2012a, Kakamega, Harare2012a, b and 2013, ART2012 and 2013, Chitedze and Nanga. Average across site yield averages ranged from 4.35 t ha^{-1} in GART to 10.70 t ha^{-1} in ART2012. The trial mean was 7.54 t ha^{-1}. The minimum yields ranged from 2.91 t ha^{-1} in Harare2012b to 7.35 t ha^{-1} in ART2012 while the maximum ranged from 5.67 t ha^{-1} in GART to 17.22 t ha^{-1} in ART2012. Yield was highest in ART2012 and lowest in GART.

Nine CIMMYT hybrids, eight released from 2007 to 2010 and one from 2001 ranked in the top 10, yielding from 8.62 t ha^{-1} to 9.14 t ha^{-1} based on average performance across sites (Table 3.3). SC727 ranked sixth with a yield average of 8.71 t ha^{-1}. Other commercial checks ranked at 56 for SC635 (6.68 t ha^{-1}) and 64 for SC513 (6.09 t ha^{-1}). Hybrids that ranked from 61 to 70 yielded from 5.22 t ha^{-1} to 6.22 t ha^{-1} that was less than the trial mean and the yield of the 10 best yielding hybrids.

Number of days to mid-anthesis significantly varied among hybrids across 12 of the 13 locations at $P \leq 0.001$ (Table 3.4). The number of days to mid-anthesis did not significantly vary in Nanga at $P \leq 0.05$. Average days to mid-anthesis across sites ranged from 60.1 in Serere to 90.1 in Chiredzi. Generally the hybrids matured relatively faster in Serere compared to all sites and latest in Chiredzi. Hybrids did not show significant variation in ASI in Harare2012a, c and 2013, Bulindi2012a and ART2013 ($P \leq 0.05$).

Table 3.3 Single site analysis of variance in grain yield (t ha^{-1}) under optimal conditions in ESA showing top 10 and 10 least yielders

Name	Rank	Across	Bulindi 2012a	Harare 2012a	Kakamega 2012	Chiredzi 2012	Serere 2012	ART Farm 2012	Chitedze 2012	Nanga 2012	GART 2012	Harare 2012b	Harare 2012c	ART Farm 2013	Harare 2013	Release (Years)
CKH08051	1	9.14	7.63	8.86	12.51	5.32	9.28	13.56	14.12	7.12	4.66	9.78	8.67	11.75	11.55	2008
CKH10717	2	8.83	8.56	8.69	12.72	5.35	7.82	12.04	12.49	7.95	5.20	9.68	9.00	10.38	10.98	2010
CKH10723	3	8.79	9.25	11.16	12.01	5.73	8.26	13.35	12.67	5.92	4.38	7.49	7.71	10.75	11.12	2010
CKH0749	4	8.76	8.69	8.62	12.30	5.31	8.65	13.11	13.06	7.09	4.26	8.14	8.08	10.59	12.06	2007
CKH08032	5	8.72	7.71	9.44	12.40	5.03	10.50	12.74	8.49	8.08	5.29	8.82	7.74	10.07	11.64	2009
SC727	6	8.71	11.80	5.80	13.53	5.10	10.95	8.37	12.68	8.30	3.50	8.64	10.15	10.45	12.28	2010
CZH0713	7	8.70	8.71	7.61	10.87	5.55	9.06	12.43	13.88	6.11	5.67	8.24	8.14	11.49	10.30	2008
CKH08049	8	8.69	10.16	8.33	13.32	5.37	9.40	13.00	12.06	7.62	4.41	6.78	7.73	9.77	10.27	2009
CKH0790	9	8.62	8.95	8.45	10.46	4.65	8.99	11.68	13.15	8.48	4.36	8.25	6.73	11.29	11.22	2007
CZH00027	10	8.62	9.88	8.62	11.00	5.24	8.23	11.92	12.26	8.69	4.40	7.92	6.65	11.44	10.44	2001
CZH0735	61	6.22	6.82	7.08	6.12	4.90	7.76	8.67	6.81	6.89	3.66	5.38	4.96	7.50	7.04	2008
CZH03005	62	6.18	4.98	7.28	7.94	4.20	6.41	9.39	7.01	5.65	4.00	4.94	5.50	7.84	8.24	2004
CZH056	63	6.14	7.10	6.70	5.98	4.16	7.26	9.06	6.99	4.75	3.44	5.24	5.63	9.37	7.73	2006
SC513	64	6.09	5.55	4.32	8.10	4.64	6.73	11.42	8.44	6.95	3.33	3.55	5.76	7.41	7.01	1997
CZH04002	65	6.00	7.16	5.77	4.43	4.27	8.46	8.08	8.40	5.93	3.43	3.98	4.95	8.00	7.71	2005
CZH0524	66	5.95	7.17	5.16	7.50	4.05	5.59	7.52	7.82	5.78	3.62	4.76	6.58	6.30	8.97	2006
KDH3	67	5.90	6.51	5.41	5.79	4.08	9.23	9.32	7.36	5.93	4.02	3.10	4.68	6.98	6.39	2006
KDH4	68	5.85	5.71	7.73	6.01	3.85	6.18	8.67	7.44	5.58	4.09	3.91	4.32	7.98	5.71	2007
CZH095	69	5.49	5.56	3.68	5.74	3.37	6.91	8.93	5.57	4.05	3.78	2.91	3.59	9.06	9.07	2010
CZH00002	70	5.24	6.15	5.37	5.85	3.79	5.99	7.35	4.95	5.09	4.46	4.35	6.72	7.16	5.02	2001
Mean		**7.54**	**7.76**	**7.45**	**9.60**	**4.98**	**8.12**	**10.70**	**10.08**	**6.76**	**4.35**	**6.85**	**7.07**	**9.46**	**9.56**	
LSD (0.05)		**0.47**	**2.19**	**2.54**	**3.20**	**1.11**	**2.54**	**2.13**	**2.89**	**1.67**	**1.28**	**2.52**		**1.69**	**1.54**	
MSe		**1.10**	**0.00**	**1.85**	**1.10**	**0.16**	**1.66**	**1.81**	**3.48**	**0.56**	**0.00**	**1.89**	**1.31**	**1.23**	**0.60**	
h^2		**0.954**	**0.65**	**0.47**	**0.74**	**0.33**	**0.45**	**0.67**	**0.76**	**0.66**	**0.39**	**0.60**	**0.59**	**0.35**	**0.80**	
P			***	***	***	**	**	***	***	***	**	***	ns	***	***	
Minimum		**5.24**	**4.66**	**3.68**	**3.54**	**3.37**	**5.59**	**7.35**	**4.95**	**4.05**	**3.13**	**2.91**	**3.59**	**6.30**	**5.02**	
Maximum		**9.14**	**11.80**	**11.16**	**13.53**	**6.06**	**11.70**	**17.22**	**16.91**	**9.08**	**5.67**	**9.78**	**10.15**	**11.75**	**12.72**	

h^2 is heritability, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$, LSD (0.05) Leas significant diference

There were significant variations recorded in Serere ($P \leq 0.05$), in Chitedze ($P \leq 0.01$) and in Harare2012b, ART2012, GART, Nanga, Kakamega and Chiredzi ($P \leq 0.001$). Average ASI across sites ranged from 0.0 d to 1.8 d with a trial mean of 1.3 d. Minimum ASI ranged from -6.1 d in Harare2012b to -0.1 d in Bulindi2012a while the maximum ranged from 2.0 d at ART2013 to 6.4 d in Chiredzi.

Barrenness measured as the number of ears per plant did not vary in Harare2012 a, b and c, GART, Bulindi2012a, Chiredzi and Serere. Variations were observed ($P \leq 0.05$) in Harare2013, at $P \leq 0.01$ in Chitedze and at $P \leq 0.001$ in ART2012 and 2013 and Kakamega. The number of ears per plant on average ranged from 0.77 to 1.10 across all sites with a trial mean of 0.93. The minimum number of ears per plant recorded across the 13 sites ranged from 0.53 in Bulindi2012a to 0.90 in Kakamega while the maximum ranged from 0.87 ears plant^{-1} in Chiredzi to 2.08 ears plant^{-1} in Bulindi2012a.

Plant height was not recorded in Nanga and Chitedze. Height varied significantly ($P \leq 0.001$) for all sites except Harare2012b as shown in Table 3.5. Average plant height ranged from 200.6 cm in Chiredzi to 286.9 cm in ART2012. The trial mean was 209.1 cm. Minimum plant height ranged from 165.1 cm in Chiredzi to 242.7 cm in ART2012 while the maximum ranged from 233.9 cm in Serere to 307.2 cm in ART2013. Plants were generally tallest in ART2012 and 2013 and shortest in Chiredzi.

For the 10 top yielding hybrids across locations, across site average days to mid-anthesis ranged from 71.7 d to 75.2 d for the CIMMYT hybrids and 75.9 d for the Check SC727 (Table 3.6). Even though the hybrids matured later than the least yielding 10 hybrids, they still matured within the range of cultivated commercial varieties. This was significantly later than hybrids ranked from 61 to 70 that ranged from 64.9 d to 73.6 d. The other checks reached mid-anthesis at 72.4 d for SC635 and 70.8 d for SC513. The top 10 yielders were significantly taller at 213.0 cm to 227.0 cm than the 10 lowest yielding hybrids that ranged from 190.7 cm to 209.2 cm. There were no significant variations between the 10 best and 10 poorest yielders for ASI, root and stem lodging, number of ears per plant, husk cover, ear rots, diseases and ear aspect (Table 3.6).

Table 3.4 Analysis of variance for flowering and barrenness under optimal conditions

Trait	Site	Trial mean	LSD (0.05)	MSe	h^2	P	Minimum	Maximum	Across site mean
Days to 50 % Anthesis (d)	Harare2012a	74.3	4.6	5.5	0.80	***	60.5	79.4	71.5
	Harare2012b	72.1	3.8	3.8	0.86	***	64.2	76.0	
	Harare2012c	75.2	3.4	3.0	0.90	***	67.9	80.3	
	ART2012	70.4	2.2	2.0	0.95	***	62.0	76.1	
	Chitedze	70.5	2.0	2.7	0.90	***	64.8	77.9	
	GART	70.7	3.0	0.6	0.79	***	66.2	75.6	
	Nanga	85.2	.	.	0.95	ns	77.6	88.9	
	Bulindi2012a	62.7	4.1	0.0	0.89	***	58.7	68.2	
	Kakamega	76.1	2.5	178.9	0.94	***	66.7	82.7	
	Chiredzi	90.1	3.0	8	0.92	***	80.3	95.9	
	Serere	60.1	2.4	444.7	0.86	***	53.7	65.3	
	ART2013	73.0	2.3	2.0	0.91	***	65.9	79.4	
	Harare2013	78.4	2.2	1.3	0.92	***	72.5	83.9	
Anthesis-silking interval (d)	Harare2012a	1.6	3.9	3.7	0.30	ns	-3.1	6.0	1.3
	Harare2012b	0.7	2.8	2.1	0.56	***	-6.1	4.3	
	Harare2012c	0.3	.	3.9	0.00	ns	-3.1	4.5	
	ART2012	0.0	2.1	1.7	0.52	***	-2.6	3.1	
	Chitedze	0.7	1.5	0.9	0.45	**	-1.3	3.4	
	GART	1.8	1.5	0.0	0.51	***	-0.6	3.2	
	Nanga	0.5	1.6	0.1	0.78	***	-2.4	5.6	
	Bulindi2012a	1.8	2.9	0.0	0.10	ns	-0.1	5.0	
	Kakamega	0.2	1.8	0.0	0.69	***	-2.3	3.0	
	Chiredzi	2.2	2.9	0.0	0.58	***	-1.4	6.4	
	Serere	1.3	2.4	0.0	0.33	*	-1.2	4.4	
	ART2013	0.1	1.8	1.2	0.20	ns	-1.4	2.0	
	Harare2013	0.1	1.5	0.6	0.10	ns	-1.0	2.1	
Ears per plant (number)	Harare2012a	1.03	.	0.02	0.58	ns	0.58	1.89	0.9
	Harare2012b	1.02	0.42	0.05	0.35	ns	0.74	1.69	
	Harare2012c	1.05	0.31	0.03	0.17	ns	0.82	1.39	
	ART2012	0.97	0.16	0.01	0.77	***	0.74	1.34	
	Chitedze	1.03	0.17	0.01	0.40	**	0.85	1.28	
	GART	0.96	0.43	0.00	0.00	ns	0.60	1.40	
	Nanga	.	.	.	.	.	.	.	
	Bulindi2012a	0.98	0.50	0.00	0.07	ns	0.53	2.08	
	Kakamega	1.10	0.26	0.00	0.51	***	0.90	1.58	
	Chiredzi	0.77	0.13	0.00	0.21	ns	0.65	0.87	
	Serere	1.03	0.25	0.00	0.00	ns	0.82	1.28	
	ART2013	1.01	0.50	0.01	0.71	***	0.83	1.27	
	Harare2013	0.99	0.20	0.01	0.27	*	0.83	1.27	

h^2 is heritability, MSe is Mean square error, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$, LSD (0.05) Least significant difference

Table 3.5 Analysis of variance for plant height under optimal conditions

Trait	Site	Trial mean	LSD (0.05)	MSe	h^2	P	Minimum	Maximum	Across site mean
Plant height (cm)	Harare2012a	212.2	27.9	257.7	0.54	***	177.4	249.8	209.1
	Harare2012b	201.0	31.1	255.3	0.65	*	171.5	234.3	
	Harare2012c	203.3	27.9	196.1	0.63	***	173.2	243.9	
	ART2012	286.9	21.4	194.2	0.79	***	242.7	323.9	
	Chitedze	.	.	.	.	.	.	.	
	GART	207.8	25.9	18.8	0.45	***	179.9	244.5	
	Nanga	.	.	.	.	.	.	.	
	Bulindi2012a	222.4	19.0	0.0	0.57	***	191.1	248.4	
	Kakamega	254.1	30.2	12.1	0.76	***	221.9	288..0	
	Chiredzi	200.6	28.6	258.6	0.48	***	165.1	243.2	
	Serere	204.7	18.2	126.5	0.48	***	167.4	233.9	
	ART2013	264.2	26.0	257.6	0.70	***	227.0	307.2	
	Harare2013	246.4	24.8	155.3	0.87	***	209.0	297.0	

h^2 is heritability, MSe is Mean square error, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$, LSD (0.05) Least significant difference

Table 3.6 Across site analysis of variance in secondary traits under optimal conditions showing top 10 and least 10 yielding hybrids

Name	**Rank**	**Mid-anthesis**	**ASI**	**Plant height**	**Root Lodging**	**Stem lodging**	**Ears/ plant**	**Husk cover**	**Ear rot**	**GLS**	**Rust**	**ET**	**Leaf senescence**	**Grain texture**	**Grain moisture**	**MSV**	**Ear aspect**	**Release**
		d	d	cm	%	%	#	%	%	1-5	1-5	1-5	1-10	1-5	%	1-5	1-5	Year
CKH08051	1	74.5	1.4	219.9	7.2	6.9	1.06	5.8	5.8	1.7	1.6	2.0	19.2	3.5	18.2	2.1	2.7	2005
CKH10717	2	74.6	1.0	219.4	10.3	6.3	0.94	4.9	5.3	1.7	1.6	2.2	18.6	3.4	19.3	2.1	2.6	2010
CKH10723	3	73.8	1.0	213.4	14.4	5.6	0.98	5.2	5.6	2.1	1.6	2.3	21.3	3.5	17.3	2.0	2.8	2010
CKH0749	4	75.0	1.6	221.2	13.5	7.3	0.92	4.1	4.8	2.1	1.7	2.3	19.2	3.6	19.9	1.8	2.6	2007
CKH08032	5	71.7	1.1	208.2	4.1	2.5	1.01	3.9	9.5	1.6	1.7	2.5	19.8	3.8	17.5	1.8	2.7	2009
SC727	6	75.9	1.0	230.6	6.6	5.5	0.86	6.0	5.7	1.8	1.9	2.5	19.3	4.3	18.4	2.0	2.5	2010
CZH0713	7	73.2	0.8	213.0	6.2	5.5	1.08	4.3	6.0	2.1	1.9	2.1	20.8	3.4	17.4	2.1	2.7	2008
CKH08049	8	73.9	0.8	216.5	6.2	6.2	0.92	4.4	5.0	1.7	1.6	1.8	18.6	3.3	16.7	2.3	2.6	2009
CKH0790	9	75.2	1.7	218.1	11.0	9.7	0.90	4.9	4.5	2.0	1.7	2.1	18.6	3.6	18.7	1.8	2.5	2007
CZH00027	10	74.0	1.0	227.0	7.9	7.0	0.99	6.8	5.7	1.8	1.9	2.3	19.5	3.2	17.8	1.9	2.6	2001
SC635	56	72.4	2.2	209.1	8.8	6.3	0.89	7.9	8.5	1.8	2.0	2.4	20.6	3.9	16.3	1.8	3.0	2003
CZH0735	61	67.0	1.5	190.7	9.1	5.6	0.90	4.4	5.9	2.4	1.8	2.5	23.8	3.0	15.8	1.8	3.1	2008
CZH03005	62	68.9	1.4	206.2	4.4	4.4	0.93	5.2	5.4	1.9	1.8	2.7	23.2	3.4	16.4	2.0	2.9	2004
CZH056	63	73.6	0.7	209.2	7.6	3.7	0.99	4.0	4.6	1.9	1.5	2.0	16.8	3.6	18.4	2.0	2.6	2006
SC513	64	70.8	1.8	204.3	15.3	4.7	0.79	6.4	12.3	1.7	2.3	2.4	22.4	3.6	16.1	2.4	3.2	1997
CZH04002	65	73.2	1.3	204.8	7.1	2.1	0.92	4.1	5.8	2.1	1.5	2.3	20.5	3.0	18.7	2.0	2.6	2005
CZH0524	66	66.2	1.4	191.8	10.3	7.1	0.89	3.9	5.3	2.0	1.8	2.7	22.1	2.9	15.4	1.9	3.1	2006
KDH3	67	66.0	1.9	195.6	11.5	7.7	0.89	5.1	6.5	2.6	1.9	2.9	22.8	2.9	15.4	2.3	3.1	2006
KDH4	68	64.9	1.8	193.4	9.4	5.4	0.92	6.1	6.6	2.6	2.0	2.8	28.2	2.8	15.0	2.1	3.3	2007
CZH095	69	71.7	0.9	201.1	3.7	4.0	1.02	5.4	4.2	1.9	1.8	2.5	19.3	2.9	16.9	1.6	2.5	2010
CZH00002	70	68.3	2.2	191.8	6.4	3.8	0.86	6.2	9.0	1.7	1.7	2.7	24.0	2.5	16.3	2.7	2.9	2001
Mean		**71.5**	**1.3**	**209.1**	**8.0**	**5.1**	**0.93**	**5.5**	**6.1**	**1.8**	**1.8**	**2.4**	**20.1**	**3.2**	**17.4**	**2.0**	**2.7**	
LSD (0.05)		**2.9**	**0.4**	**4.8**	**3.8**	**3.0**	**0.05**	**1.6**	**1.4**	**0.2**	**0.2**	**0.2**	**1.3**	**0.4**	**0.8**	**0.3**	**0.1**	
MSe		**93.6**	**1.5**	**219.1**	**64.8**	**24.7**	**0.01**	**10.5**	**15.4**	**0.1**	**0.0**	**0.1**	**7.8**	**1.3**	**5.1**	**0.2**	**0.1**	
h^2		**0.98**	**0.80**	**0.94**	**0.01**	**0.69**	**0.51**	**0.51**	**0.53**	**0.73**	**0.09**	**0.67**	**0.70**	**0.89**	**0.86**	**0.00**	**0.88**	
Min		**64.9**	**0.3**	**187.7**	**3.2**	**0.2**	**0.79**	**3.1**	**2.1**	**1.4**	**1.4**	**1.8**	**16.8**	**2.2**	**15.0**	**1.6**	**2.2**	
Max		**75.9**	**2.9**	**230.6**	**24.5**	**13.5**	**1.08**	**9.9**	**12.3**	**2.6**	**2.4**	**3.0**	**28.2**	**4.3**	**19.9**	**2.8**	**3.3**	

h^2 is heritability, MSe is Mean square error, * $P \le 0.05$, ** $P \le 0.01$, *** $P \le 0.001$, ns non significance at $P \le 0.05$, LSD (0.05) Least significant difference

3.3.2 Multi Environment Trial Evaluation (META) analysis

3.3.2.1 Genetic correlations for grain yield among sites

Genetic correlations for the 14 sites ranged from -0.82 to 0.99 (Table 3.7). Most of the sites had high correlations (above 0.6) with other sites. Bulindi2012b had the lowest correlations with all sites ranging from -0.82 to 0.38. Bulindi2012b had the lowest average yield of < 3 t ha^{-1} indicating the trial must have experienced some stresses. Based on the recommendations by Weber *et al*. (2012) that trials yielding < 3 t ha^{-1} under optimal conditions should not be combined with trials yielding > 3 t ha^{-1}, and due to the low genetic correlations with other sites, Bulindi2012b was excluded from further analysis.

3.3.2.2 Complete linkage cluster analysis

From the linkage cluster analysis conducted Bulindi2012b was different from the rest of the sites at a distance of 3.08 (Figure 3.3). GART also differed from the rest of the sites at a distance of 1.54. The rest of the trials formed one major cluster that sub-divided into three sub-clusters. ART2012 and 2013, Harare2013 and Chitedze formed the first sub-cluster at distances less < 1.00. The four sites had high mean yields of 10.7 t ha^{-1}, 9.46 t ha^{-1}, 10.08 t ha^{-1} and 9.56 t ha^{-1} respectively. Chiredzi2012 clustered with Harare2012a, b and c and Kakamega but generally differed from the rest of the sub-cluster. The third sub-cluster was made up of Nanga, Serere and Bulindi2012a. From the complete linkage cluster analysis, Bulindi2012b was an outlier and based on this as well, the site was excluded from further analysis.

Table 3.7 Genetic correlations for grain yield among optimal locations in ESA

Location	Bulindi2012a	Harare2012	Kakamega 2012	Chiredzi2012	Serere2012	Bulindi2012b	ART2012	Chitedze2012	Nanga2012	GATR2012	Harare2012b	Harare2012c	ART2013	Harare2013
Bulindi2012a	-													
Harare2012a	0.86	-												
Kakamega2012	0.88	1.00	-											
Chiredzi2012	1.00	1.00	0.97	-										
Serere2012	0.82	0.40	0.61	1.00	-									
Bulindi2012b	0.10	-0.18	-0.03	-0.03	-0.27	-								
ART2012	0.68	0.77	0.88	0.96	0.60	-0.35	-							
Chitedze2012	0.73	0.90	0.83	0.74	0.48	-0.12	0.72	-						
Nanga2012	0.70	0.85	0.66	0.96	0.67	-0.03	0.57	0.55	-					
GART2012	0.43	0.55	0.61	0.71	0.79	0.38	0.20	0.26	0.92	-				
Harare2012b	0.45	1.00	0.99	1.00	0.61	0.20	0.82	0.79	0.83	0.66	-			
Harare2012c	0.69	0.90	1.00	1.00	0.66	0.06	0.82	0.78	0.66	0.74	1.00	-		
ART2013	0.73	0.87	0.92	1.00	0.97	-0.82	1.00	0.89	0.76	0.29	1.00	0.94	-	
Harare2013	0.38	0.47	0.61	0.90	0.46	-0.39	0.76	0.70	0.46	0.23	0.75	0.69	1.00	

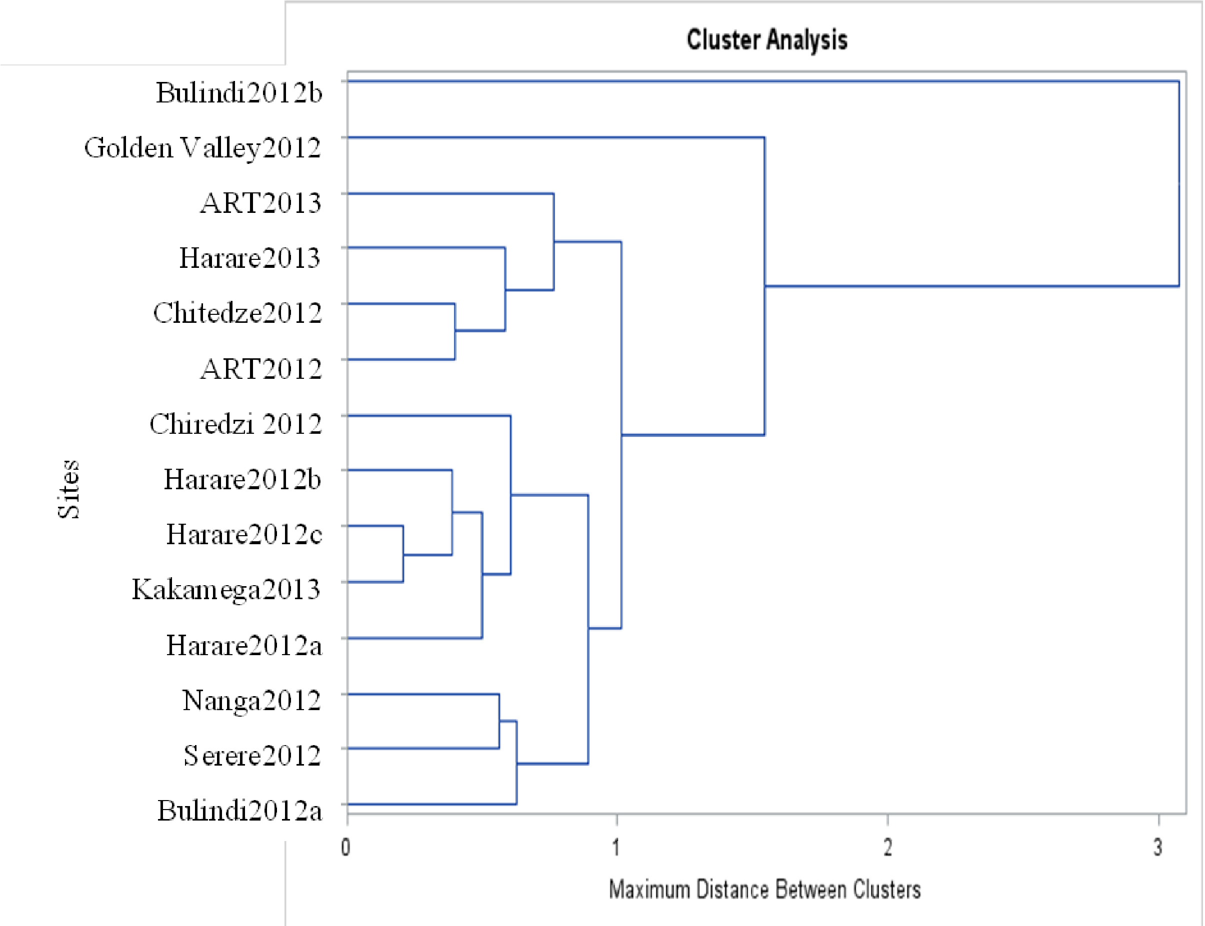


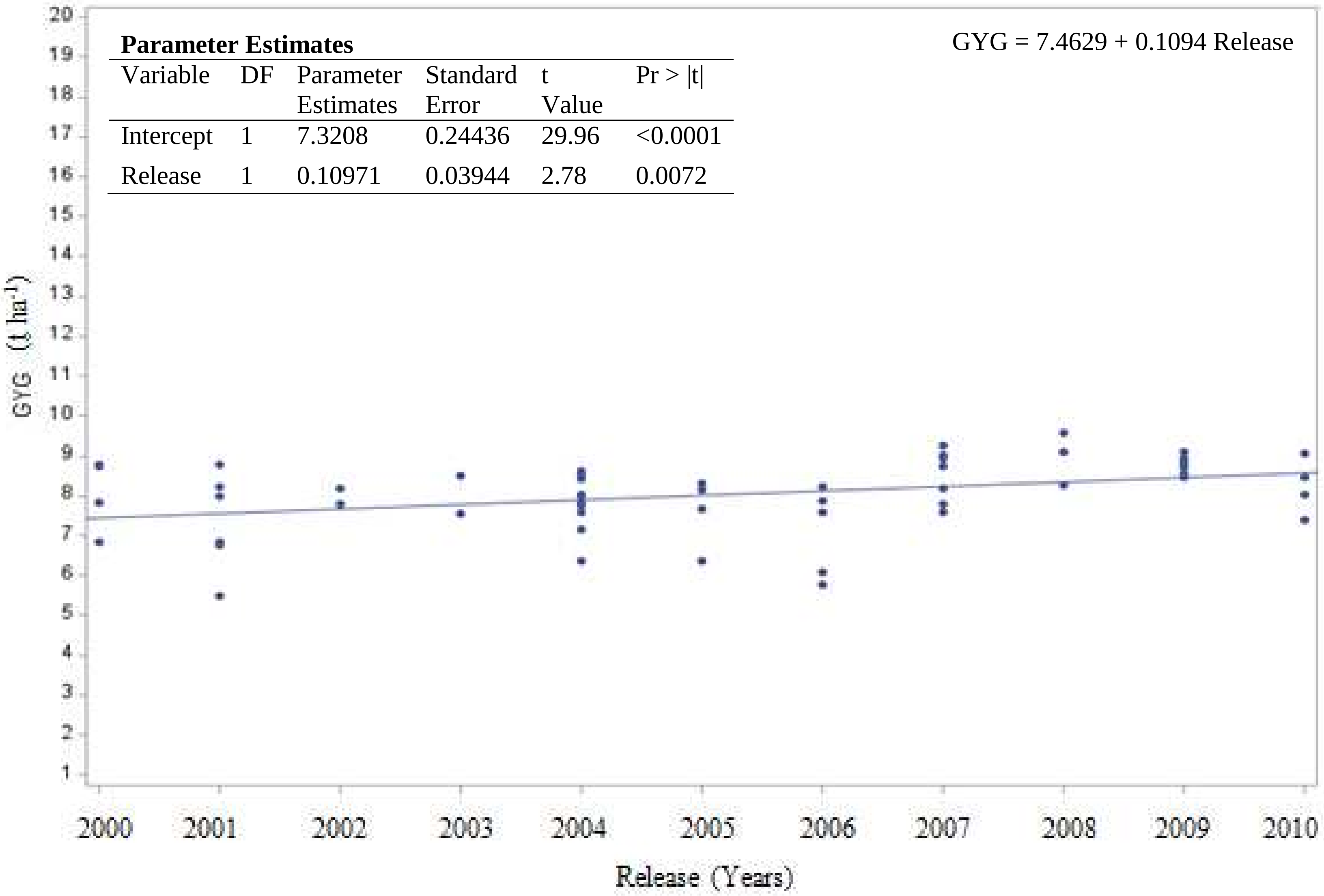
Figure 3.3 Complete linkage cluster analysis of optimal sites based on phenotypic correlations

3.3.3 Evaluation of genetic gain

The study showed significant genetic gain in hybrids developed by the CIMMYT ESA drought tolerance breeding programme over the 11 year period from 2000 to 2010. Grain yield of the CIMMYT hybrids under optimal conditions across 13 sites showed an estimated net genetic gain of 109.4 kg ha^{-1} $year^{-1}$ (1.4% $year^{-1}$) over the 11 years (Figure 3.4) at $P \leq 0.01$. There were changes other than in yield that were observed for different secondary traits over the same period of time.

There was no significant change in number of days to mid-anthesis at $P \leq 0.05$ (Figure 3.5). New hybrids yielded more, utilising the same maturity range as the old hybrids. New hybrids had, on average, shorter ASI compared to the old hybrids. An estimated net decrease rate of 0.08 d $year^{-1}$ ($P \leq 0.001$) was recorded (Figure 3.6). This means that the high yielding new varieties had shorter ASI, giving the new hybrids better synchrony in terms of male and female flowering.

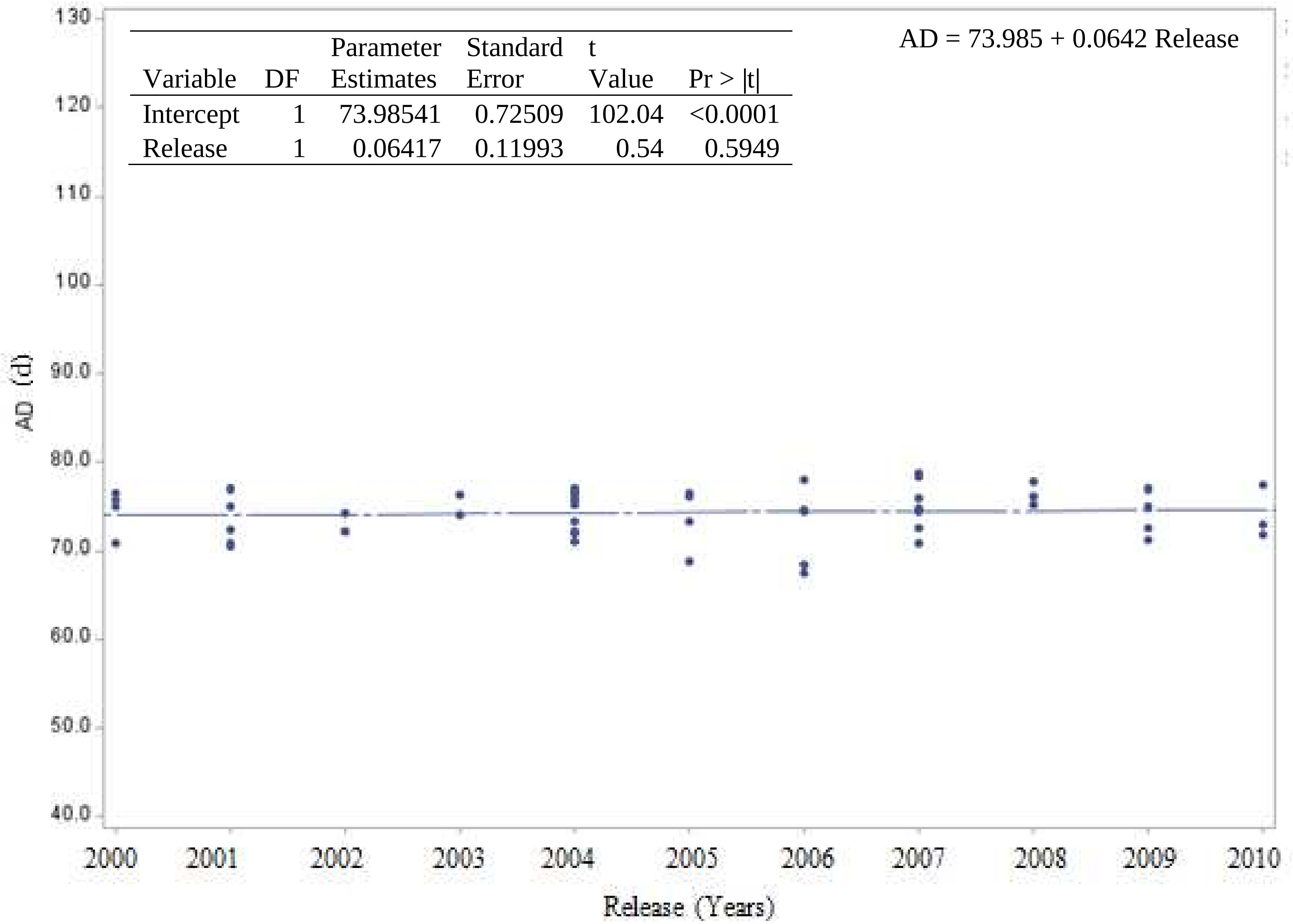
Plant height did not change over the 11 years at $P < 0.05$ (Figure 3.7). The number of ears per plant, an important trait that determines barrenness in a plant, did not show net change in the number of ears per plant ($P \leq 0.05$) as well (Figure 3.8). The grain yield/ grain moisture ratio increased over the 11 years at a rate of 0.0047 $year^{-1}$ at $P < 0.01$.



Parameter Estimates

Variable	DF	Parameter Estimates	Standard Error	t Value	Pr > |t|
Intercept	1	7.3208	0.24436	29.96	<0.0001
Release	1	0.10971	0.03944	2.78	0.0072

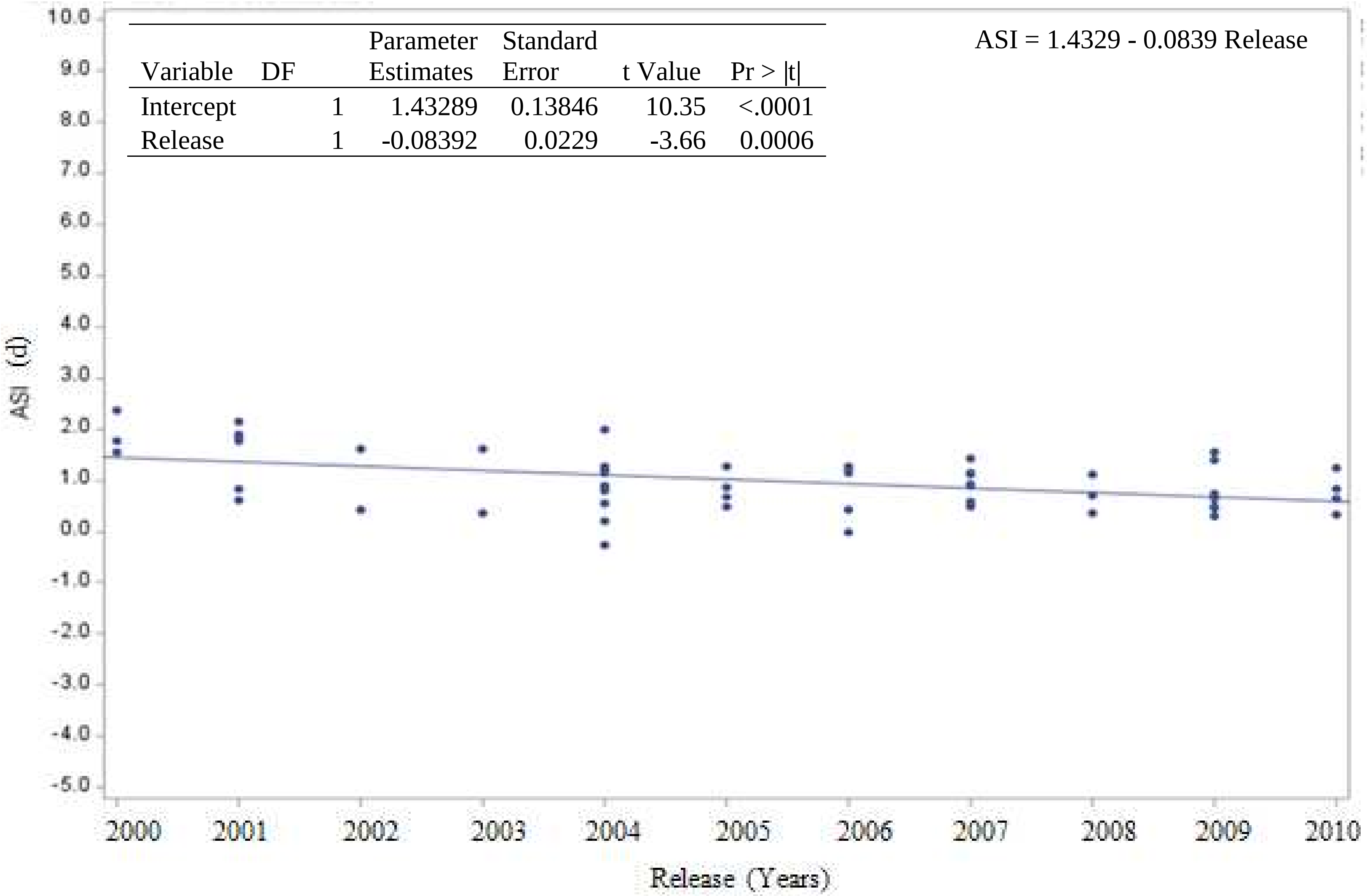
Figure 3.4 Genetic gain in yield in CIMMYT hybrids released from 2000 to 2010 tested across 13 sites under optimal conditions



Variable	DF	Parameter Estimates	Standard Error	t Value	Pr > |t|
Intercept	1	73.98541	0.72509	102.04	<0.0001
Release	1	0.06417	0.11993	0.54	0.5949

AD = 73.985 + 0.0642 Release

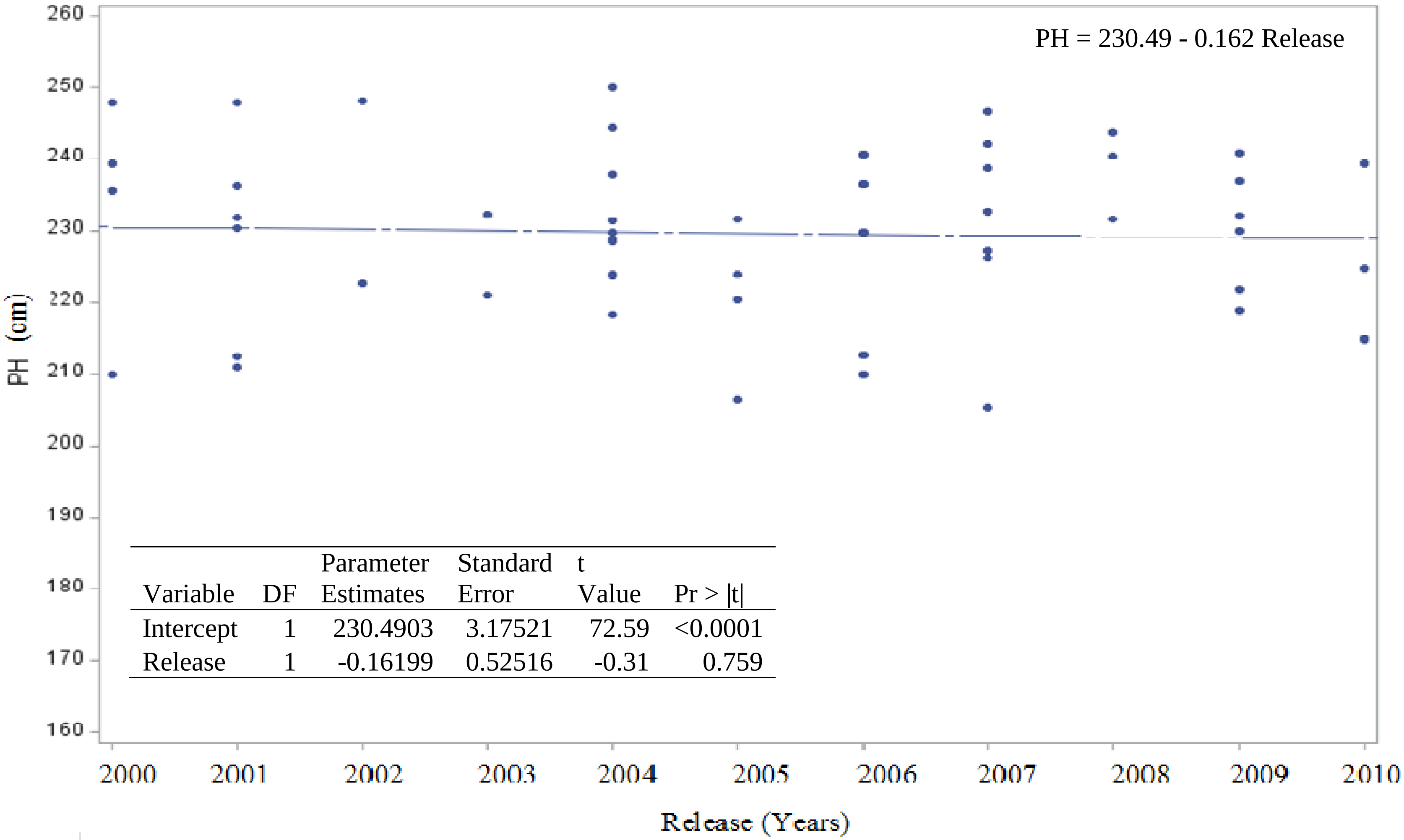
Figure 3.5 Changes in AD in CIMMYT hybrids released from 2000 to 2010 tested across 13 sites under optimal conditions



Variable	DF	Parameter Estimates	Standard Error	t Value	Pr > |t|
Intercept	1	1.43289	0.13846	10.35	<.0001
Release	1	-0.08392	0.0229	-3.66	0.0006

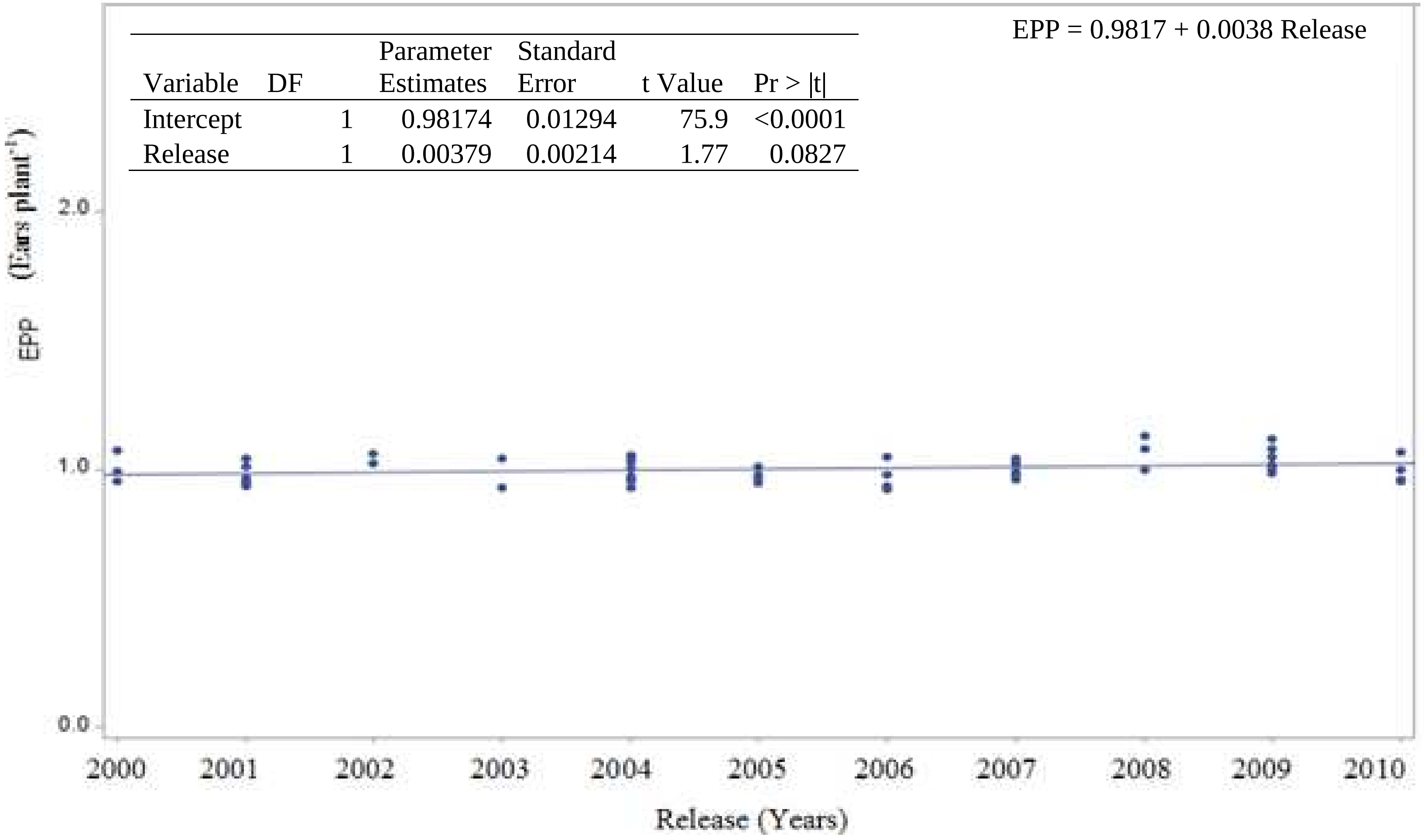
ASI = 1.4329 - 0.0839 Release

Figure 3.6 Changes in plant ASI in CIMMYT hybrids released from 2000 to 2010 tested across 13 sites under optimal conditions



PH = 230.49 - 0.162 Release

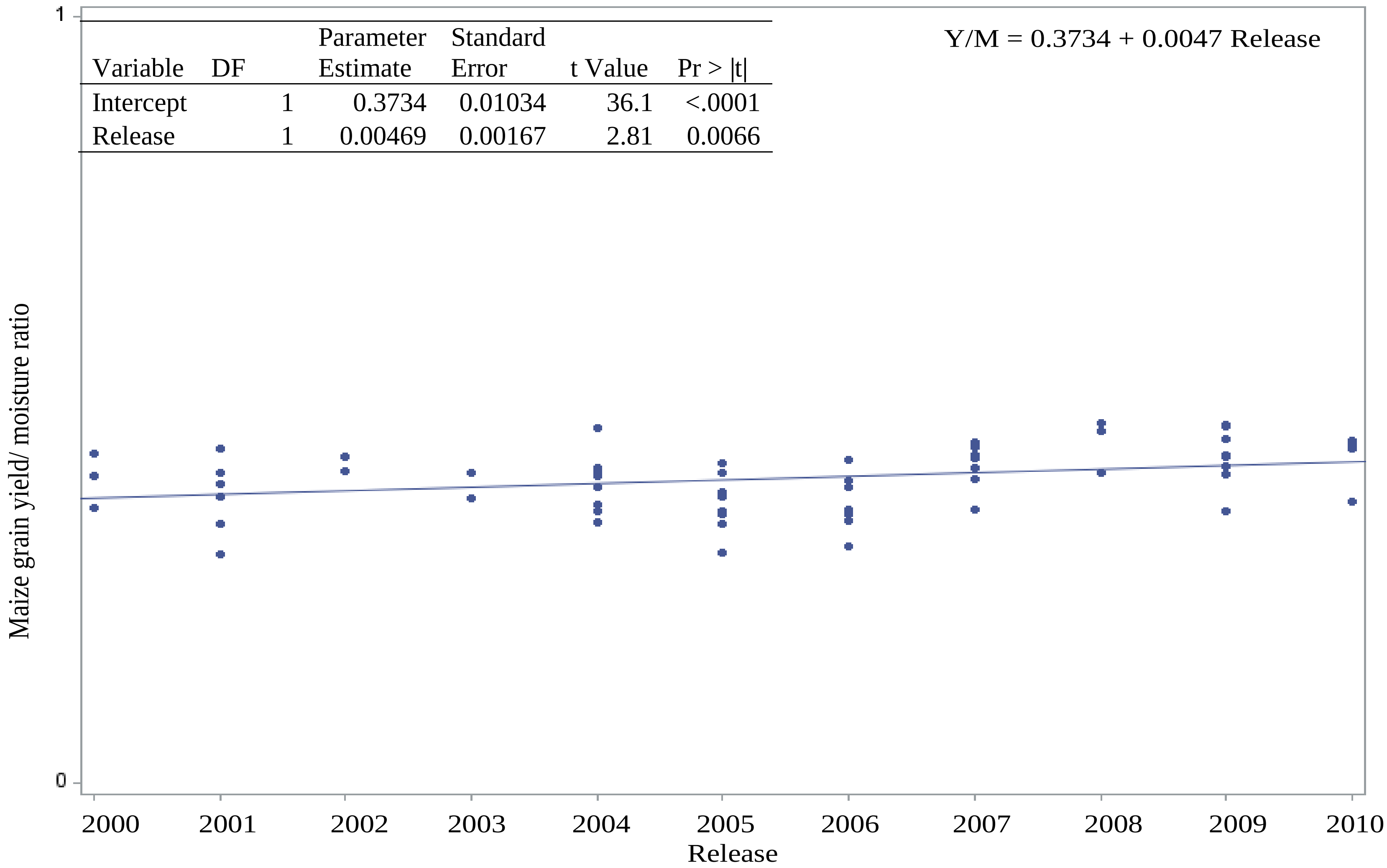
Variable	DF	Parameter Estimates	Standard Error	t Value	Pr > |t|
Intercept	1	230.4903	3.17521	72.59	<0.0001
Release	1	-0.16199	0.52516	-0.31	0.759



Variable	DF	Parameter Estimates	Standard Error	t Value	Pr > |t|
Intercept	1	0.98174	0.01294	75.9	<0.0001
Release	1	0.00379	0.00214	1.77	0.0827

EPP = 0.9817 + 0.0038 Release

Figure 3.8 Changes in number of ears per plant in CIMMYT hybrids released from 2000 to 2010 tested across 13 sites under optimal conditions



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.3734	0.01034	36.1	<.0001
Release	1	0.00469	0.00167	2.81	0.0066

Figure 3.8 Changes in grain yield/ grain moisture ratio CIMMYT hybrids released from 2000 to 2010 under optimal conditions

3.3.4 Dissection of genetic gain in CIMMYT drought tolerant hybrids under optimal conditions

To see the response of yield to changes in secondary traits, genetic correlations of secondary traits and yield were analysed. The correlations of number of days to mid-anthesis with grain yield showed a weak negative correlation of -0.18 (Table 3.8). Yield was lower when flowering was delayed even though in general the late flowering hybrids (late maturing hybrids) yielded better than the early maturing hybrids. Late flowering hybrids utilise a longer season and generally yield better than short season hybrids but in both the long and short season hybrids delayed flowering can result in reduced yield. Grain yield was lower in hybrids that had a wider or high anthesis-silking interval. Anthesis-silking interval and grain yield had a negative correlation of -0.25.

A positive correlation (r = 0.64) was recorded for grain yield and plant height. Taller hybrids yielded better than short hybrids under optimal conditions. Increased prolificacy or increased number of ears per plant resulted in increased yield. Grain yield and the number of ears per plant correlated positively at 0.25. Hybrids with low senescence rate yielded better than hybrids with high rates (r = -0.34). ASI was higher in hybrids that flowered early (r = -0.11). Taller hybrids generally had shorter ASI (r = -0.31). Barrenness increased with increased anthesis-silking interval. Number of ears per plant were lower at high ASI (r = -0.15).

Table 3.8 Genetic correlations of grain yield and secondary traits and across all traits

	GYG	AD	ASI	PH	EPP	SEN	GYMOI
GYG	-						
AD	-0.18***	-					
ASI	-0.25***	-0.11***	-				
PH	0.64***	-0.02^{ns}	-0.31***	-			
EPP	0.25***	-0.21***	-0.15***	0.16***	-		
SEN	-0.34***	-0.30***	0.02^{ns}	0.03^{ns}	-0.06^{ns}	-	
Y/M	0.93***	0.22^{ns}	-0.00^{ns}	0.30*	0.49***	-0.43***	-

* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, ns non significance at P ≤ 0.05, GYG is grain yield, AD is days to mid-anthesis, ASI is anthesis-silking interval, PH is plant height, EPP is number of ears per plant, SEN is leaf senescence, GYMOI is the grain yield/grain moisture content ratio

The number of ears per plant was higher in hybrids that flowered earlier (r = -0.21). Barrenness was lower in taller plants with a correlation of 0.16. Senescence (SEN) did not show any significant relationship with ASI, plant height and the number of ears per plant. Plant height and number of days to male flowering did not show a significant relationship. Grain yield/ grain moisture content ratio showed no significant relationship with days to mid-anthesis and anthesis-silking interval, positively and highly correlated with grain yield, positively correlated with plant height and number of ears per plant but negatively correlated with senescence.

3.4 Discussion

This study showed a positive net estimated genetic gain in yield of 109.4 kg ha^{-1} year^{-1} (1.4% year^{-1}) under optimal conditions. The rate of genetic gain is commendable considering the period of study that was from 2000 to 2010, 11 years, compared to other studies that spanned 20, 30 up to more than 60 years. In West Africa Badu-Apraku *et al*. (2013) reported estimated genetic gain of 40 kg ha^{-1} (1.3%) year^{-1} under optimal conditions for 50 varieties selected from a period of 20 years. In Ontario, Tollenaar (1989) reported a net estimated genetic gain under optimum conditions of 1.9% per year. Ci *et al*. (2011) reported an estimated gain of 94.7 kg ha^{-1} year^{-1} for hybrids in China selected from a 30 year period. Duvick (2004) reported a gain of 196 kg ha^{-1} year^{-1} in F1 hybrids and in 2005 reported a gain of 115 kg ha^{-1} year^{-1} in the US from the 1934 to 2004. This study therefore recorded comparably high estimated genetic gain considering the period studied.

The gain recorded was genetic because all the entries in the trial were planted side by side in each trial site as recommended by Duvick (2004) and Tollenaar and Lee (2006). This ensured that entries in the trial at any given place or time received the same agronomic management under the same environment at the same time such that variation among entries is attributed to genetic differences. In other studies historical data has been used to show the influence of secondary traits on yield and trends in yield improvement (Barrero Farfan *et al*., 2013) but the changes in yield includes improvements in both genetic make-up as well as agronomic practices.

Genetic gain results from improvement in phenology and physiology of the varieties (Duvick, 2005a; Lee and Tollenaar, 2007). From the evaluation of secondary traits in this study, a number of changes were observed. There were no changes in the season length, plant height and the number of ears per plant over the 11 years. Number of days to mid-anthesis did not show significant change over the 11 years in this study but in a separate study by Badu-Apraku *et al.* (2013) an increase in number of days to mid-anthesis of 0.02% year^{-1} was reported under optimal conditions while other studies did not record a particular trend or change for days to mid-anthesis (Duvick, 2005a; 2005b; Tollenaar and Lee, 2011). According to the crop phenology, days to mid-anthesis marks half the life time of a maize variety. The new hybrids still matured in the same season range as old hybrids but yielded better. The correlation of grain yield against days to mid-anthesis was negative at -0.18.

Senescence was relatively slower in the new hybrids compared to the old hybrids and negatively correlated with grain yield (-0.34). The stay green, visual and/or functional, or reduced senescence was reported to increase dry matter accumulation in the new varieties (Duvick, 2005b; Lee and Tollenaar, 2007; Tollenaar and Lee, 2011). This should have contributed towards grain yield improvement in the current study. Stay green allows more photosynthesis and provides an extended grain filling period. Grain moisture was higher in the newer varieties, a possible indication that the grain filling period was extended in new hybrids compared to old hybrids.

ASI showed an average decrease of about 0.8 days in 10 years according to the regression of ASI against time. In some earlier studies ASI did not change or show any trend (Tollenaar and Lee, 2011) while in others it showed a linear trend and was shorter in the new varieties (Duvick, 2005b). ASI had a negative correlation of -0.25 with yield hence lower in newer hybrids compared to old hybrids. Shorter ASI improves pollination and grain set. This will improve grain yield levels. Findings from the current study agree with other studies where ASI tended to be shorter in new hybrids compared to old hybrids (Duvick, 2005a). Through indirect selection when selecting for yield, ASI can be reduced as it is closely linked to yield (Monneveux *et al.*, 2006).

In some studies, plant height was observed to be lower in new hybrids (Duvick, 2005a) and this agrees with the trend observed in this study. Plant height did not significantly change but showed an average decrease of 1.6 cm in 10 years. The decrease in plant height over the 11 years could be a result of improved dry matter partitioning towards ear development (Duvick, 2005a; Lee and Tollenaar, 2007). The correlation of grain yield with plant height was strong and positive (0.64) suggesting that yield was higher in taller plants. These findings agree with findings by Badu-Apraku *et al.* (2013). However, over the 11 year period evaluated in this study no significant change in plant height was recorded.

There was no significant change in the number of ears per plant even though the hybrids showed a trend to increase over the 11 years. In some studies the number of ears per plant increased

(Bruce *et al.*, 2002; Duvick, 2005a; Campos *et al.*, 2006; Monneveux *et al.*, 2006; Lee and Tollenaar, 2007) even though others (Tollenaar and Lee, 2011) showed no change or any tendency. The correlation between grain yield and the number of ears per plant was positive (0.25), with yield increasing with increasing number of ears per plant. No trends could be established for root and stem lodging, leaf angle and tassel size.

Grain yield/ grain moisture content ratio increased over the 11 years and positively correlated with yield at 0.93. An increase in the grain yield/ grain moisture content ratio corresponded to increase in yield as reported in a separate study by Ngure (1995). Grain yield/ grain moisture content ratio can be used as a secondary trait for selecting for improved yield (Ngure, 1995).

Most of the traits that did not show significant change over the 11 years showed a trend and had significant correlations with grain yield. Because the study covered a short period of 11 years, the period might have been limited to effectively show significant changes but the trends shown in the regression graphs and the correlations give an indication of some changes that should have contributed towards improved grain yield.

The improvement in yield or gain in yield was therefore a result of a combination of improvements in the phenology and physiology of hybrids that included visual and/or functional stay green and better synchrony at flowering due to reduced ASI, reduced barrenness or higher number of ears per plant and increased grain yield/ grain moisture content ratio which is in agreement with other studies (Ngure, 1995; Tollenaar and Wu, 1999; Duvick, 2005a; Duvick, 2005b; Lee and Tollenaar, 2007; Tollenaar and Lee, 2011).

3.5 Conclusions

This study recorded a significant estimated genetic gain in yield of 109.4 kg ha^{-1} year^{-1} (1.4% year^{-1}) over the 11 year period under optimal conditions. The gain is higher than the gains recorded in other studies under optimal conditions. This was associated with phenological and physiological changes that altered the structure and development of the hybrids, as well as physiological processes such as grain filling, dry matter partitioning, senescence and the season length. The new varieties produced better under the same conditions and within the same season length as the old varieties and commercial checks. New CIMMYT drought tolerant hybrids yielded better than old hybrids.

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Chapter 4

Genetic gain for tolerance to drought stress in the CIMMYT Eastern and Southern Africa maize breeding programme

Abstract

In East and Southern Africa drought and low nitrogen are the major constraints in food production, including maize. Breeding for drought tolerance in maize in CIMMYT started in the 1970s. Varieties developed by CIMMYT for the region were screened and tested under optimum and stress conditions and in regional trials. Some varieties were disseminated to farmers through stakeholders in the public and private sector. This study investigated the genetic gain in drought tolerance in hybrids developed by CIMMYT and released from 2000 to 2010 through multi-environment trials evaluated from 2011 to 2013. Newer varieties yielded better under drought compared to the older varieties. The study indicated a genetic gain of 22.7 kg ha^{-1} year^{-1} or 0.85% year^{-1} and 32.5 kg ha^{-1} year^{-1} or 0.85% year^{-1} under random and managed drought stress respectively. Days to mid-anthesis showed a trend of increasing under both random and managed drought stress at rates of 0.05 d year^{-1} and 0.10 d year^{-1} respectively. A decrease in ASI was recorded under both random and managed drought stress at rates of 0.08 d year^{-1} and 0.16 d year^{-1} respectively. Successful breeding produced hybrids that performed better under optimal conditions and expressed some levels of drought stress tolerance.

4.1 Introduction

4.1.1 Maize production and challenges in sub-Saharan Africa

Of the land under maize production in the world, 150 million ha is grown under rainfed conditions and is highly susceptible to drought. In sub-Saharan Africa (SSA) maize production is affected by recurrent periodic and severe droughts. Drought and creeping desertification have reduced maize production in the region (Reeves, 1997). In Kenya alone, 1.4 million ha is reported to be affected by drought (Njoroge *et al.*, 1997). Drought is unpredictable (Campos *et al.*, 2006), a major factor in genotype by environment interaction (GXE) (Campos *et al.*, 2004) and is difficult to manage. Drought is the most important challenge on the livelihoods of people in SSA (Mungoma and Mwambula, 1997; Bänziger *et al.*, 2006; Bänziger and Aurus, 2007; Kassie *et al.*, 2012). In some countries in SSA some farmers consider the use of improved varieties very risky in terms of reliability of yields under drought conditions (Kassie *et al*., 2012). Because of the irregular seasonal and spatial distribution and at times no rainfall, the effect of drought on resource poor farmers is severe, especially when compounded by low fertility, poor crop management, biotic stresses and the lack of government programmes to finance farmers (Santos *et al*., 1997, Zambezi and Mwambula, 1997). Drought is reported to cause yield losses of 10 to 50% on 80% of the maize land area in the Southern Africa Development Community (SADC) region (Zambezi and Mwambula, 1997, Short and Edmeades, 1991). The yields in SSA remain low, yet the population is increasing. Despite SSA's high adoption rates of developed maize varieties (55% in 1996) the average yields in Southern Africa, excluding South Africa, is low at 1.1 t ha^{-1}, 1.7 t ha^{-1} in West Africa and 1.5 t ha^{-1} in East Africa (Smale *et al*., 2011).

UNESCO (1999) described drought as complex, unpredictable, difficult to manage and synonymous with Africa, affecting more victims compared to other forms of disasters. Considering that more than 300 million vulnerable people in Africa depend on maize for food, feed and livelihoods (Rovere *et al*., 2010) a drought disaster will affect a significant proportion of this population. Drought occurs frequently in ESA. A study by Rovere *et al*. (2010) showed, based on the probability of failed seasons, that ESA faces 20% to more than 40% of years in which the season is likely to fail due to drought, with most of the area in the more than 40% category. Faced with this scenario and climate change, developing drought tolerant varieties is a possible intervention.

Early maturing varieties can be used with the hope to escape drought as a way of attempting to produce some maize under low rainfall conditions (Mungoma and Mwambula, 1997). However, it is important to breed for drought tolerance (Mungoma and Mwambula, 1997; Bänziger and Aurus, 2007) to provide some varieties that can produce reasonable higher grain yield under drought stress compared to the non-drought tolerant local varieties. Varieties with efficient uptake and/or use of resources such as water, nutrients and canopy radiation can potentially improve yields. In earlier studies by Dwyer *et al*. (1992) and Duvick (1997), newer hybrids were reported to have improved tolerance to soil moisture stress compared to older hybrids. Tollenaar and Lee (2006) reported that yield response varied with the environment and season. Increased grain yield could be a result of the interaction of the genetic potential of a variety with the ability to tolerate limited resource stress and other general stresses. 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). Resource poor farmers who are faced with frequent droughts can benefit from improved varieties that have been selected in breeding programmes for better stress tolerance compared to older hybrids (Tollenaar and Wu, 1999). Such varieties can insure the farmers against drought.

Frequency and severity of drought is predicted to increase due to climate change and deforestation, reducing global food production (Lobell *et al*., 2008; Rovere *et al*., 2010; 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 be handy, especially when faced with the predicted increase in likelihood of a failed season due to drought. Selection for stress tolerance under drought was noted to result in indirect selection for low N stress tolerance (Lafitte and Edmeades, 1995; Zambezi and Mwambula, 1997; Bänziger and Lafitte, 1997) giving the varieties some compound stress tolerance. One of the important goals of breeders has been to enhance stability of performance of maize under stress. Through breeding, significant yield increases have been recorded due to drought tolerance.

One of the main objectives of CIMMYT's global maize programme (GMP) in SSA is breeding for tolerance to drought and low N. Some drought and low N tolerant materials have been developed. Newer hybrids have been reported to have tolerance to soil moisture stress (Dwyer *et al*., 1992, Duvick, 1997) and low soil N (McCullough *et al*., 1994) compared to the older hybrids. The CIMMYT GMP has developed a number of drought tolerant varieties. Success has been recorded in some SSA countries including Malawi (Smale *et al*., 2011; Kassie *et al*., 2012). The drought tolerant varieties have been tested in the regional trials each year and some were released through the national programmes and private sector. 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 and low N tolerance in CIMMYT hybrids released by the ESA maize programmes in regional trials from 2000 to 2011.

4.1.2 Objectives

The major objective was to evaluate the net genetic gain in yield and the changes in secondary traits of CIMMYT hybrids tested by ESA maize programme with respect to drought tolerance. The specific objectives of this study were to:

i) evaluate genetic gain in yield in CIMMYT hybrids under drought stress released from 2000 to 2010

ii) evaluate changes in secondary traits of CIMMYT hybrids under drought stress released 2000 to 2010

iii) dissect genetic gain in CIMMYT hybrids under drought stress released from 2000 to 2010

4.2 Materials and methods

4.2.1 Germplasm

The same 67 hybrids checked with three commercial varieties (Appendix I) described in Chapter 3 section 3.2 were evaluated under low random and -managed drought stress. SC513, SC635 and SC727 were used as checks. None of the checks were purported to be drought tolerant.

4.2.2 Trial sites

The trial was evaluated in 11 sites under random and managed drought stress (Appendix III) between 2012 and 2013. Random stress sites were planted during the normal rainy season in areas that receive total annual rainfall below 800 mm annum^{-1} (in Kadoma), below 450 mm annum^{-1} (Chiredzi) and around 500 mm annum^{-1} in Potchefstroom, that 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. The random drought sites in Kadoma and Potchefstroom were totally rainfed but in Chiredzi irrigation is necessary for crop establishment and maintenance up to two weeks before flowering. A random drought stress site was planted in Kadoma, Zimbabwe on 27 December 2011 and another one in Potchefstroom in December 2011. On 31 December 2011 a random drought trial was planted in Chiredzi (21° 0' 58"S, 31° 34' 17E"). In March 2012, a managed drought site was planted in Kiboko, Kenya (2° 12' 52"N, 37° 43' 26$^{"}$E). Two random drought sites were planted in Kadoma in December 2012 and two in Chiredzi on the 4th of January 2013. In May 2013 two managed drought sites were planted in Chiredzi. Chiredzi Research Station is characterised by red clayey loam soils and Kadoma some red clay soils. The rainfall distribution is shown in Appendix VI.

4.2.3 Trial management

At all sites experiments were planted in an alpha-lattice design with three replications in Chiredzi and Kiboko and two replications in Kadoma and Potchefstroom. Entries were planted in two row plots with a final plant density of 53 000 plants ha^{-1} in Chiredzi and 66 600 plants ha^{-1} in Kiboko. In Kiboko hybrids were planted in two 4 m rows spaced at 0.75 m interrow and 0.20 m in the row with one plant per station or hill. At harvesting, the end plants were discarded to eliminate border effects, leaving a net plot length of 3.5 m in Chiredzi and 3.6 m in Kiboko. In Chiredzi and Kadoma all plots received 28 kg N ha^{-1}, 56 kg P ha^{-1} and 28 kg K ha^{-1} at sowing as Compound D (7 N 14 P_2O_5 7 K_2O 6.5 S). A second application of 120 kg N ha^{-1} was done approximately three weeks after sowing (V6 stage, Ritchie *et al*., 1993) and a final application of 120 kg N ha^{-1} at five to six weeks after planting. In Kiboko all plots received 54 kg N ha^{-1} and 65 kg P ha^{-1} as Diammonium phosphate (DAP) 18:46:0 at planting and 60 kg N ha^{-1} as calcium ammonium nitrate (CAN) as a top dress.

In Chiredzi, trials received 50 mm irrigation at planting and 20 mm seven days later for emergence. At three weeks after planting 50 mm irrigation was applied for thinning and the first top dressing with ammonium nitrate. Ten days after top dressing, 50 mm irrigation was applied. The last 50 mm irrigation was applied at five to six weeks after planting with the second top dressing with ammonium nitrate. In Kadoma and Potchefstroom the trials were totally rainfed from planting until harvesting. In Chiredzi during the rainy season the crop sometimes received rainfall in addition to irrigation but during the second season of the year from May to November usually no rainfall is received and as such, it is possible to control the amount of water the crop receives. Unlike the other random drought stress sites where the experiments were totally rainfed, Chiredzi receives annual rainfall below 400mm per annum that is not sufficient to establish and maintain a crop. The Chiredzi random sites were therefore irrigated at planting and for crop establishment up to five to six weeks after planting.

Managed drought sites were planted in Chiredzi during the dry season, usually from May to July and in Kiboko in March. Since no rainfall is usually received in these areas at this time, the crop was fully irrigated up to two weeks before flowering to ensure that drought stress to coincide with the reproductive stage. Water management in the random drought sites and the managed drought sites therefore differed.

Recommended plant disease, weed and insect control measures were used. While all other conditions were maintained at optimal levels water or soil moisture was controlled.

4.2.4 Data recorded

Traits listed in Table 4.1 were recorded. The listed traits were recorded as described in Appendix IV. Days to mid-anthesis and to mid-silking were recorded. Plant height from the base of the plant to the first branch of the tassel, ear height, plant stand, stem and root lodging and husk cover were recorded within two to three weeks to harvesting. Chlorophyl content measured using the SPAD meter and senescence were recorded at two, four and six weeks after harvesting. At harvesting the number of ears harvested from each plot, ear rot incidence from each plot, ear aspect, ear texture, field weight for each plot, grain weight for each plot and grain moisture were recorded at harvesting.

Table 4.1 Traits recorded under managed and random drought stress

Secondary traits (phenology)	Secondary traits (stress tolerance)	Yield components	Direct yield assessment
Days to mid- anthesis	SPAD	Plant stand	Field weight and/-or grain weight
Days to mid- silking	Senescence / stay green	Number of ears	Grain moisture
Plant height	Number of ears per plant / barrenness	Ear aspect	
Ear height		Grain texture	
Root and stem lodging			

4.2.5 Statistical analysis

Data was processed and analysed as described in Chapter 3 section 3.2.6. Data recorded under random- and managed drought stress was analysed separately since water management varied between the two management environments even though the locations were the same in some instances, as in Chiredzi.

4.3 Results

4.3.1 Analysis of variance of hybrid performance under random drought stress trials

Grain yield standardised to 12.5% varied significantly among hybrids at $P \leq 0.05$ in Kadoma2012 and Chiredzi2013b, and at $P \leq 0.01$ in Kadoma2013a and 2013b and at $P \leq 0.001$ Chiredzi2012 and 2013b but did not vary in Potchefstroom at $P \leq 0.05$ (Table 4.2). Across all seven sites yield average was 2.80 t ha^{-1}. The average for the six sites ranged from 1.83 t ha^{-1} in Kadoma2013b to 3.59 t ha^{-1} in Chiredzi2013b.

Table 4.2 Analysis of variance of yield and secondary traits under random drought stress

Trait	Site	Trial mean	LSD (0.05)	MSe	h^2	p	Minimum	Maximum	Across site mean
Grain yield (t ha-1)	Chiredzi2012	3.03	1.00	0.74	0.32	***	1.65	4.22	2.80
	Kadoma2012	3.08	1.11	0.33	0.34	*	1.81	4.48	
	Potchefstroom2012	3.32	2.21	0.13	0.33	ns	1.01	5.79	
	Kadoma2013a	1.88	0.88	0.23	0.42	**	0.92	2.90	
	Kadoma2013b	1.83	0.74	0.15	0.47	**	1.12	2.67	
	Chiredzi2013a	3.41	1.20	1.01	0.21	***	2.35	4.47	
	Chiredzi2013b	3.59	1.57	3.31	0.27	*	2.20	4.85	
Mid-anthesis (d)	Chiredzi2012	75.07	2.33	2.36	0.85	***	70.82	79.27	65.80
	Kadoma2012	62.75	3.58	4.56	0.68	***	56.76	67.74	
	Potchefstroom2012	73.63	4.70	1.30	0.42	**	68.87	80.08	
	Kadoma2013a	64.87	3.46	3.06	0.7	***	58.65	68.36	
	Kadoma2013b	64.38		3.98	0.75	ns	58.39	70.90	
	Chiredzi2013a	59.02	3.64	5.29	0.69	***	50.88	64.64	
	Chiredzi2013b	59.45	1.13	0.90	0.94	***	53.77	63.23	
Anthesis-silking interval (d)	Chiredzi2012	1.19	4.14	4.27	0.33	*	-7.89	5.13	0.50
	Kadoma2012	0.33	2.27	1.28	0.58	***	-1.56	4.69	
	Potchefstroom2012	0.61	2.75	0.20	0.3	ns	-2.40	5.68	
	Kadoma2013a	3.06	3.70	3.48	0.27	ns	-1.50	5.75	
	Kadoma2013b	0.18	1.44	0.78	.	***	-1.66	2.70	
	Chiredzi2013a	0.44	2.10	1.79	0.78	***	-1.41	4.51	
	Chiredzi2013b	2.42	4.37	4.67	0.61	ns	-0.08	5.84	
Ears per plant (#)	Chiredzi2012	0.67	0.23	0.02	0.53	ns	0.40	0.95	0.78
	Kadoma2012	1.06	0.51	0.07	.	***	0.77	1.71	
	Potchefstroom2012	0.95	0.44	0.00	0.53	ns	0.43	1.62	
	Kadoma2013a	0.87	0.32	0.03	0.26	**	0.51	1.20	
	Kadoma2013b	0.83	0.32	0.03	0.2	*	0.58	1.17	
	Chiredzi2013a	0.71	0.14	0.01	0.35	***	0.49	0.81	
	Chiredzi2013b	0.72	0.18	0.02	0.62	ns	0.43	0.96	

h^2 is heritability, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$, MSe is Mean square error, LSD is least significant difference

The minimum yield in the sites ranged from 0.92 t ha-1 in Potchefstroom to 2.35 t ha-1 in Chiredzi2013a. Kadoma2013b on average yielded the least. Average across site grain yield among the top 10 yielding hybrids ranged from 3.09 t ha-1 to 3.38 t ha^{-1} (Table 4.4). The yield range among the 10 lowest yielding hybrids was lower than the top 10 yielders ranging from 2.08 t ha-1 to 2.54 t ha-1.

The hybrids significantly varied in days to mid-anthesis at $P \leq 0.001$ in Chiredzi2012, 2013a and 2013b and Kadoma2012 and 2013a, at $P \leq 0.01$ in Potchefstroom but did not vary in Kadoma2013b at $P \leq 0.05$ (Table 4.2). Average days to mid-anthesis ranged from 59.0 to 73.6. The trial mean was 65.8 d. The minimum days to mid-anthesis ranged from 50.9 in Chiredzi2013a to 75.1 in Chiredzi2012 while the maximum ranged from 63.2 in Chiredzi2013b to 80.1 in Potchefstroom. Mid-anthesis was delayed in Chiredzi2012. There were no significant differences in days to mid-anthesis between the top 10 yielding and the 10 lowest yielding hybrids (Table 4.4).

Variation in ASI was significant at $P \leq 0.05$ in Chiredzi2012 and at $P \leq 0.001$ in Chiredzi2013a and Kadoma2012 and 2013b. There were no significant differences at $P \leq 0.05$ in Chiredzi2013b and Kadoma2013a and Potchefstroom. Mean ASI for the trial was 0.5 d, which ranged from 0.18 d to 3.06 d (Table 4.2). Minimum ASI ranged from -7.9 d in Chiredzi2012 to -0.8 d in Chiredzi2013b and the maximum up to 5.84 in Chiredzi2013b. Even though the average ASI is 0.5 d, there were relatively high stress levels across the seven sites that resulted in wide ASI ranges. For instance, in Chiredzi 2012 ASI ranged from -7.89 d to 5.13 d (13.73 d) and from -2.40 d to 5.68 d (8.08 d) in Potchefstroom as the highest within sites. In the 10 lowest yielding hybrids ASI ranged from -0.8 d to 2.6 d while in the 10 highest yielding hybrids ranged from -0.8 d to 2.5 d (Table 4.4).

There were no significant variations in barrenness in Potchefstroom and Chiredzi2012 and 2013b (Table 4.2). Barrenness, measured as the number of ears per plant significantly varied at $P \leq 0.05$ in Kadoma2013b, at $P \leq 0.01$ in Kadoma2013a and at $P \leq 0.001$ in Chiredzi2013a and Kadoma2012. The average number of ears per plant ranged from 0.67 to 1.06 with a trial mean of 0.78. Barrenness was least in Kadoma2012. Barrenness was lower in the top 10 yielding hybrids (0.68 to 0.90 ears per plant) compared to 0.62 to 0.86 ears per plant for the 10 lowest yielding hybrids (Table 4.4). Plant height did not significantly vary among hybrids at

Kadoma2012 and 2013b. There was variation in plant height at $P \leq 0.001$ in Chiredzi2012 and 2013b (Table 4.3). Mean plant height ranged from 179.6 cm in Kadoma2013a to 233.2 cm in Potchefstroom2012. The trial mean was 192.1 cm. There were no significant differences between the 10 best yielding hybrids and the 10 lowest yielding hybrids for plant height, root and stem lodging, grain texture, ear rots and ear aspect (Table 4.4). Senescence was not recorded in Kadoma. Senescence among hybrids varied at $P \leq 0.01$ in Chiredzi2013a and at $P \leq 0.001$ in Chiredzi2012 and 2013a (Table 4.3). There was no significant variation in senescence scores in Potchefstroom ($P \leq 0.05$). The trial mean was 4.3 ranging from 3.0 to 5.0. The minimum senescence scores recorded across the three sites ranged from 1.5 to 4.5 while the maximum ranged from 3.9 to 5.8.

Grain moisture was higher (12.7% to 16.5%) in the top yielding hybrids and ranged from 12.8% to 14.9% in the 10 lowest yielding hybrids (Table 4.4). The general performance of the top 10 yielding hybrids across the seven sites was commendable.

Table 4.3 Analysis of secondary traits under random drought stress

Trait	Site	Trial mean	LSD (0.05)	MSe	h^2	p	Minimum	Maximum	Across site mean
Plant height (cm)	Chiredzi2012	203.54	38.86	664.85	0.13	***	151.17	234.58	192.10
	Kadoma2012	181.44	29.11	329.97	0.34	ns	145.25	220.48	
	Potchefstroom2012	233.27	35.08	0.01	.	**	208.18	276.74	
	Kadoma2013a	179.56	33.12	326.79	0.06	*	148.40	213.10	
	Kadoma2013b	183.27	30.62	243.87	0.4	ns	151.62	212.81	
	Chiredzi2013a	224.20	19.48	212.66	0.47	**	203.61	252.21	
	Chiredzi2013b	220.58	33.83	529.78	0.34	***	186.84	284.05	
Senescence (0-10)	Chiredzi2012	2.98	0.77	0.42	0.95	***	2.15	3.94	4.30
	Kadoma2012	.	.	.	.	.	.	.	
	Potchefstroom2012	3.32	2.17	0.21	0.40	ns	1.54	5.48	
	Kadoma2013a	.	.	.	.	.	.	.	
	Kadoma2013b	.	.	.	.	.	.	.	
	Chiredzi2013a	5.01	0.58	0.18	0.42	**	4.49	5.65	
	Chiredzi2013b	4.97	0.65	0.59	0.54	***	4.05	5.77	

h^2 is heritability, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$, MSe Mean square error, LSD (0.05) Least significant difference at ($P < 0.05$)

Table 4.4 Across site analysis of variance for grain yield and secondary traits under random drought showing the top 10 and bottom 10 ranking hybrids

Entry	Name	Grain yield t/ha	Rank Rank	Anthesis Date d	ASI d	Plant height cm	Ear height cm	Root lodging %	Ears plant^{-1} #	Husk cover %	Ear rot %	Senescence 1-10	Grain texture 1-5	Grain moisture %	Plant stand #	Ear aspect 1-5	Release
1	**SC727**	3.37	12	69.7	-0.8	215.0	128.9	3.1	0.68	3.1	5.6	3.9	3.7	16.3	28.0	3.7	2010
2	**CZH0724**	3.38	11	66.2	0.1	197.7	119.8	3.6	0.81	-0.3	4.4	4.2	3.2	15.7	29.3	3.5	2008
3	**CKH10717**	3.24	23	67.8	0.0	195.6	117.6	5.0	0.73	5.7	3.8	4.2	3.2	16.0	30.4	3.4	2010
4	**CZH0511**	3.30	16	65.9	0.4	187.1	95.9	16.7	0.78	7.7	1.2	4.1	3.2	14.8	30.4	3.2	2006
5	**CKH0749**	3.23	16	68.7	0.9	202.5	117.7	9.0	0.76	2.1	6.7	4.0	3.5	16.5	29.9	3.5	2007
6	**CKH08049**	3.15	18	67.1	-0.3	195.5	115.9	6.6	0.72	-0.1	3.8	4.4	3.1	14.6	30.0	3.5	2009
7	**CZH0524**	3.18	17	62.8	2.5	183.5	108.5	14.2	0.88	5.5	1.3	4.1	3.2	12.7	25.6	3.4	2006
8	**CZH02018**	3.09	28	66.9	-0.4	195.5	113.8	14.7	0.80	-0.5	3.6	4.0	2.8	15.2	28.7	3.4	2003
9	**CZH03005**	3.10	22	63.0	0.8	198.9	106.4	5.7	0.90	2.6	4.8	4.4	3.4	13.3	27.1	3.7	2004
10	**CZH03022**	3.17	20	66.2	-0.8	196.8	111.5	7.6	0.78	4.0	3.6	4.4	3.1	14.2	30.5	3.5	2004
57	**SC513**	2.52	47	66.3	1.3	189.5	102.9	5.3	0.64	16.1	5.6	4.2	3.1	13.8	27.5	3.4	1997
59	**SC635**	2.45	51	65.6	1.5	198.5	107.1	3.2	0.65	0.4	3.8	4.3	3.5	13.3	29.8	3.6	2003
61	**CZH0713**	2.54	44	66.5	-0.4	196.4	104.1	9.5	0.78	3.4	4.0	4.8	3.1	14.3	29.9	3.7	2008
62	**CZH0735**	2.44	52	62.8	1.2	173.8	96.0	6.8	0.86	5.4	4.4	4.7	3.1	12.8	26.9	3.9	2008
63	**CZH0536**	2.39	57	67.5	-0.3	196.4	106.8	2.1	0.70	8.1	0.8	4.3	3.5	14.2	26.3	3.5	2006
64	**CZH99026**	2.34	53	66.7	1.7	196.6	118.3	9.0	0.75	-0.1	0.9	4.7	2.5	14.3	31.2	3.4	2000
65	**CZH04001**	2.45	48	66.3	0.1	183.9	101.8	6.9	0.79	0.2	5.9	4.3	3.1	14.9	27.4	3.8	2005
66	**CZH99025**	2.36	52	66.3	1.4	200.0	122.3	16.3	0.62	6.0	7.4	4.4	2.6	14.5	29.6	3.7	2000
67	**CZH00011**	2.31	57	63.6	2.6	174.9	101.3	10.1	0.78	30.9	4.5	4.6	3.2	12.9	28.2	3.7	2001
68	**CZH04032**	2.28	53	66.2	1.1	180.4	98.8	6.6	0.66	2.3	4.4	4.5	3.5	13.4	31.5	3.8	2005
69	**CZH04034**	2.30	55	66.8	-0.8	208.1	112.2	8.4	0.67	-0.1	3.7	4.3	3.2	12.8	27.3	3.5	2005
70	**CZH056**	2.08	59	66.5	0.0	188.2	100.6	12.0	0.84	0.1	6.4	4.1	3.3	14.0	22.1	3.7	2006
Mean		**2.80**	**36**	**65.8**	**0.5**	**192.1**	**108.5**	**7.5**	**0.78**	**3.9**	**4.4**	**4.3**	**3.1**	**14.1**	**28.3**	**3.5**	
LSD (0.50)		**0.65**		**1.2**	**1.2**	**13.5**	**9.4**	**7.1**	**0.09**	**10.8**	**5.3**	**0.6**	**0.3**	**1.2**	**1.8**	**0.5**	
h^2		**0.56**	**.**	**0.92**	**0.72**	**0.75**	**0.89**	**0.27**	**0.36**	**.**	**0.42**	**0.45**	**0.87**	**0.71**	**0.52**	**0.51**	
Minimum		**2.08**	**11**	**60.9**	**-1.9**	**166.5**	**81.4**	**0.8**	**0.62**	**-0.5**	**-0.3**	**3.8**	**2.1**	**12.2**	**22.1**	**3.0**	
Maximum		**3.38**	**59**	**69.7**	**2.6**	**215.0**	**129.6**	**18.1**	**0.96**	**30.9**	**15.1**	**4.8**	**3.8**	**16.5**	**31.5**	**3.9**	

h^2 is heritability, ASI anthesis silking interval, LSD (0.05) Least significant difference at ($P < 0.05$)

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

Grain yield, days to mid-anthesis, ASI, number of ears per plant, plant height and senescence all significantly varied in the different locations. Grain yield among hybrids varied at $P \leq 0.001$ in Kiboko2012, Chiredzi2013a and 2013b and at $P \leq 0.05$ in Chiredzi2012 with average grain yield ranging from 0.89 t ha^{-1} in Chiredzi2012 to 5.23 t ha^{-1} in Chiredzi2013a (Table 4.5). The trial mean yield was 3.95 t ha^{-1}. The minimum yield recorded across the three managed drought stress sites ranged from 0.18 t ha^{-1} in Chiredzi2012 to 2.81 t ha^{-1} in Chiredzi2013a while the maximum ranged from 1.42 t ha^{-1} in Chiredzi2012 to 7.55 t ha^{-1} Chiredzi2013b. Chiredzi2012 recorded the lowest yields.

Days to mid-anthesis varied at all sites at $P \leq 0.001$, ranging on average from 63.8 in Chiredzi2012 to 94.1 in Chiredzi2013b. The trial mean was 75.7 d. The maximum days to mid anthesis ranged from 71.7 in Chiredzi2012 to 105.5 in Chiredzi2013b. Days to mid-anthesis was delayed in Chiredzi2013b. Stress delayed anthesis in some entries in Chiredzi2013a and b.

Significant variation in ASI ($P \leq 0.001$) was recoded in Kiboko2012, Chiredzi2013a and 2013b, and at $P \leq 0.01$ in Chiredzi2012. Average ASI ranged from 2.1 d in Chiredzi2013a to 7.9 d in Chiredzi2012. The trial average was 4.0 d. Minimum ASI ranged from -2.1 d in Chiredzi2013b to 1.5 d in Chiredzi2012. The maximum ASI for the three sites ranged from 5.4 d in Chiredzi2013a to 18.0 d in Chiredzi2012. Chiredzi2012 had the widest range of ASI from 1.5 d to 18.0 d indicating high levels of stress.

Barrenness varied at $P \leq 0.01$ in Chiredzi2013a and at $P \leq 0.001$ in Kiboko2012 and Chiredzi2012 and 2013b. Measured as the number of ears per plant, barrenness on average was highest in Chiredzi2012 (0.29) and lowest in Chiredzi2013b (0.87) with a trial mean of 0.64. Highest levels of barrenness recoded ranged from 0.01 ears plant^{-1} in Chiredzi2012 to 0.76 ears plant^{-1} in Chiredzi2013b. The lowest levels of barrenness ranged from 0.70 ears plant^{-1} to 0.99 ears plant^{-1}. Chiredzi2012 had the widest range of number of ears plant, from 0.01 to 0.70.

Plant height varied significantly across all sites, at $P \leq 0.05$ in Chiredzi2012, $P \leq 0.01$ in Chiredzi2013a and at $P \leq 0.001$ in Kiboko2012 and Chiredzi 2013b. Average plant height ranged from 162.4 cm in Chiredzi2012 to 226.2 cm in Chiredzi2013a. The across site average plant height was 203.4 cm. Plants were generally shortest in Chiredzi2012.

Table 4.5 Analysis of variance of yield and secondary traits under managed drought stress

Trait	Site	Trial mean	LSD (0.05)	MSe	h^2	p	Minimum	Maximum	Across site mean
Grain yield (t ha^{-1})	Kiboko2012	4.19	1.47	0.60	0.64	***	2.21	6.14	3.95
	Chiredzi2012	0.89	0.58	0.12	0.18	*	0.18	1.42	
	Chiredzi2013a	5.23	1.37	0.21	0.63	***	2.81	6.98	
	Chiredzi2013b	2.24	1.58	0.64	0.47	***	2.76	7.55	
Days to mid-Anthesis (d)	Kiboko2012	68.4	2.3	1.4	0.94	***	62.0	74.1	75.7
	Chiredzi2012	63.8	3.8	5.6	0.88	***	53.6	71.7	
	Chiredzi2013a	92.9	2.4	1.7	0.96	***	84.3	101.5	
	Chiredzi2013b	94.1	3.3	1.6	0.92	***	85.0	105.5	
Anthesis-silking interval (d)	Kiboko2012	2.3	2.4	0.0	0.64	***	0.3	8.1	4.0
	Chiredzi2012	7.9	6.7	11.7	0.48	**	1.5	18.0	
	Chiredzi2013a	2.1	3.0	0.3	0.49	***	-0.9	5.4	
	Chiredzi2013b	2.9	4.5	0.0	0.46	***	-2.1	13.7	
Ears per plant (number)	Kiboko2012	0.79	0.20	0.01	0.27	***	0.41	0.96	0.64
	Chiredzi2012	0.29	0.24	0.03	0.60	***	0.01	0.70	
	Chiredzi2013a	0.85	0.10	0.00	0.39	**	0.74	0.99	
	Chiredzi2013b	0.87	0.10	0.00	0.21	***	0.76	0.97	
Plant height (cm)	Kiboko2012	208.1	10.1	0.0	0.79	***	186.4	223.6	203.4
	Chiredzi2012	162.4	22.5	328.5	0.30	*	135.9	185.1	
	Chiredzi2013a	226.2	23.2	0.0	0.51	**	201.7	252.7	
	Chiredzi2013b	216.7	19.8	410.1	0.58	***	189.3	243.1	
Senescence (1-10)	Kiboko2012	.	.	.	.	.	.	.	5.1
	Chiredzi2012	5.8	0.5	0.1	0.77	***	5.2	6.8	
	Chiredzi2013a	5.0	0.6	0.3	0.69	***	3.9	6.1	
	Chiredzi2013b	4.8	0.6	0.3	0.50	***	4.0	5.6	

h^2 is heritability, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$, MSe Mean square error, LSD (0.05) Least significant difference

Senescence was not recorded in Kiboko. Variation was recorded in all the other three sites at $P \leq 0.001$ with average senescence scores ranging from 4.8 in Chiredzi2013b to 5.8 in Chiredzi2012. The across site average score was 5.1. Rates of senescence were lower in Chiredzi2013a and b. Chiredzi2012 had the highest levels of senescence and Chiredzi2013b had the lowest.

The top 10 hybrids yielded significantly higher than the 10 lowest yielding hybrids at 5.52 t ha^{-1} to 6.56 t ha^{-1} compared to 3.11 t ha^{-1} to 4.22 t ha^{-1}(Table 4.6). For the top 10 and 10 lowest yielders the days to mid-anthesis was in the same range, even though slightly earlier in the least yielding hybrids. ASI was higher among lowest yielding CIMMYT hybrids ranging from 0.1 d to 6.5 d compared to the top 10 hybrids that ranged from 0.6 d to 3.9 d.

From the above results, Chiredzi2012 experienced the highest stress levels compared to the other sites. The yields were lowest, plants were shortest while anthesis-silking interval, barrenness and senescence were highest in Chiredzi2012 compared to the other sites.

The top 10 hybrids were relatively taller at 206.3 cm to 228.4 cm compared to 200.5 cm to 225.4 cm in the 10 lowest yielding hybrids. Root and stem lodging was variable but did not show a pattern. There was no distinct variation between the top 10 hybrids and the 10 lowest yielding hybrids. Barrenness, ear rot, senescence, grain texture, plant stand and plant aspect did not vary between the best and least yielders. Ear aspect was better for the top 10 hybrids that were bigger and better filled compared to the 10 least yielding hybrids. Even though for some traits there was no significant variation between the top yielders and the lowest yielders, the top 10 hybrids were superior in yield and other secondary traits, including ASI and ear aspect.

Table 4.6 Across site summary for grain yield and secondary traits under managed drought stress showing the top 10 and bottom 10 ranking hybrids

Name	Rank	Across	Grain yield Kiboko	Grain yield Chiredzi 2013	Grain yield Chiredzi 2013	Days to mid-anthesis	ASI	Plant height	Root lodging	Stem lodging	Ears plant^{-1}	Husk cover	Ear rot	Senescence	Grain texture	Plant stand	Ear aspect	Plant aspect	Release
	Rank	t/ha	t/ha	t/ha	t/ha	d	d	cm	%	%	#	%	%	1-10	1-5	#	1-5	1-5	
CKH08032	1	6.56	6.22	6.27	7.18	82.4	0.6	211.3	2.3	9.6	0.96	7.5	5.3	4.8	4.3	28.8	2.9	2.5	2008
CZH0819	2	5.96	4.43	5.88	7.55	81.0	2.1	215.0	1.4	4.2	0.86	0.1	8.8	5.3	3.6	26.6	3.1	2.6	2008
CKH08105	3	5.75	4.50	6.41	6.33	81.7	1.7	211.4	0.1	7.3	0.82	3.5	7.6	5.1	4.1	28.2	3.4	3.0	2008
CZH00027	4	5.72	3.68	6.79	6.69	83.5	1.8	228.4	6.9	10.2	0.81	8.4	11.2	4.7	3.6	28.1	3.2	3.3	2001
CZH03006	5	5.67	4.07	6.24	6.71	80.2	3.5	224.2	3.1	7.2	0.86	3.7	3.4	5.4	3.7	28.2	3.1	2.6	2004
CZH0616	6	5.65	5.84	5.77	5.35	78.5	0.7	206.3	1.8	2.5	0.91	5.4	5.0	4.4	4.0	27.6	2.8	2.8	2007
CZH0928	7	5.65	3.89	6.98	6.08	77.7	1.3	211.2	-0.3	8.8	0.88	2.1	12.1	5.0	3.8	28.5	3.2	3.1	2010
CZH03028	8	5.61	5.91	5.59	5.32	81.5	3.9	227.8	1.6	7.8	0.83	6.6	7.4	4.5	3.8	27.9	3.0	3.0	2004
CZH0724	9	5.59	5.55	5.88	5.35	81.6	2.2	224.6	4.0	6.0	0.86	5.4	3.2	4.4	4.1	27.6	2.9	3.1	2008
CZH03013	10	5.52	5.38	4.90	6.30	84.9	0.8	215.3	4.0	5.3	0.80	6.8	6.4	4.2	3.5	26.9	3.0	3.0	2004
SC513	58	4.28	3.22	5.52	4.11	80.2	2.8	213.6	16.5	16.3	0.72	1.7	9.9	5.5	4.0	26.6	3.5	3.1	1997
CZH0837	61	4.22	3.74	3.98	4.93	78.1	2.4	225.4	-0.2	4.4	0.75	8.5	10.3	5.0	3.6	23.5	3.0	3.0	2009
CZH0615	62	4.18	2.60	5.07	4.88	79.0	3.9	200.5	4.3	7.4	0.69	4.8	8.9	5.7	3.8	28.4	3.6	3.1	2007
CZH0823	63	4.18	2.40	4.77	5.36	79.2	1.5	207.3	1.5	4.5	0.72	17.4	7.7	5.4	4.0	26.3	3.3	3.5	2009
KDH3	64	4.17	3.24	4.54	4.72	74.4	1.7	201.5	2.4	2.3	0.82	5.3	8.8	5.8	3.7	28.4	3.6	3.1	2007
CZH056	65	4.07	4.17	3.87	4.17	84.3	2.2	213.5	1.9	5.7	0.79	3.8	2.8	4.1	3.7	23.3	3.2	2.8	2006
CZH04001	66	4.02	2.84	4.90	4.33	84.3	1.3	212.4	0.0	12.1	0.79	0.2	2.6	5.2	3.6	27.9	3.6	3.0	2005
CZH095	67	3.55	2.24	3.14	5.26	83.1	1.3	219.4	0.1	12.7	0.90	2.5	3.5	4.6	3.1	20.4	3.2	2.8	2010
CZH0524	68	3.51	4.61	3.16	2.76	75.3	2.3	217.9	0.6	2.9	0.78	5.3	12.9	4.6	4.0	16.8	3.0	3.1	2006
SC635	69	3.50	2.71	3.97	3.84	86.2	6.5	217.6	7.8	16.2	0.58	7.0	11.4	4.5	4.2	27.5	3.9	3.1	2003
CZH0536	70	3.11	3.44	2.81	3.10	83.9	0.1	219.6	4.9	8.2	0.81	1.9	9.2	4.6	3.9	17.4	2.8	2.6	2006
Mean		**4.91**	**4.24**	**5.23**	**5.24**	**81.6**	**2.2**	**217.0**	**4.3**	**8.3**	**0.82**	**4.1**	**6.1**	**4.9**	**3.7**	**27.2**	**3.1**	**3.0**	
LSD (0.05)		**0.65**	**1.49**	**1.36**	**1.58**	**108.8**	**0.5**	**10.9**	**1.0**	**2.8**	**0.08**	**0.0**	**0.7**	**0.4**	**0.1**	**0.1**	**0.4**	**0.1**	
h2		**0.6**	**0.6**	**0.6**	**0.5**	**0.9**	**0.5**	**0.7**	**0.3**	**0.7**	**0.3**	**.**	**.**	**0.8**	**.**	**0.8**	**0.3**	**.**	
Minimum		**3.11**	**2.24**	**2.81**	**2.76**	**74.4**	**0.1**	**199.0**	**-1.5**	**-0.1**	**0.58**	**-0.2**	**2.0**	**4.1**	**2.9**	**16.8**	**2.8**	**2.5**	
Maximum		**6.56**	**6.22**	**6.98**	**7.55**	**87.9**	**6.5**	**237.9**	**18.0**	**31.2**	**0.96**	**17.4**	**13.4**	**5.9**	**4.3**	**28.8**	**3.9**	**3.5**	

h^2 heritability, ASI anthesis-silking interval, LSD (0.05) Least significant difference at $P < 0.05$

4.3.3 Multi-environment trials analysis

4.3.3.1 Genetic correlations for grain yield among random and managed drought sites

Genetic correlations for random drought stress sites ranged from -0.10 for Kadoma2013a and Chiredzi2012 to 0.99 for Chiredzi2013a and b (Table 4.7). Chiredzi2012 had low correlations of -0.10 with Kadoma2013a and 0.38 with Chiredzi2013b. Chiredzi2013a had a low negative correlation with Kadoma2013b of -0.22. The rest of the correlations among all sites were moderate to high.

Table 4.7 Genetic correlations for grain yield under random drought sites

Site	Chiredzi 2012	Kadoma 2012	Potchef-stroom	Kadoma 2013a	Kadoma 2013b	Chiredzi 2013a	Chiredzi 2013b
Chiredzi2012							
Kadoma2012	0.57						
Potchefstroom	0.55	0.30					
Kadoma2013a	-0.10	0.47	0.24				
Kadoma2013b	0.58	0.29	0.51	0.58			
Chiredzi2013a	0.98	0.72	0.43	0.40	-0.22		
Chiredzi2013b	0.38	0.78	0.72	0.65	0.95	1.00	

Genetic correlations for managed drought sites ranged from -0.44 for Chiredzi2012 and 2013b to 0.92 for Chiredzi2013a and b (Table 4.8). Chiredzi2012 had negative correlations with the other three sites, the weakest with Chiredzi2013a at -0.06 and -0.18 with Kiboko. Yields in Chiredzi2012 were low compared to the other sites with an average of 0.89 t ha $^{-1}$.

Table 4.8 Genetic correlations for grain yield among sites under managed drought stress

Site	Chiredzi2012	Kiboko2012	Chiredzi2013a	Chiredzi2013b
Chiredzi2012				
Kiboko2012	-0.18			
Chiredzi2013a	-0.06	0.34		
Chiredzi2013b	-0.44	0.25	0.92	

4.3.3.2 Complete linkage cluster analysis among random and managed drought sites

From the cluster analysis of the random drought stress sites, Kadoma2013b clustered along with Potchefstroom2012 (Figure 4.1). The cluster varied from the next cluster with Chiredzi2013b, Kadoma2012 and 2013a at a distance less than 1.

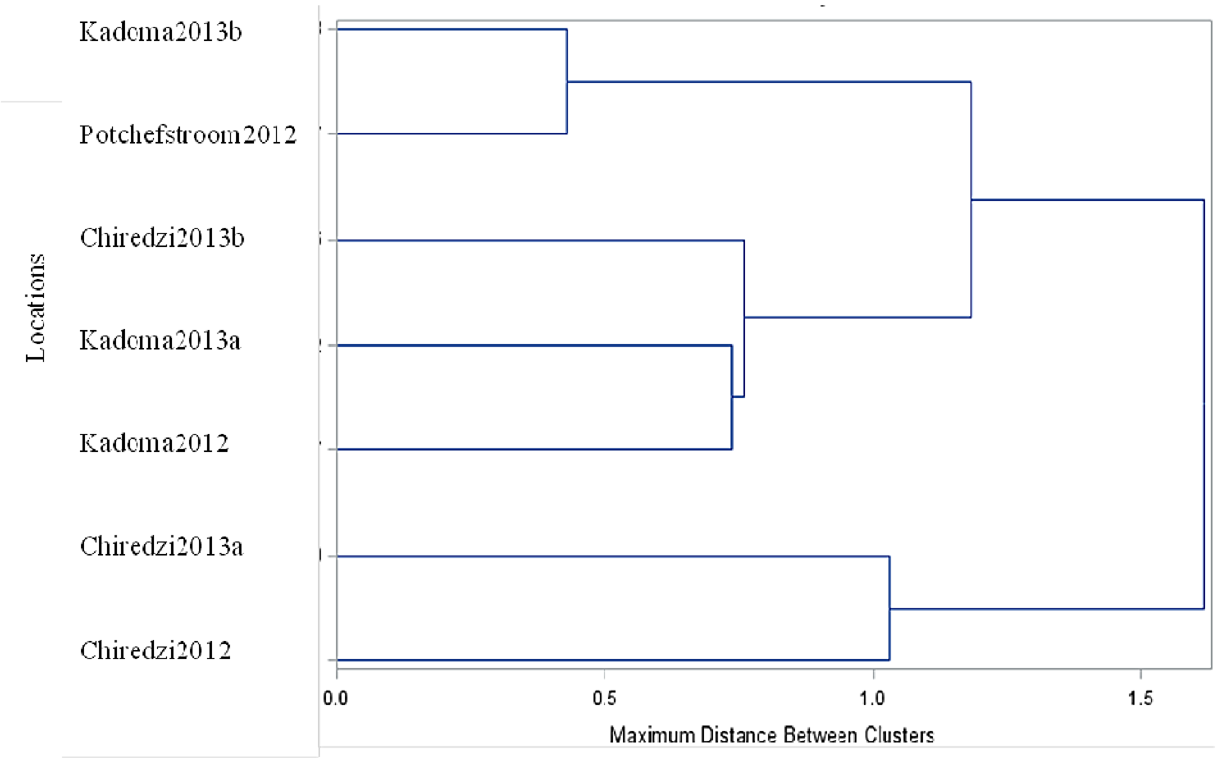


Figure 4.1 Complete linkage cluster analysis of random drought sites

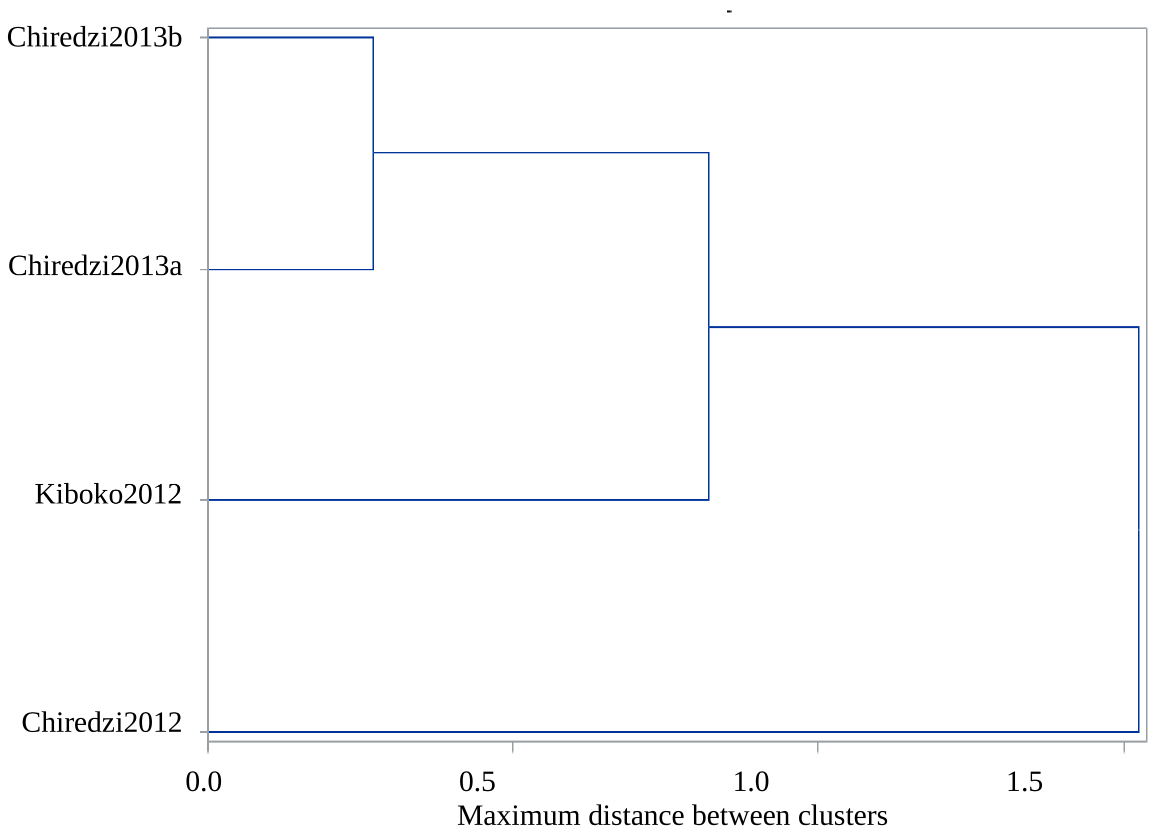


Figure 4.2 Complete linkage cluster analysis of managed drought stress sites

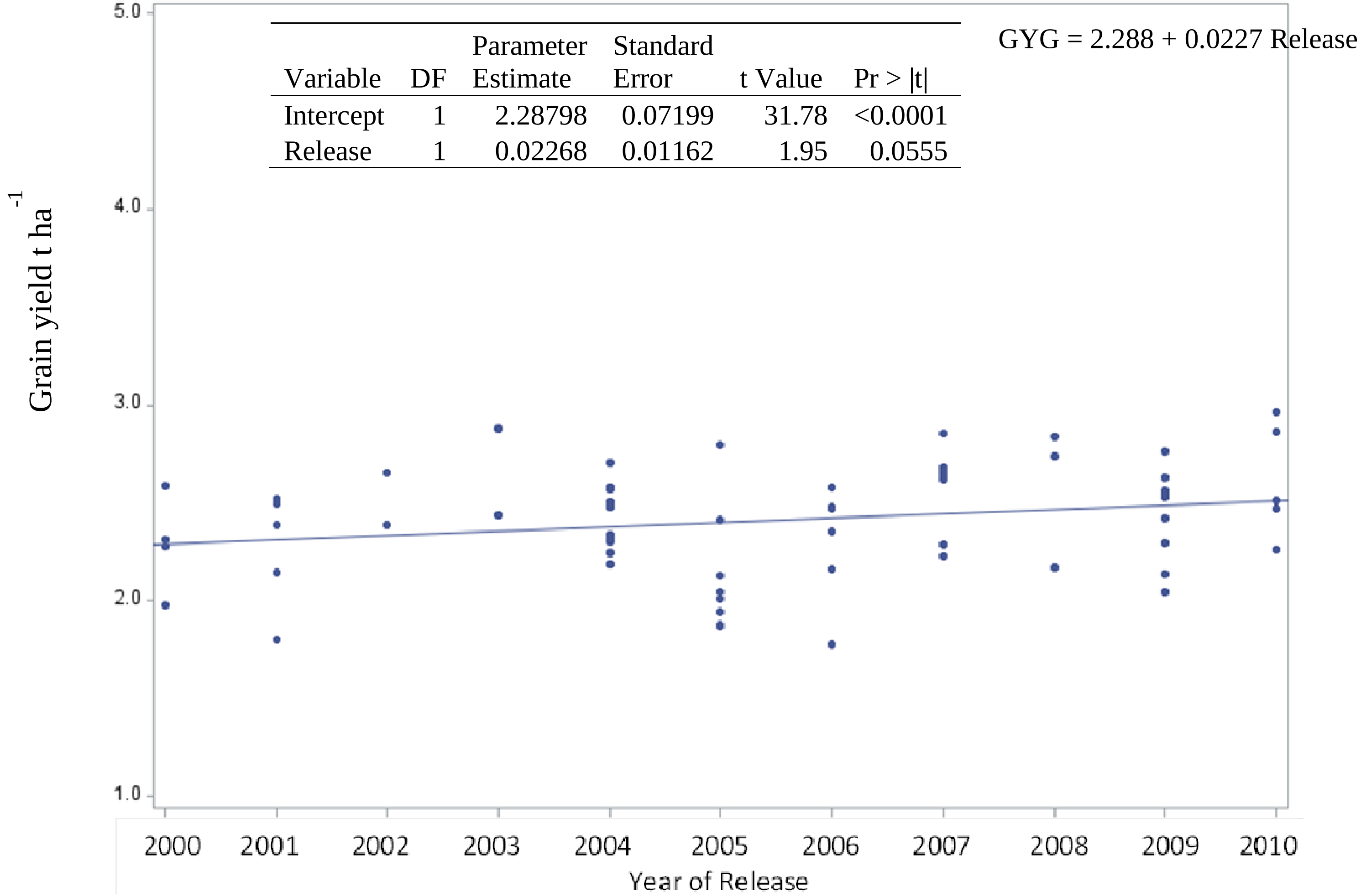
Chiredzi2013b varied from Kadoma2012 and 2013a at a distance less than 1.0. Chiredzi2012 and 2013a varied from the rest of the sites at a distance of more than 1.5.

Under managed drought stress Chiredzi2013a and 2013b and Kiboko2012 clustered together even though Kiboko2012 differed from the other two sites (Figure 4.2). Chiredzi2012 did not cluster with the rest of the managed drought sites. Considering the negative correlations and the yield levels that varied from the rest of the trial sites, Chiredzi2012 was not included in further analysis.

4.3.4 Analysis of genetic gain for yield and secondary traits under random drought stress

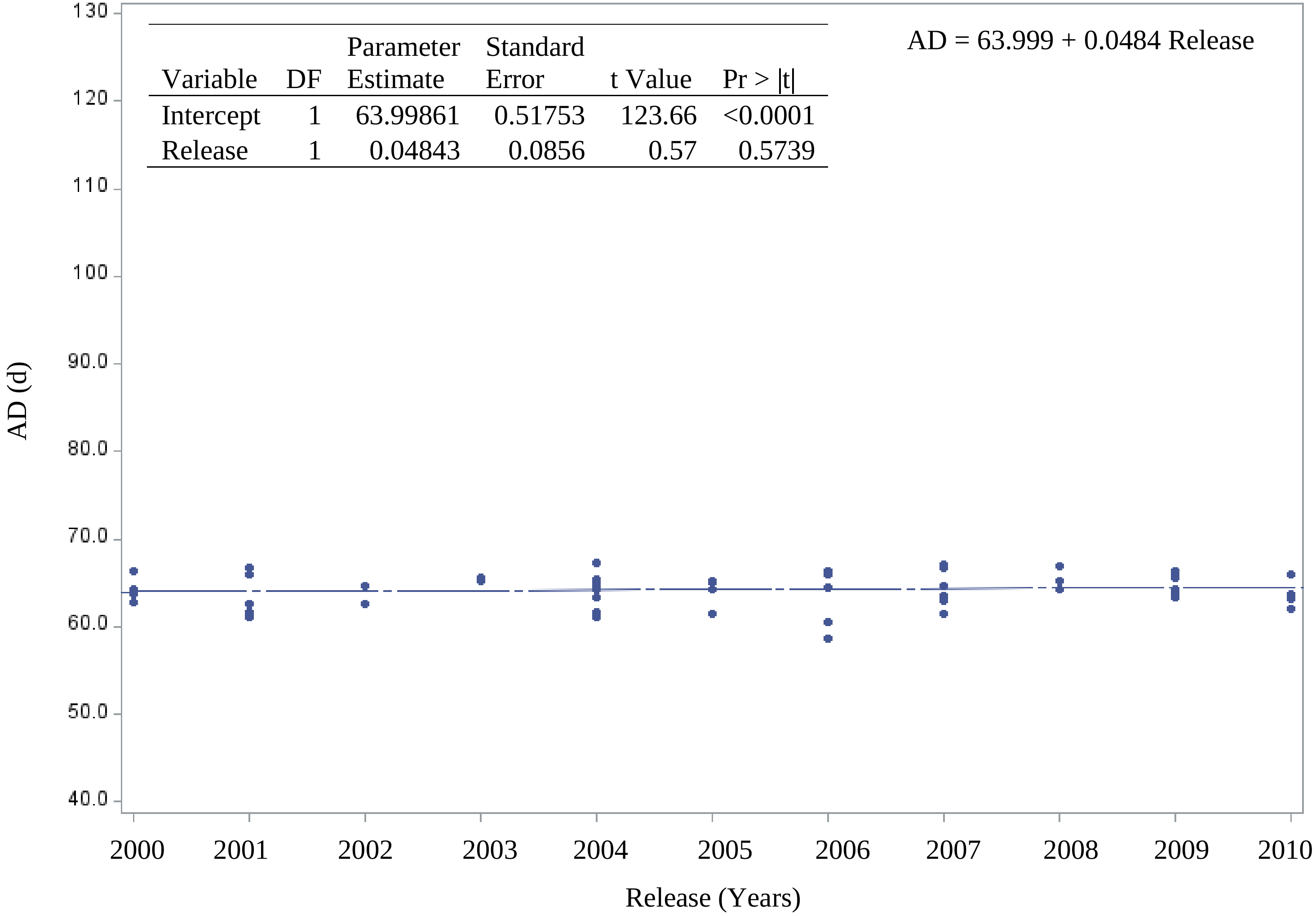
From a regression analysis of maize grain yield against time of release there was a net positive genetic gain estimated at 22.7 kg ha^{-1} year^{-1} (0.85% year^{-1}) (Figure 4.3) under random drought stress over the 11 year period from 2000 to 2010. The new hybrids yielded better than the old hybrids. This change was associated with changes in some secondary traits of the maize hybrids. There was no change in number of days to mid-anthesis over the 11 years ($P \leq 0.05$). The new hybrids flowered in the same range of days as the old hybrids and commercial checks that reached up to 68.2 d for commercial SC727. ASI showed a trend to decrease at a rate of -0.08 d year^{-1} (Figure 4.5) but the change was not significant ($P \leq 0.05$).

From 2000 to 2010 the number of ears per plant increased at a rate of 0.004 year^{-1} (Figure 4.6) at $P \leq 0.05$. There was no significant change in plant height ($P \leq 0.05$) even though it showed a trend of decreasing over the 11 year period at an estimated rate of -0.39 cm year^{-1} (Figure 4.7). There was no significant change in senescence over the 11 years at $P \leq 0.05$ but showed a trend to decrease at a rate of 0.01 scores year^{-1} (on a 0-10 scale) as shown in Figure 4.8. There was no significant change in the grain yield/ grain moisture content ratio over the 11 years (Figure 4.9).



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	2.28798	0.07199	31.78	<0.0001
Release	1	0.02268	0.01162	1.95	0.0555

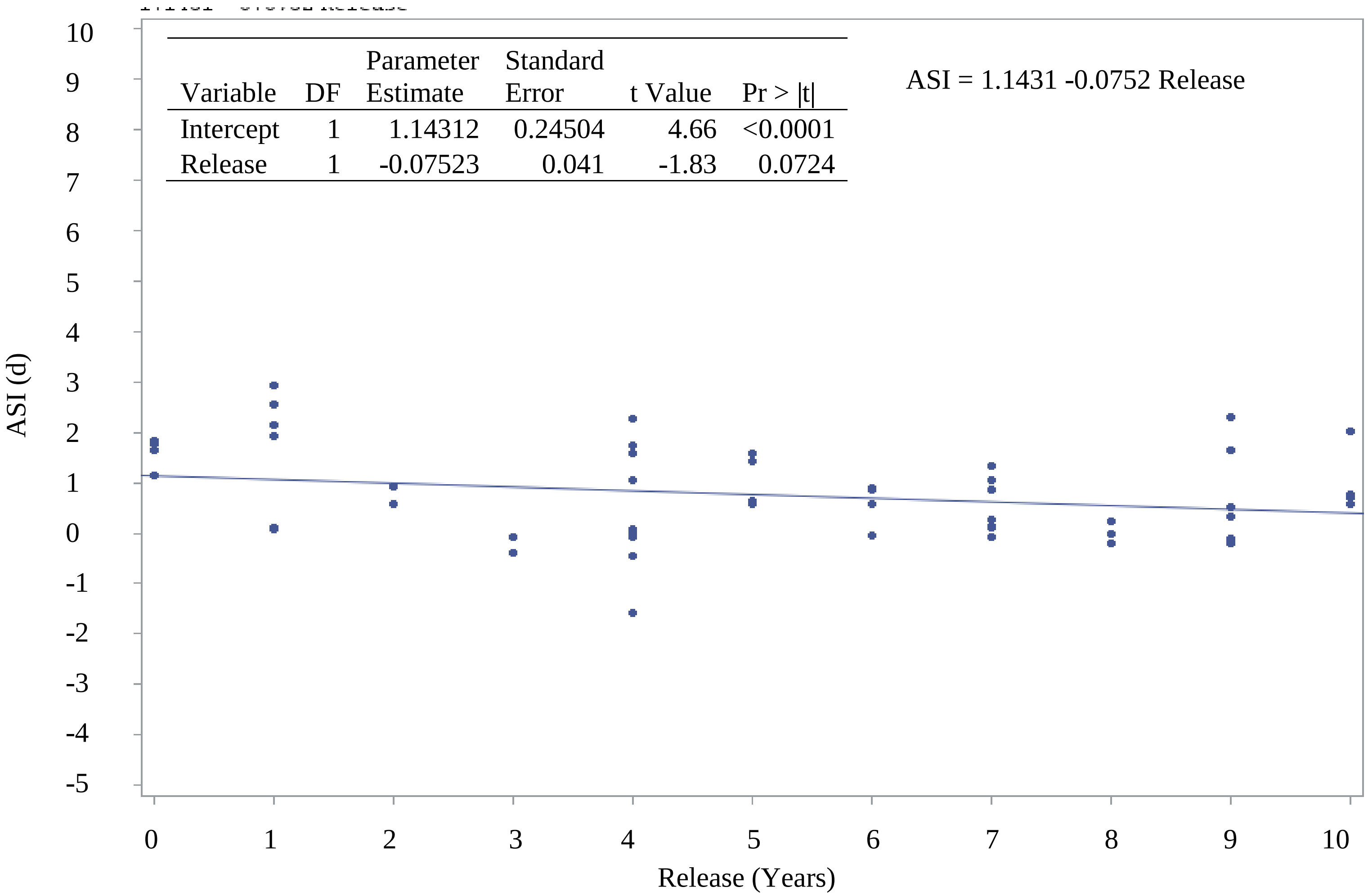
Figure 4.3 Genetic gain in yield (drought tolerance) in CIMMYT hybrids released from 2000 to 2010 tested under random drought stress



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	63.99861	0.51753	123.66	<0.0001
Release	1	0.04843	0.0856	0.57	0.5739

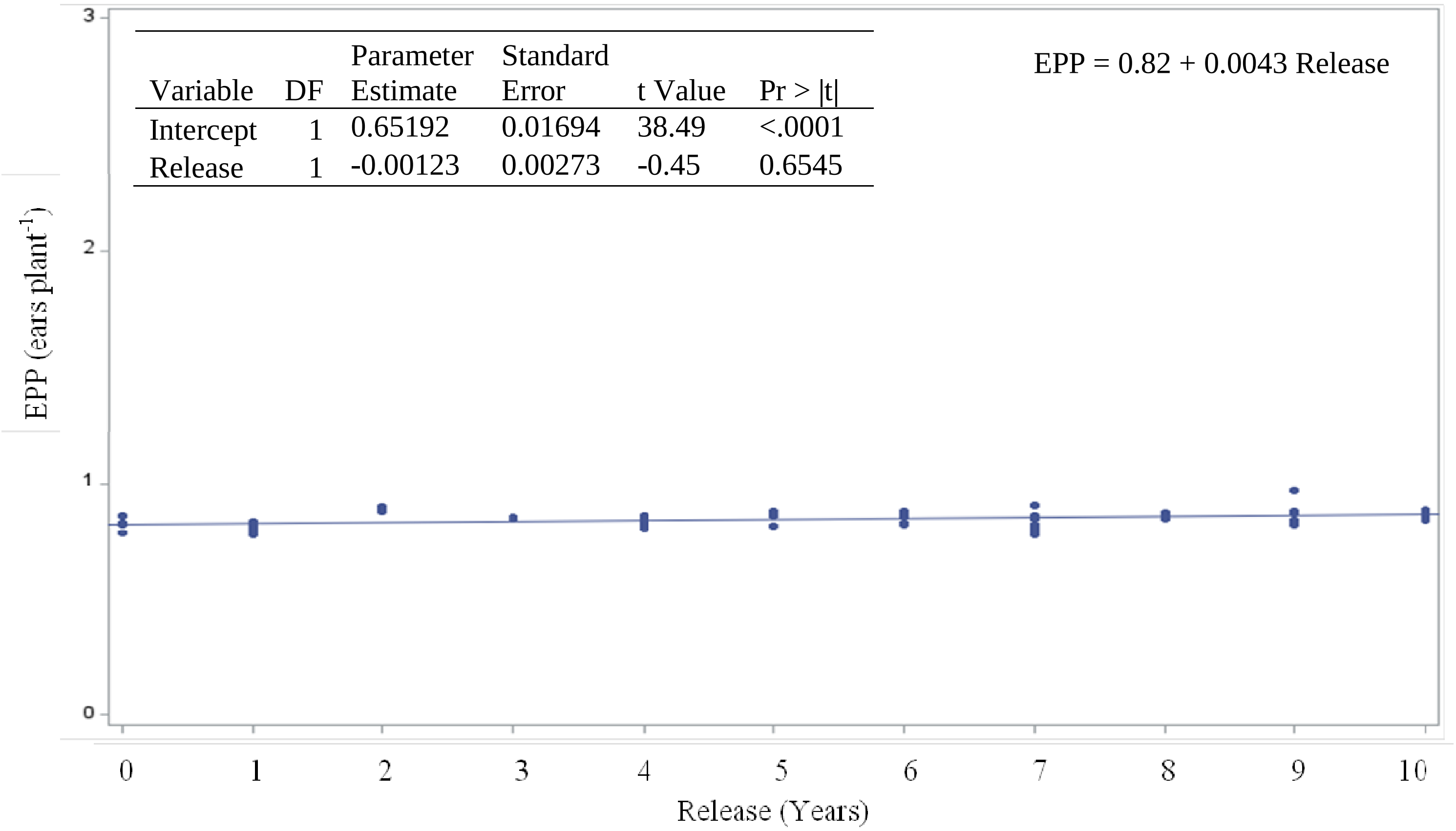
AD = 63.999 + 0.0484 Release

Figure 4.4 Changes in days to mid-anthesis in CIMMYT hybrids released from 2000 to 2010 (random drought stress)



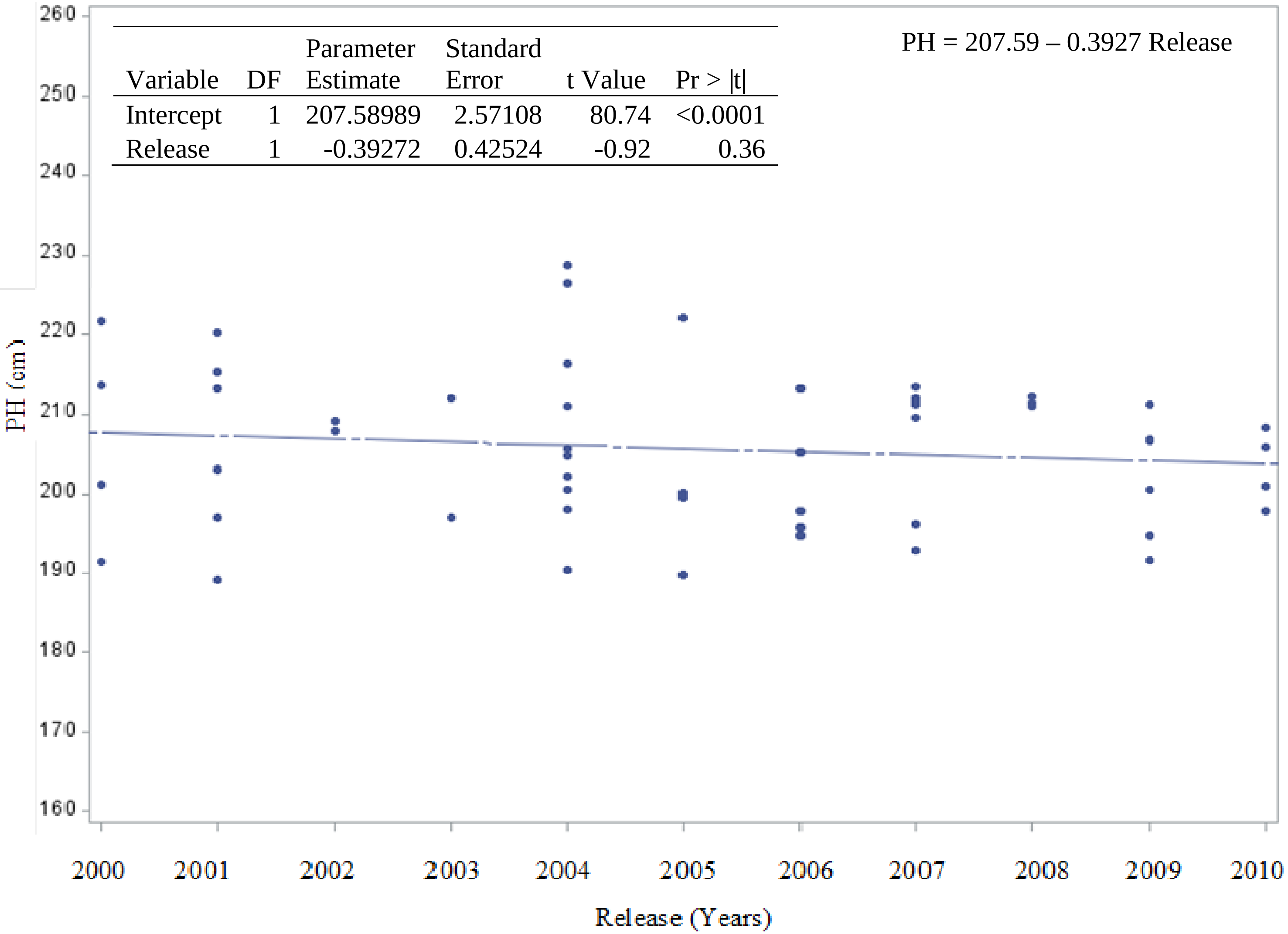
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.14312	0.24504	4.66	<0.0001
Release	1	-0.07523	0.041	-1.83	0.0724

Figure 4.5 Changes in ASI in CIMMYT hybrids released from 2000 to 2010 (random drought stress)



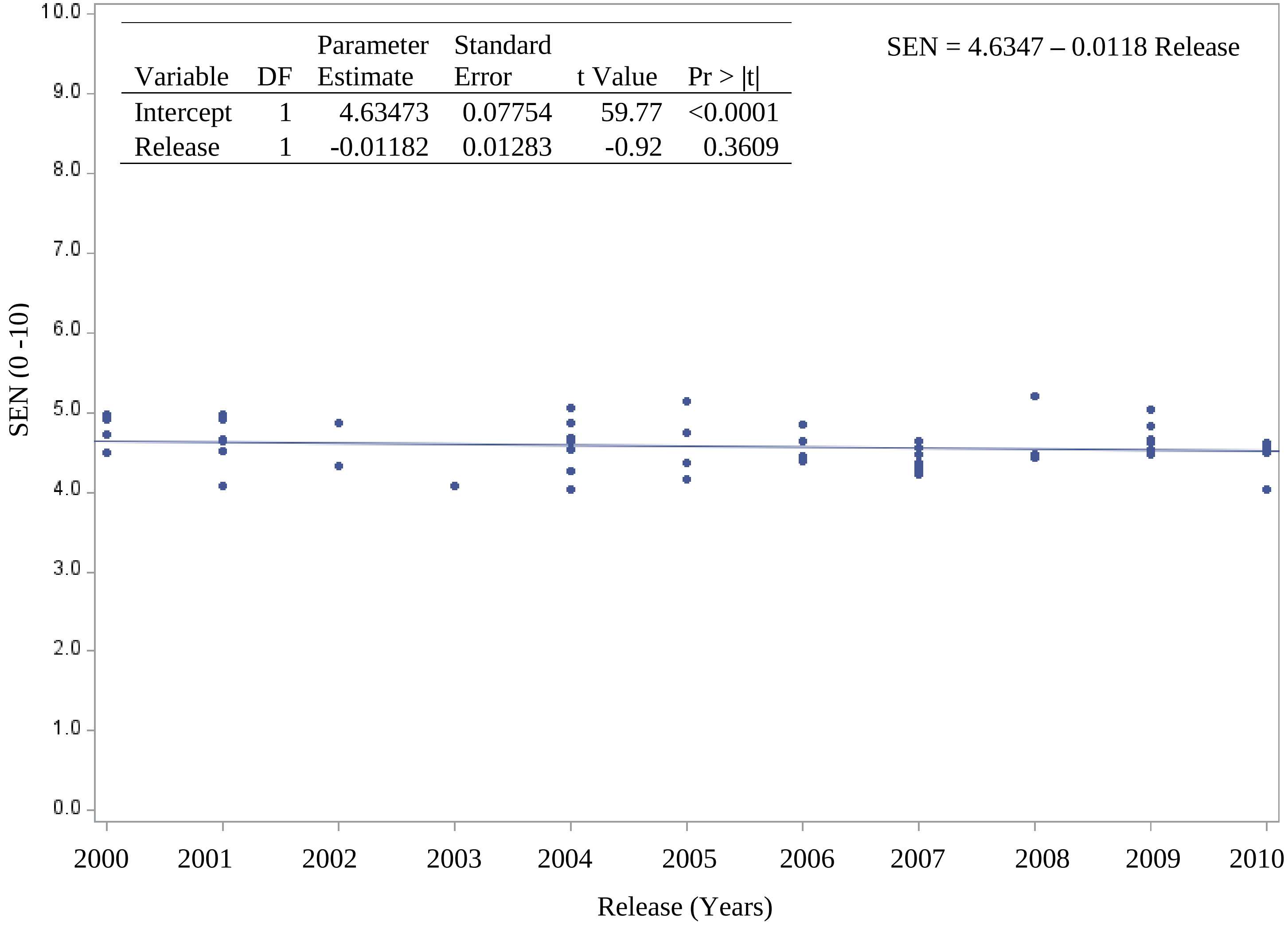
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.65192	0.01694	38.49	<.0001
Release	1	-0.00123	0.00273	-0.45	0.6545

Figure 4.6 Changes in number of ears per plant in CIMMYT hybrids released from 2000 to 2010 (random drought stress)



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	207.58989	2.57108	80.74	<0.0001
Release	1	-0.39272	0.42524	-0.92	0.36

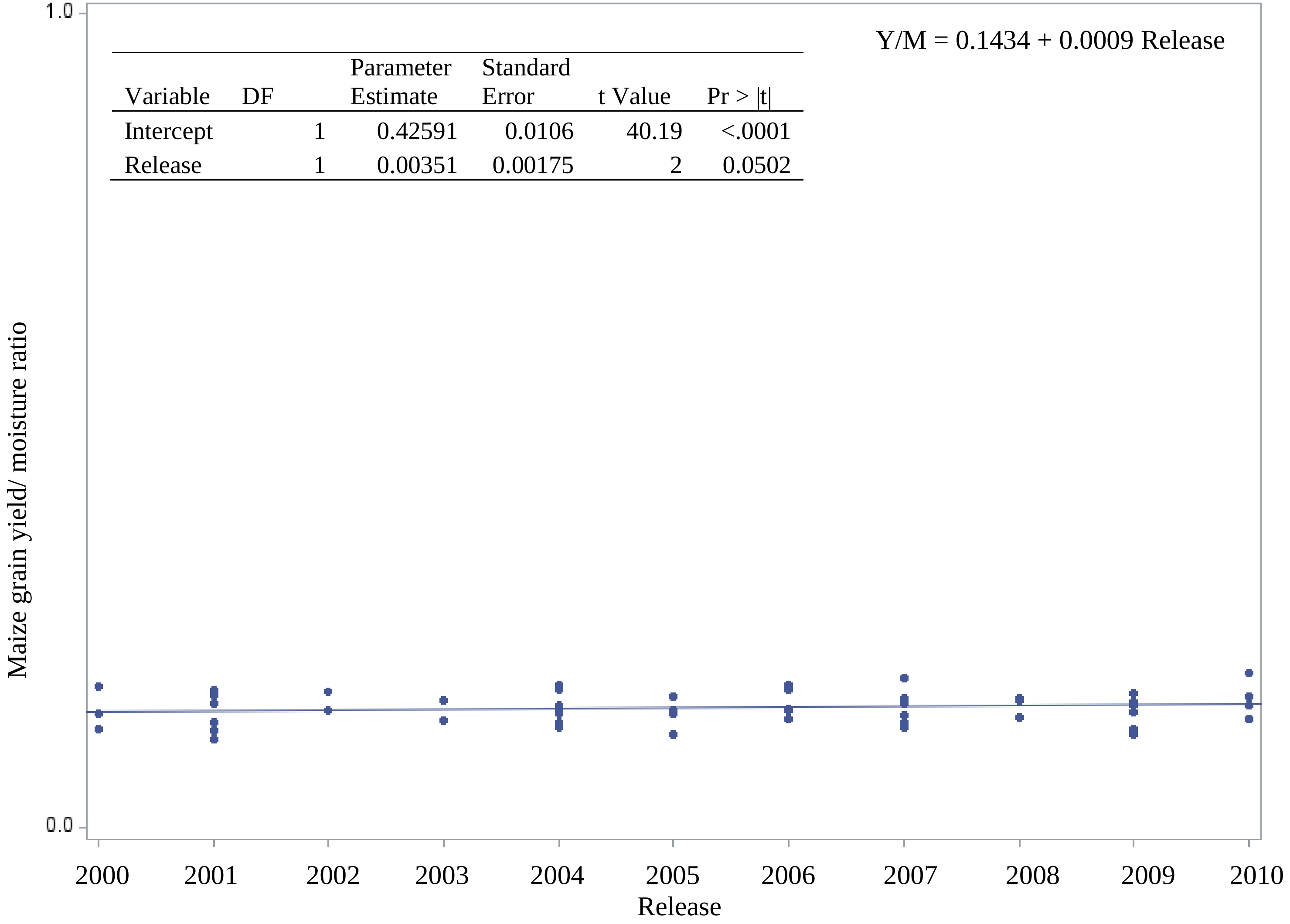
Figure 4.7 Changes in plant height in CIMMYT hybrids released from 2000 to 2010 (random drought stress)



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	4.63473	0.07754	59.77	<0.0001
Release	1	-0.01182	0.01283	-0.92	0.3609

SEN = 4.6347 – 0.0118 Release

Figure 4.8 Changes in senescence in CIMMYT hybrids released from 2000 to 2010 (random drought stress)


Y/M = 0.1434 + 0.0009 Release

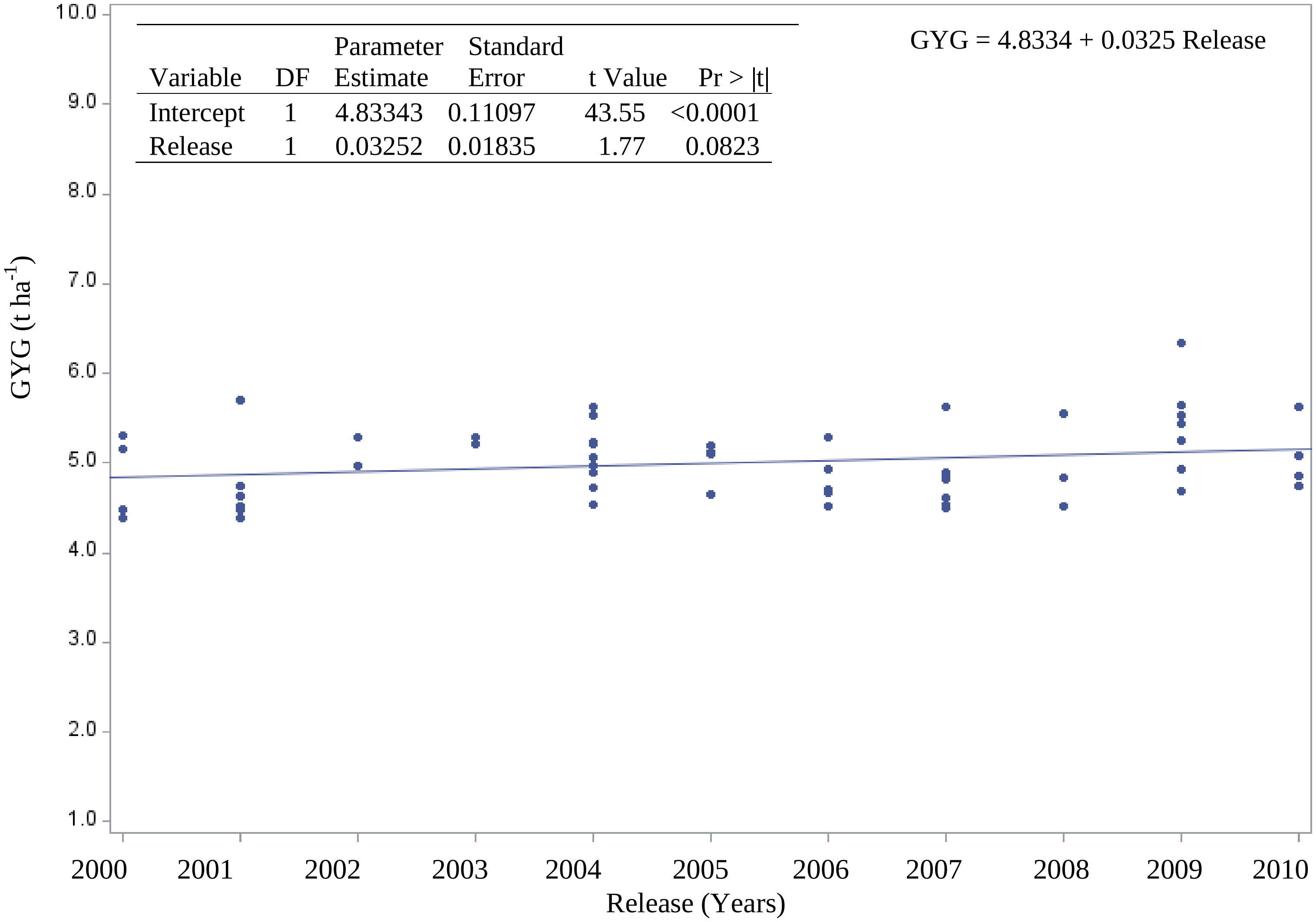
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.42591	0.0106	40.19	<.0001
Release	1	0.00351	0.00175	2	0.0502

Figure 4.9 Changes in grain yield/ grain moisture content ratio in CIMMYT hybrids released from 2000 to 2010 (random drought stress)

4.3.5 Analysis of genetic gain for yield and secondary traits under managed drought stress

Under managed drought stress a genetic gain in yield of 32.5 kg ha^{-1} year^{-1} (0.85% year^{-1}) was recorded (Figure 4.10). This converts to an estimated net increase of 325 kg ha^{-1} over a 10 year period. Phenological changes were also recorded. Days to mid-anthesis did not significantly change over the 11 years at $P \leq 0.05$ but showed a tendency to increase at a rate of 0.10 d year^{-1} (Figure 4.11). New hybrids flowered within the same range as the old hybrids and commercial checks but yielded better than the old hybrids. A net decrease of, on average, 0.16 d year^{-1} was recorded for ASI under managed drought stress (Figure 4.12) at $P \leq 0.01$. New hybrids had relatively shorter ASI compared to the old hybrids.

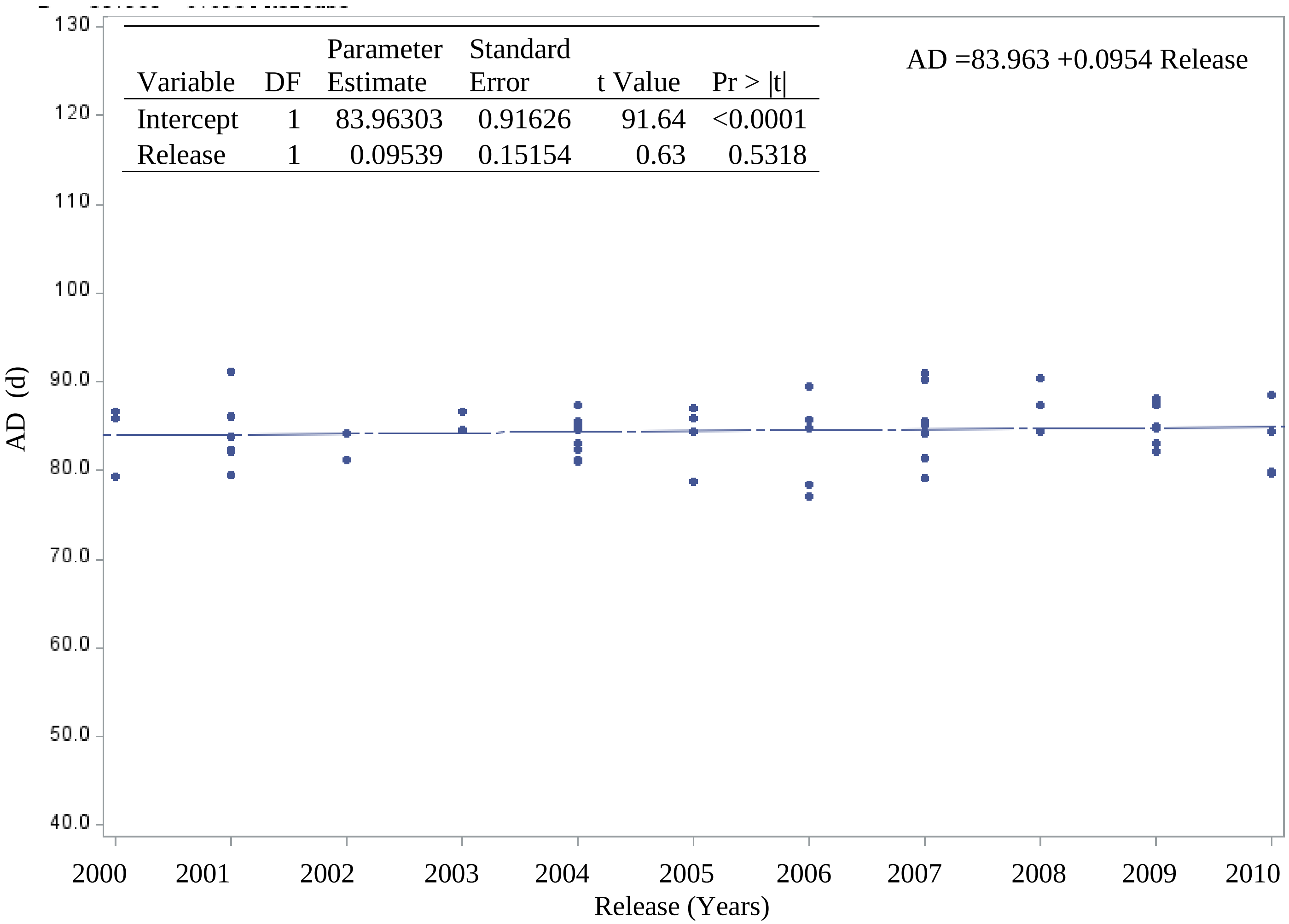
Barrenness was lower in the new hybrids. New hybrids had a relatively higher number of ears per plant that increased at a rate of 0.004 (Figure 4.13) which is significant at $P \leq 0.01$. Plant height showed a trend to increase at an average rate of 0.12 cm year^{-1} over the 11 years (Figure 4.14) but there was no significant change at $P \leq 0.05$. Senescence rate did not significantly change ($P \leq 0.05$) over the 11 years but showed a tendency to increase at a rate of 0.01 scores year^{-1} (Figure 4.15). The grain yield/ grain moisture content ratio did not change significantly over the 11 years (Figure 4.16). Under managed drought stress new hybrids that yielded better than old hybrids had shorter ASI or improved synchronization at flowering and increased number of ears per plant.



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	4.83343	0.11097	43.55	<0.0001
Release	1	0.03252	0.01835	1.77	0.0823

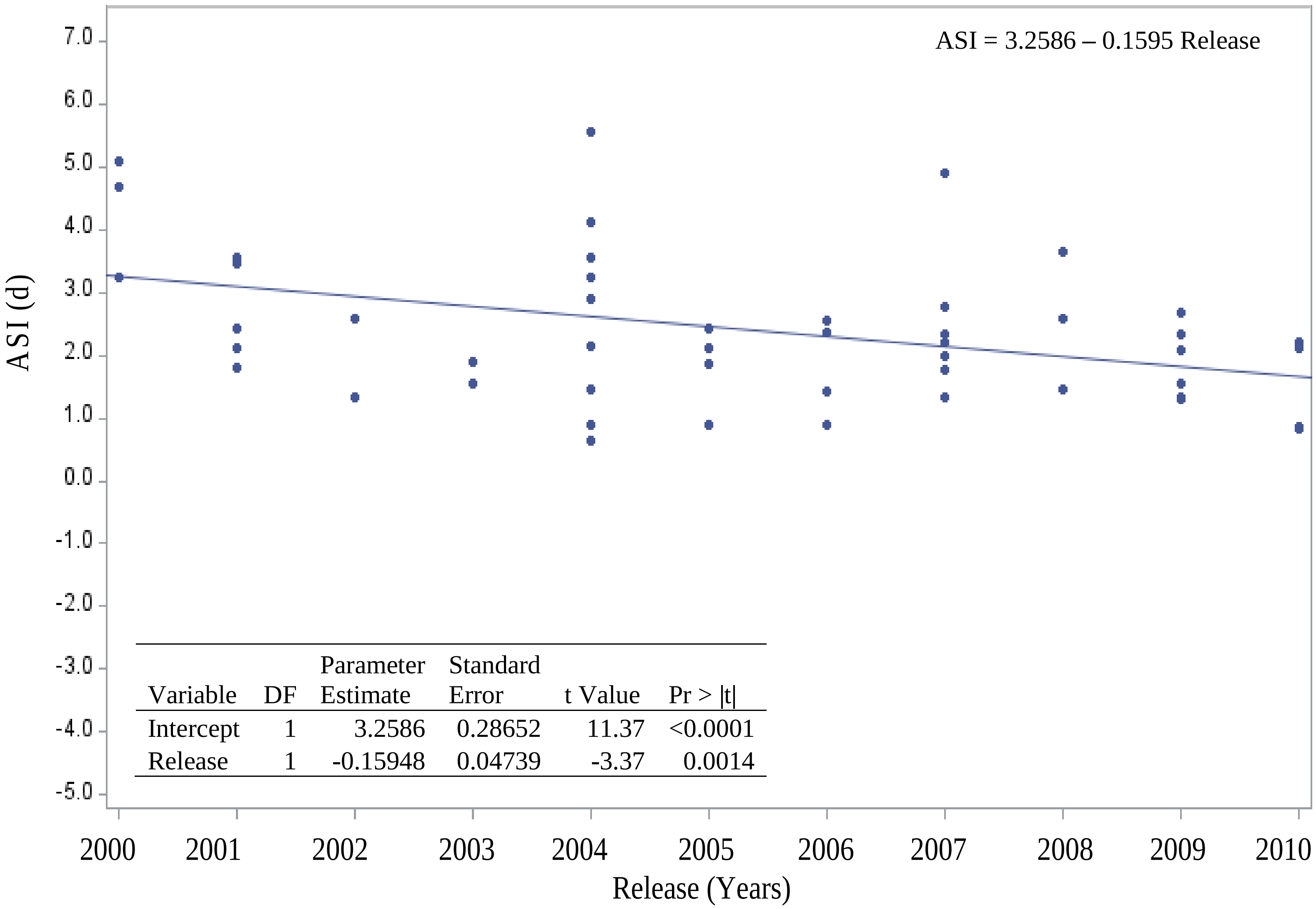
GYG = 4.8334 + 0.0325 Release

Figure 4.10 Genetic gain in yield for drought tolerance in CIMMYT hybrids released from 2000 to 2010 under managed drought stress



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	83.96303	0.91626	91.64	<0.0001
Release	1	0.09539	0.15154	0.63	0.5318

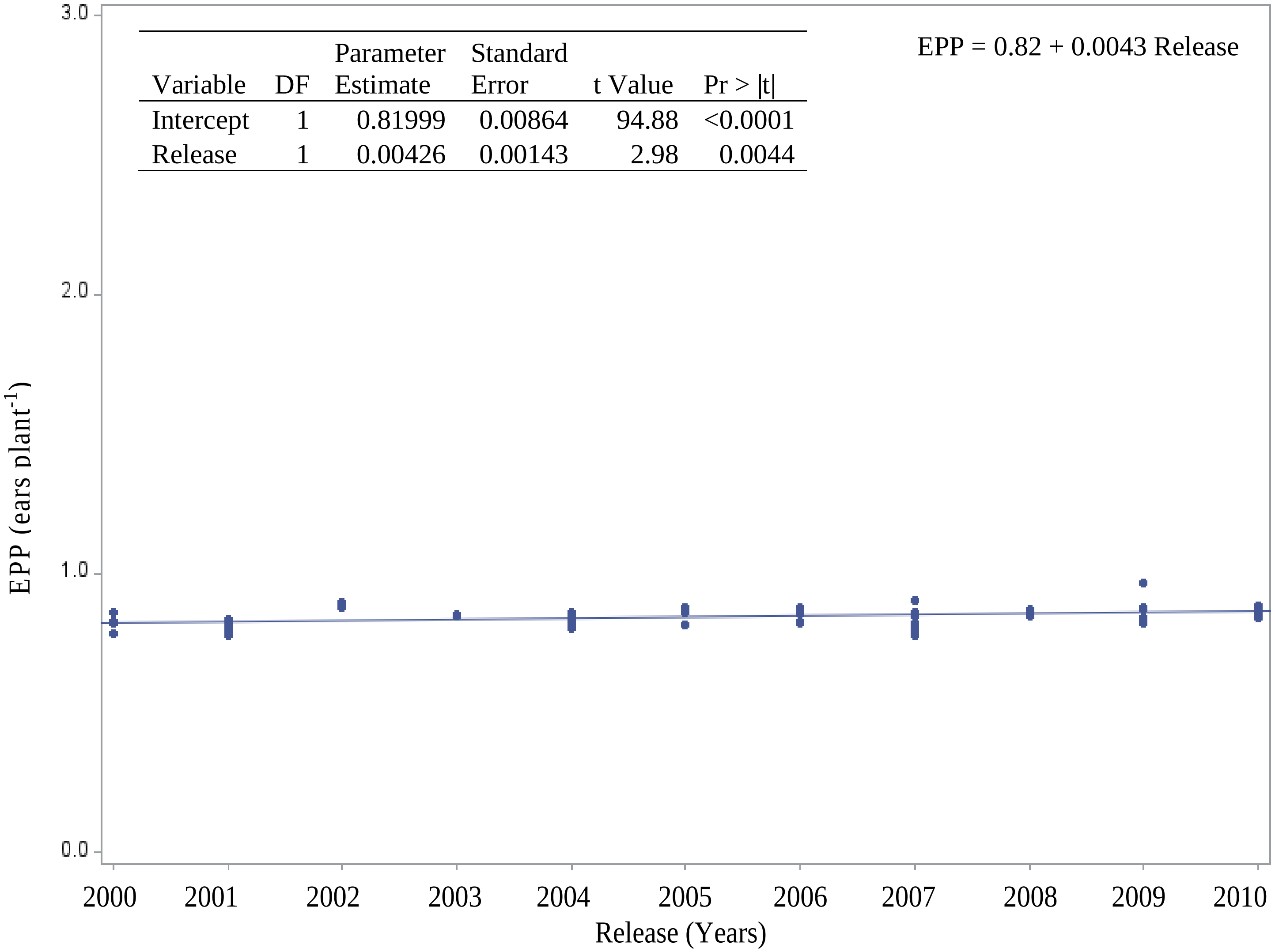
Figure 4.11 Changes in days to mid-anthesis in CIMMYT hybrids released from 2000 to 2011 under managed drought stress



ASI = 3.2586 – 0.1595 Release

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	3.2586	0.28652	11.37	<0.0001
Release	1	-0.15948	0.04739	-3.37	0.0014

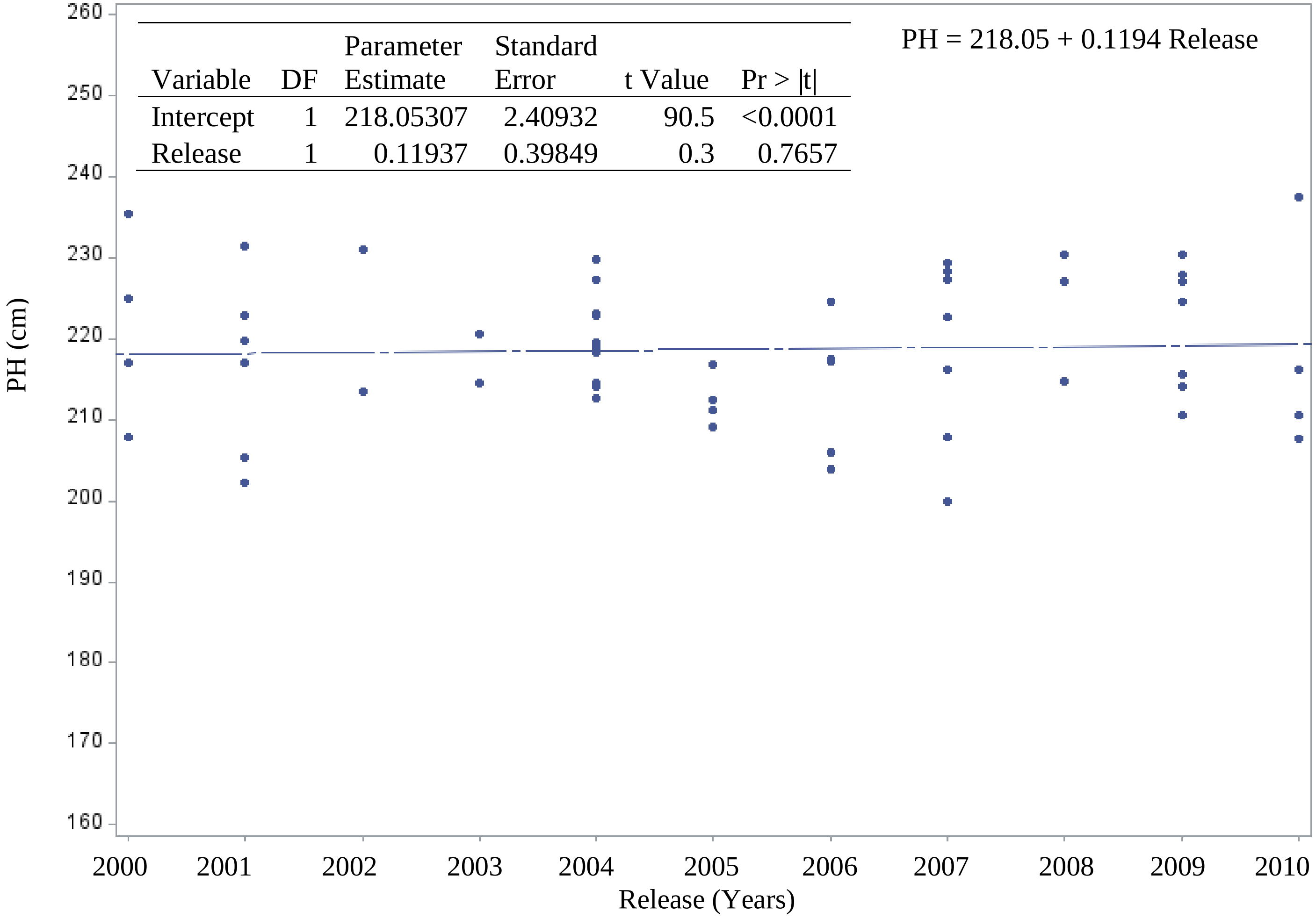
Figure 4.12 Changes in ASI in CIMMYT hybrids released from 2000 to 2011 under managed drought stress



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.81999	0.00864	94.88	<0.0001
Release	1	0.00426	0.00143	2.98	0.0044

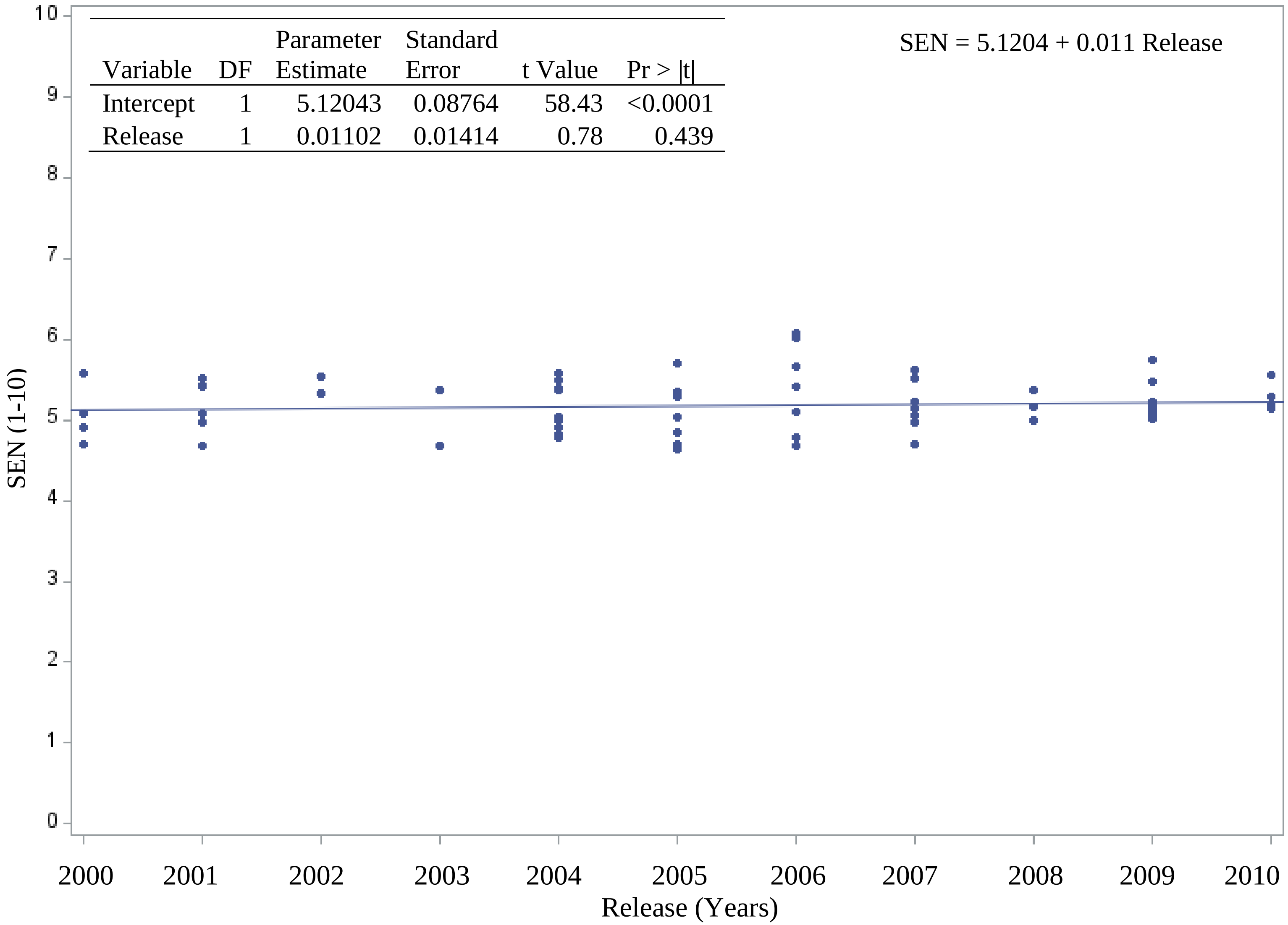
EPP = 0.82 + 0.0043 Release

Figure 4.13 Changes in number of ears per plant in CIMMYT hybrids released from 2000 to 2011 under managed drought stress



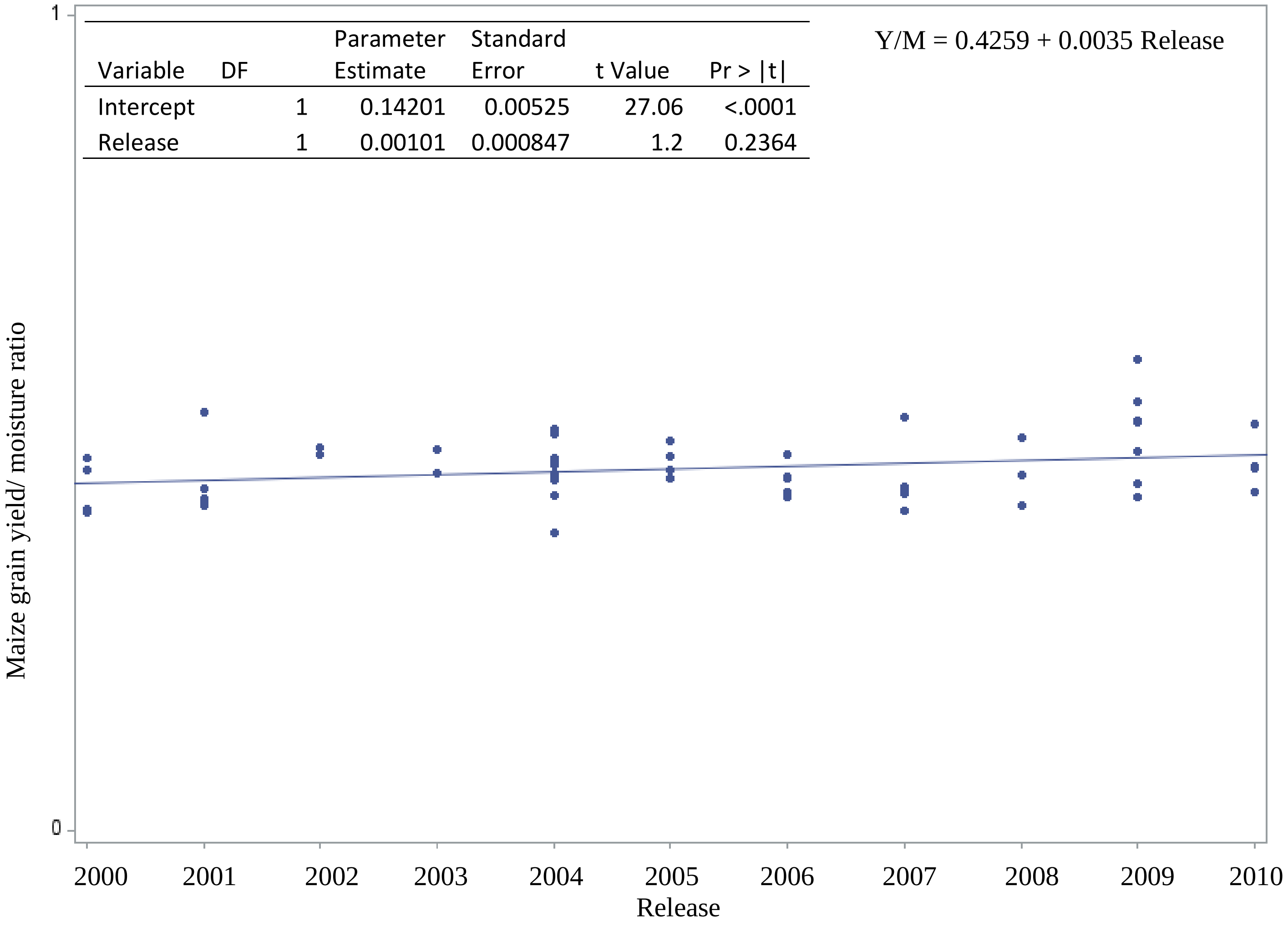
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	218.05307	2.40932	90.5	<0.0001
Release	1	0.11937	0.39849	0.3	0.7657

Figure 4.14 Changes in plant height in CIMMYT hybrids released from 2000 to 2011 under managed drought stress



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	5.12043	0.08764	58.43	<0.0001
Release	1	0.01102	0.01414	0.78	0.439

Figure 4.15 Changes in senescence in CIMMYT hybrids released from 2000 to 2011 tested under managed drought stress



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.14201	0.00525	27.06	<.0001
Release	1	0.00101	0.000847	1.2	0.2364

Figure 4.16 Changes in senescence in CIMMYT hybrids released from 2000 to 2011 tested under managed drought stress

4.3.6 Dissection of genetic gain in yield of CIMMYT hybrids released from 2000 to 2010

4.3.6.1 Correlation of yield and secondary traits under random drought stress

The change in yield was associated with some physiological and phenological changes. The following observations were made from an investigation of the relationship of grain yield and some secondary traits. The correlation between grain yield and days to mid-anthesis was negative at -0.374 (Table 4.9). Correlations for grain yield with anthesis-silking interval and senescence were both negative at -0.319 and -0.312 respectively. Plant height and the number of ears per plant had positive correlations with yield of 0.357 and 0.473 respectively even though plant height did not significantly change over the 11 years under random drought stress. Under random drought stress yield was higher in hybrids that flowered early, that had reduced barrenness, with shorter anthesis-silking interval and low senescence rates.

Table 4.9 Genetic correlations of grain yield and secondary traits under random drought stress

	GYG	**AD**	**ASI**	**PH**	**EPP**	**SEN**	**Y/M**
GYG	-						
AD	-0.37***	-					
ASI	-0.32***	0.22***	-				
PH	0.36***	-0.16***	-0.24***	-			
EPP	0.47***	-0.42***	-0.27***	-0.08*	-		
SEN	-0.31**	-0.60***	-0.02^{ns}	-0.05^{ns}	0.01^{ns}	-	
Y/M	0.67***	-0.82***	-0.31*	-0.31**	0.95***	-0.085^{ns}	-

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$, GYG is grain yield, AD is days to mid-anthesis, ASI is anthesis-silking interval, PH is plant height, EPP is number of ears per plant, SEN is leaf senescence, Y/M is grain yield/ grain moisture content ratio

Anthesis-silking interval was higher in hybrids that flowered late (r = 0.220) and in shorter hybrids (r = -0.235). Days to mid-anthesis had negative correlations with plant height at -0.158, number of ears per plant at -0.424 and senescence at -0.595. Shorter plants that were probably stunted flowered late, had fewer ears or were more barren and senesced at a faster rate compared to the taller or normal plants. Barrenness was higher at high anthesis-silking interval that was shown by the negative correlation (-0.266). There was no significant correlation between senescence and anthesis-silking interval as well as plant height and the number of ears per plant. The correlation of grain yield/ grain moisture content ratio with yield and the number of ears per plant was positive at 0.67 and 0.95 respectively.

4.3.6.2 Correlations of grain yield and secondary traits under managed drought stress

Grain yield showed positive correlations of 0.298 (Table 4.10) with days to mid-anthesis, 0.521 with plant height and 0.493 with the number of ears per plant and negative correlations of -0.229 with anthesis-silking interval and -0.394 with senescence. Under managed drought stress grain yield was higher in taller hybrids that had reduced barrenness or increased number of ears per plant, shorter anthesis-silking interval and low rates of senescence. The positive correlation between days to mid-anthesis and grain yield indicates possible benefits of a longer season but from the genetic gain analysis, there was no net change with respect to maturity range.

Table 4.10 Correlation for grain yield and secondary traits under managed drought stress

	GYG	AD	ASI	PH	EPP	SEN
GYG	-					
AD	0.298***	-				
ASI	-0.229***	0.087*	-			
PH	0.521***	0.293***	-0.179***	-		
EPP	0.493***	0.248***	-0.064ns	0.123**	-	
SEN	-0.394***	-0.063ns	0.128*	-0.357***	-0.113*	-
Y/M	0.99***	-0.20^{ns}	0.06^{ns}	0.13^{ns}	0.99***	0.75***

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$, GYG is grain yield, AD is days to mid-anthesis, ASI is anthesis-silking interval, PH is plant height, EPP is number of ears per plant, SEN is leaf senescence, Y/M is grain yield/ grain moisture content ratio

The relationship between days to mid-anthesis and anthesis-silking interval was very weak even though positive at r = 0.087. The hybrids that flowered late were taller (r = 0.293) with reduced barrenness (r = 0.248). There was no significant relationship between days to mid-anthesis and senescence at $P \leq 0.05$. Anthesis-silking interval was higher in shorter hybrids (r = -0.179) and in hybrids that had high senescence rates (r = 0.128) but both relationships were weak. There was no significant relationship between anthesis-silking interval and barrenness or the number of ears per plant. Senescence was lower in taller hybrids (r = -0.357) and higher in shorter plants that might have been stunted due to drought stress. Barrenness was lower in the hybrids that attained normal growth (tall) having a high number of ears per plant (r = 0.123) and higher in hybrids with high senescence rate (r = -0.113). Grain yield/grain moisture content ratio positively correlated with grain yield and the number of ears per plant (barrenness).

4.4 Discussion

In this study under random drought stress, an estimated net genetic gain of 22.7 kg ha^{-1} year^{-1}

(0.85% year^{-1}) over the 11 years from 2000 to 2010 was recorded while 32.5 kg ha^{-1} year^{-1} (0.85% year^{-1}) was recorded under managed drought stress. New hybrids yielded significantly better than old hybrids. This is good progress for ESA. In Iowa, Central USA, a genetic gain of 74 kg ha^{-1} year^{-1} was reported over a period of 70 years from 1934 to 1991. Genetic gain was reviewed across different stresses including optimal conditions, drought and water logging stress (Duvick, 1997). In WCA under drought stress an estimated genetic gain of 13.5 kg ha^{-1} (1.1%) year^{-1} was reported by Badu-Apraku *et al*. (2013). The estimated genetic gains in ESA are comparable to other findings by Badu-Apraku *et al*. (2013) and to the preliminary estimates of 18 kg ha^{-1} (0.6-1.0%) year^{-1} under drought stress projected for SSA by Edmeades (2013). Genetic gain in maize is due to improvements in the plant system efficiency and stress tolerance (Duvick, 2005b). Improvement of yield under drought stress has been associated with changes in secondary traits closely associated with yield and some physiological processes. This study evaluated the effect of different secondary traits on grain yield.

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. When grain yield was regressed against days to mid-anthesis, a negative correlation ($r = -0.37$) was observed under random drought stress while a positive correlation ($r = 0.30$) was recorded under managed drought stress. According to Campos *et al*. (2006) low phenotypic correlation between grain yield and days to mid-anthesis suggests genetic tolerance in the materials. What can vary in such a situation could be the levels of stress tolerance such that the old hybrids had low levels of stress tolerance compared to the new varieties. Duvick (2005b) explains that selection started with the farmers and achieved some adaptation to local environments and growing conditions. This then will mean that the old hybrids may have some stress tolerance but the level of tolerance may not be as high as in the new hybrids or may be significant under mild stress levels and not under severe stress. Selections by farmers may not have been directly towards stress tolerance but may be indirectly as they selected for high yield. Selection by breeders is now directed towards both yield and stress tolerance.

Under drought stress mid-silking may be delayed and at times may not be reached (Bolaños and Edmeades, 1996). In this study ASI was shorter in the new varieties compared to the old varieties (Figures 4.5 and 4.11). Based on literature the new hybrids therefore had better chances for pollination and grain set compared to the old varieties because of the shorter ASI. ASI has shown consistent correlation with yield in earlier studies (Bolaños and Edmeades, 1993a; Bolaños and Edmeades, 1996; Campos *et al*., 2006). In the current study, correlation of grain yield and ASI was negative at -0.32 and -0.23 under random- and managed drought stress respectively. Grain yield decreased with increase in ASI. Badu-Apraku *et al*. (2013) reported a similar observation. On average the new varieties had shorter ASI and yielded better than the old hybrids that had high ASI. This finding agrees with earlier studies where ASI was observed to be shorter in new varieties and yield improvement was associated with this change (Bolaños and Edmeades, 1993a; 1993b; Bruce *et al*., 2002; Monneveux *et al*., 2005; Campos *et al*., 2006; Edmeades, 2013). There was positive genetic change for ASI under random and managed drought in the CIMMYT ESA maize breeding program from 2000 to 2010. ASI is an important trait for selection under drought stress (Cairns *et al*., 2013). This study showed that genetic variability for ASI still exists under drought stress and therefore ASI can still be used in selection for drought stress tolerance.

Drought sometimes induces barrenness. The new hybrids from the CIMMYT ESA programme showed reduced barrenness. The number of ears per plant was higher in the new varieties compared to the old varieties. The change in number of ears per plant over the 11 years under managed drought stress was 0.004. Grain yield positively correlated with the number of ears per plant at 0.47 and 0.49 under random and managed drought stress respectively, increasing with increasing number of ears per plant or with reduced barrenness. These findings agree with other studies (Bolaños and Edmeades, 1993b; Bolaños and Edmeades, 1996; Bruce *et al*., 2002; Monneveux *et al*., 2005; Campos *et al*., 2006; Monneveux *et al*., 2008; Badu-Apraku *et al*., 2013; Edmeades 2013). Even though the grain yield/ grain moisture content ratio did not change over the 11 years evaluated, it positively correlated with grain yield and the number of ears per plant under both random and managed drought stress. Grain yield/grain moisture content ratio can therefore be used a secondary trait in selecting for improved grain yield under stress environments as recommended by Ngure (1995).

A study by Badu-Apraku *et al*. (2013b) reported reduced plant height over time associated with increasing yield. In other earlier studies elsewhere plant and ear height showed irregular trends (Duvick, 2005a) and no changes in a study by Tollenaar and Lee (2011). The current study showed no significant change in plant height under random and managed drought stress over the 11 year period covered but showed a trend to decrease at a rate of 0.39 cm year^{-1} over the 11 years under random drought stress and an increase of 0.12 cm under managed drought stress. This is probably an adjustment to drought stress where by the plants improve dry matter partitioning to the ear during ear initiation, silking, seed setting and grain filling (Duvick, 2005b; Tollenaar and Lee, 2006). The correlation of yield and plant height was 0.36 and 0.52 under random and managed drought stress respectively.

There was significant genetic gain for yield under random drought stress. This was associated with changes in some secondary traits that affect final grain yield such as ASI and barrenness. The new hybrids showed better drought stress tolerance compared to the old hybrids.

4.5 Conclusions

The net estimated genetic gain in yield in this study was 22.7 kg ha^{-1} year^{-1} (0.85% year^{-1}) under random drought stress and 32.5 kg ha^{-1} year^{-1} (0.85 % year^{-1}) under managed drought stress. This was associated with a reduction in ASI of 0.16 d year^{-1} under managed drought stress and reduced barrenness. Yield strongly negatively correlated with ASI and positively with reduced barrenness, grain yield/ grain moisture content ratio, plant height and days to mid-anthesis under managed drought but negatively with days mid-anthesis under random drought stress. The correlation between yield and plant height was strong and positive. Selection for drought in the CIMMYT ESA maize breeding programme from 2000 to 2010 was effective and produced better hybrids each year.

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Chapter 5

Genetic gain for tolerance to low nitrogen stress in Eastern and Southern Africa

Abstract

Low soil fertility is one of the major constraints faced by farmers in East and Southern Africa (ESA) in crop production. The staple crop for most countries in ESA is maize and its cultivation is affected by low soil fertility. The major limiting nutrient is nitrogen (N). Efforts to breed for low N tolerance in maize started in CIMMYT ESA in 1997. The programme has produced varieties that perform better than older varieties under low N stress and have no yield penalty under optimal conditions. Some of the varieties have been disseminated throughout ESA through stakeholders in the public and private sector. This study investigated the genetic gain in low N tolerance in CIMMYT hybrids tested from 2000 to 2010 in multi-environment trials evaluated from 2011 to 2013. Newer varieties yielded better under low N conditions compared to the older varieties. The study indicated an estimated genetic gain in yield of 20.9 kg ha^{-1} year^{-1}. The new varieties were more stress tolerant, exhibiting shorter anthesis-silking interval compared to old varieties under low N stress that decreased at a rate of 0.08 d year^{-1}. That indicated redirecting of dry matter partitioning in favour of the ear. A decrease in plant height of the new hybrids at a rate of 0.49 cm year^{-1} compared to the old hybrids also indicated redirecting of dry matter partitioning to the ear during ear initiation and early development as an adaptation mechanism. Successful breeding produced hybrids that performed better, yielded better and were more stress tolerant under low N conditions compared to the old hybrids.

5.1 Introduction

5.1.1 The problem of low N use among small holder farmers

The use of fertiliser and other soil improvement practices to maintain high productivity and fertility of soils in the communal farming sector in sub-Saharan Africa (SSA) is low and limited. Tropical soils with low inherent fertility combined with low fertiliser use have resulted in low maize production in SSA (Morris *et al.*, 2007; Waddington and Heissy, 1997). Fertiliser application in SSA is reported to be less than 10 kg ha^{-1} (Morris *et al.*, 2007; FAO, 2001). Southern Africa's adoption of newly developed maize varieties was high at 55% in 1996 but average yields in the region, excluding South Africa, averaged 1.1 t ha^{-1}. Fertiliser use in Africa is low compared to other regions in the world as illustrated in Figure 5.1 (FAOSTAT, 2013).

The low fertiliser use in the SSA region has resulted in low maize yields and total productivity as shown in Figure 5.1. The wide adoption of improved varieties and low fertiliser use reflect the high fertiliser cost in Africa and cash constraints faced by farmers (Smale *et al*., 2013). In a study conducted in Zimbabwe and Malawi the average fertiliser use was 80 kg ha^{-1} and 14.8 kg ha^{-1} respectively (Zambezi and Mwambula, 1997). All these rates are well below the minimum recommended for production, even under rainfed conditions. In a study conducted in Zambia, maize grain yield under small scale farmers' conditions was observed to be low at district averages of 0.58 t ha^{-1} to 3.1 t ha^{-1} mainly due to low N use (Miti *et al*., 2010) as farmers lack cash or credit to access fertilisers (Heisey and Mwangi, 1996). In SSA fertiliser use initially rose in the 1970s to 1980s when fertilisers were subsidised and the market was controlled by the governments (Morris *et al*., 2007) but declined following the withdrawal of subsidies and liberalisation of the fertiliser market in the 1990s (Heisey and Mwangi, 1996; Smale and Jayne, 2003; Morris *et al*., 2007). Reduced fertiliser use is due to a number of reasons. The fertiliser to maize price ratio is high. The fertiliser to maize price ratio went up from 0.9 in 1986 to 2.7 in 1993 (Mungoma and Mwambula, 1997) and was reported at 2.6 in 2007 in Zambia (Miti *et al*., 2010). Fertiliser use is affected by both supply and demand factors. The fertiliser demand in SSA is weak due to high fertiliser prices, low and highly variable crop prices that make fertiliser use unprofitable and lack of knowhow on appropriate use. Supply is poor as markets and demand are dispersed, demand is low, transport costs are high and profitability is low (Morris *et al*., 2007). Low fertiliser use remains a problem in SSA that needs to be addressed.

Promoting fertiliser use was initially done through subsidies and market control but this was not sustainable for the governments (Morris *et al*., 2007). As an alternative and practical potential intervention to the problem of inherently poor soil fertility and low fertiliser use in maize production in SSA, the development of low N tolerant cultivars was proposed. Breeding for low N has been on going, but this only alleviates the problem of low productivity under low N for resource poor small holder farmers in SSA who cannot afford sufficient N fertiliser or have no access. Adding N, however, enables full exploitation of the genetic potential of the varieties (Edmeades and Bänziger, 1997).

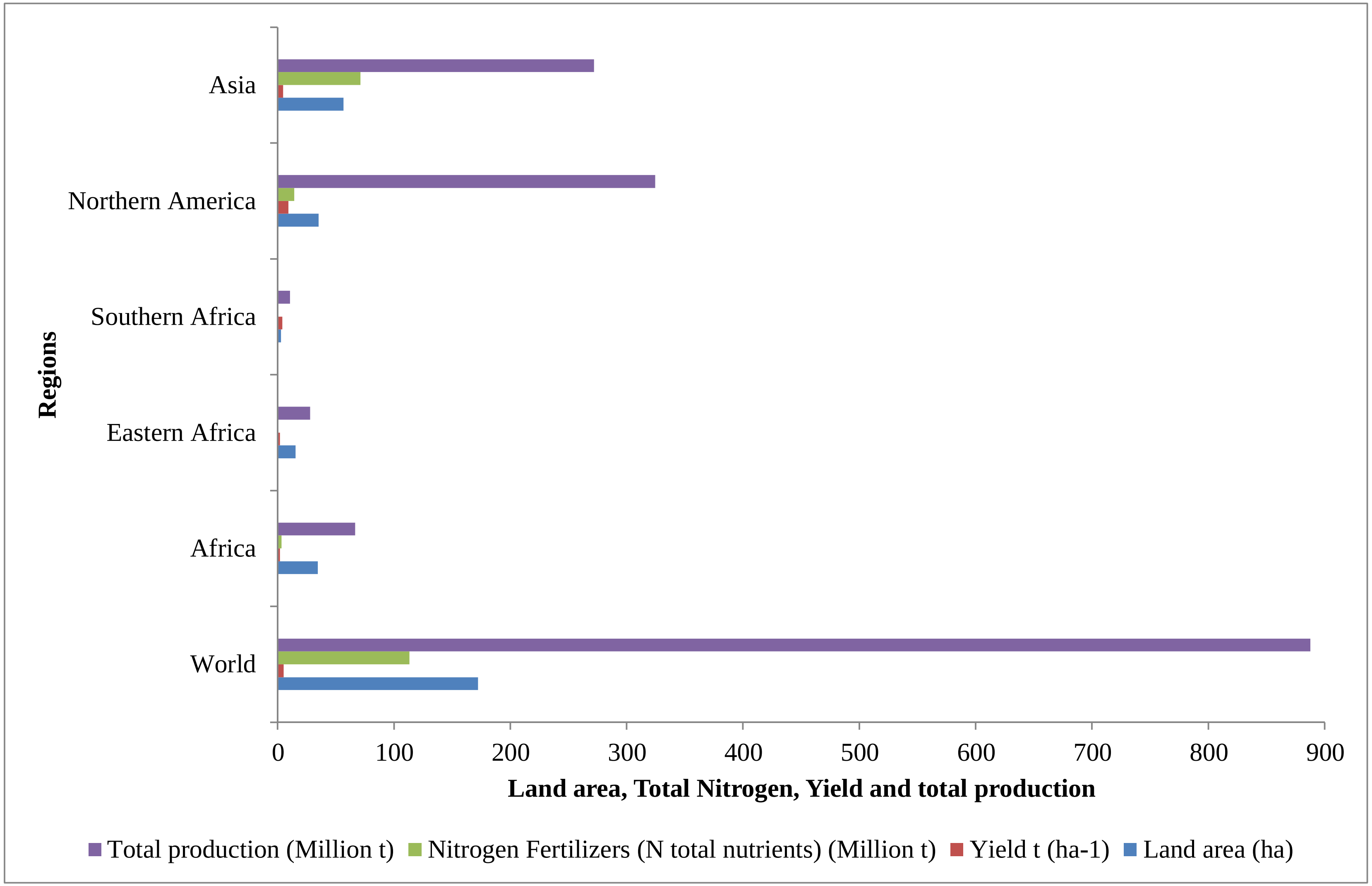


Figure 5.1 Fertiliser use in SSA (2011) compared to other regions of the world (FAOSTAT, 2013)

Improved grain yield performance under low N levels in selected low N stress tolerant hybrids can be attributed to improved N use efficiency. The low N tolerant cultivars have improved N use efficiency that comprises of improved N uptake efficiency and N utilisation efficiency (Moll *et al*., 1982). Improved performance under low N levels is a result of selection under multi-environments that include optimal, low N and other stress environments (Miti *et al*., 2010; Weber *et al*., 2012). Some varieties selected for drought tolerance have been observed to exhibit low N tolerance but the opposite has not been observed (Duvick, 1997). To complement efforts of governments in trying to improve small holder farmers' accessibility to inorganic fertiliser, breeders have been trying to mitigate the problem of low N use in maize production systems among small holder farmers in SSA through the development of maize cultivars that are tolerant to low N. Breeding for low N tolerance is a major area of focus in CIMMYT (Monneveux *et al*., 2005). New hybrids that are low N tolerant compared to the older hybrids have been developed for different regions (McCullough *et al.*, 1994). The CIMMYT breeding programme started developing low N tolerant materials in 1975 (Hess, 1997) but for the ESA programme since 1997 (Bänziger *et al*., 2006). However, the constitution of low N trials was low between 2001 and 2009 at less than 15% of the 376 early and 328 late ESA regional trials (Weber *et al*., 2012). Evaluation of the low N trials included assessment of yield and related secondary traits. The limit in proportion of low N trials could be due to challenges in designating low N plots that are permanently depleted and maintained at low N levels. Hybrids developed in the CIMMYT drought and low N breeding programme were released and some were disseminated through collaborators.

Secondary traits have been used to indirectly evaluate varieties for low N use tolerance, improving the precision of selecting for candidate genes (Monneveux *et al*., 2005). Such traits include days to mid-anthesis, days to mid-silking, plant height, ear height, green leaf number and chlorophyll content assessed using SPAD meter measurements at two, four and six weeks after mid-silking. Grain N levels and harvest index can also give an indication of N uptake during the grain filling period (Tollenaar and Wu, 1999).

One of the important goals of breeders has been to enhance stability of performance of maize under stress. Waddington and Heissy (1997) suggested that the development of N tolerant varieties complemented with the modest use of fertilisers would potentially improve yields in

Southern Africa. Increase in stress tolerance has resulted in improved yield and wider adoption of new varieties.

Tollenaar and Lee (2006) reported that yield response varies with the environment and season. Increased grain yield could be a result of the interaction of the genetic potential of a variety with the ability to tolerate limited resource stress and other general stresses. Faced with frequent droughts and limited access to fertiliser, resource poor farmers can benefit from improved varieties that have been observed in some studies conducted, to be more stress tolerant compared to older hybrids (Tollenaar and Wu, 1999) enabling the new varieties to yield better, up to around 2 t ha^{-1} under low N and water stress conditions.

The CIMMYT maize breeding programme has developed a number of drought and low N tolerant varieties. Success has been recorded in some SSA countries including Malawi (The DTMA Platform, 2009; Smale *et al.*, 2013). The drought tolerant varieties have been tested in the regional trials each year and some were released through the public and private sector. 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 aimed to establish if there has been any genetic gain in low N tolerance in CIMMYT hybrids released by the ESA maize programmes from 2000 to 2011 since the hybrids were screened for compound stress tolerance under multi-environment testing that included optimal, drought, low N and disease stress environments.

5.1.2 Aim and objectives

The aim of this study was to evaluate the net genetic gain in low N tolerance of the CIMMYT drought tolerant hybrids tested in the breeding programme from 2000 to 2010. The main objective was to evaluate genetic gain in yield and changes in secondary traits of CIMMYT drought tolerant hybrids tested by the ESA maize programme from 2000 to 2010. The specific objectives of this study were:

i) To evaluate the net genetic gain in yield under low N stress of CIMMYT hybrids tested by the ESA maize programme in regional trials from 2000 to 2010
ii) To evaluate the changes in secondary traits under low N stress of CIMMYT hybrids tested by the ESA maize programme in regional trials from 2000 to 2010

iii) To dissect the genetic gain in yield under low N in CIMMYT hybrids tested by the ESA maize programme in regional trials from 2000 to 2010

5.2 Materials and method

5.2.1 Germplasm

The same 67 hybrids checked with three commercial varieties (Appendix I) described in Chapter 3 section 3.2.were evaluated under low N. SC513, SC635 and SC727 were used as checks.

5.2.2 Trial sites

The materials were evaluated under low N stress in five sites (Appendix III) over two seasons from 2011/12 to 2012/13. Site 1, Harare2012, was evaluated at the CIMMYT station in Harare (-17^{o}50'S, 31^{o}2'E, 1483 masl) in the 2011/12 season and Site 2 Kiboko2012) was planted in March 2012 in Kiboko (2^{o}12'N, 37^{o}43'E, 1000 masl). The five sites were depleted and managed as described in Section 5.2.4. Sites 3 and 4, Harare2013a and b, were evaluated in the 2012/13 season in one low N block (-17^{o}50'S, 31^{o}1'E, 1483 masl) different from the one used for evaluation of Harare2012 but also at the CIMMYT station in Harare. CIMMYT Harare and Kiboko locations have red clay soils. Harare2012 was planted in November 2011 in Harare under low N. Harare2013a and b were planted in December 2012. Kiboko2013 was planted in March 2013 in Kiboko.

5.2.3. Field layout

The experiment was an alpha lattice design with three replications in Harare2012 in the 2011/12 season and Kiboko2012 and 2013 and two replications for Harare2013a and b in the 2012/13 season. The hybrids were planted in 2 x 4 m row plots in Harare and in 2 x 5 m row plots in Kiboko, spaced at 0.75 m between rows and 0.25 m within the row to give a plant population of 53 000 plants ha^{-1}. At harvesting the row end plants were discarded to eliminate border effects, leaving a net plot length of 3.5 m in Harare and 4.5 m in Kiboko.

5.2.4 Trial management

The experiment was evaluated on low N plots that had been depleted using a uniform wheat crop in winter and maize experiment with no N application in summer over a period of three to five seasons in Harare. Fields were depleted up to 10 ppm Nitrate N. When the experiment was carried out the low N blocks had been running for over 15 years, five, two and three years respectively for Harare2012, Harare 2013a and b and for kiboko2012 and 2013. Based on soil tests the block used for the Harare2013a and b trials was at 1.52 ppm, 2.13 ppm and 2.58 ppm nitrate-N at 0-30 cm, 31-60 cm and 61-90 cm depth respectively at the beginning of the season. In Harare all plots received 56 kg P ha^{-1} and 28 kg K ha^{-1} at sowing as single super phosphate and muriate of potash respectively. In Kiboko all plots received 90 kg P ha^{-1} as basal dressing at planting and 23 kg ha^{-1} N as top dressing. Weeds, pests and diseases were controlled where and when necessary. In Harare and Kiboko trials received 50 mm irrigation at planting and 50 mm seven days later for emergence. In Kiboko trials were also irrigated at 4 and 6 weeks after planting, at flowering and once during the grain filling period.

5.2.5 Data recorded

Data on days to mid-anthesis and days to mid-silking was recorded during flowering (Table 5.1). Normalised difference vegetation index (NDVI) was measured when the crop was 25 to 30 cm high. NDVI was recorded using a portable spectroradiometer (Greenseeker, NTech Industries, CA, USA) that was held at 60 cm above the crop canopy. Chlorophyl content (SPAD) measured by the SPAD meter (SPAD-502, Minolta, Tokyo, Japan) and senescence were recorded at two, four and six weeks after mid-silking. Traits including plant height, ear height, husk cover, root and stem lodging and plant stand at harvesting were recorded within a week or two before harvesting. At harvesting the number of ears, ear rots, ear texture, ear aspect, field weight, grain weight and grain moisture were recorded for each plot. In Kiboko further grain analysis was done that included 200 kernel sample weight and using the near infra-red (NIR) grain analyser grain oil, protein and starch content. The number of kernels per hectare (KPHA) was calculated from the average of 200 kernel sample weight for each plot. All traits were assessed according to the standards described in Appendix IV.

Table 5.1 Traits recorded in low N trials

Secondary traits (phenology)	Secondary traits (stress tolerance)	Direct yield and grain quality assessment	Yield components traits
Days to mid-anthesis Days to mid-silking Plant height Ear height Root lodging	NDVI- Normalised Difference Vegetation Index Senescence Disease scores Number of ears per plant SPAD	Field weight and / or grain weight Grain moisture KPHA Grain oil, protein and starch content	Plant stand Ear aspect Grain texture Ear rots Number of ears per plant Ear rots

5.2.6 Statistical analysis

Data was processed and analysed as described in Chapter 3 section 3.2.6.

5.3 Results

5.3.1 Analysis of variance of hybrid performance under low N stress trials

Grain yield of hybrids at all five sites standardised to 12.5% moisture content varied significantly at $P \leq 0.001$ (Table 5.2). Grain yield was significantly lower in Harare2012 and Harare2013b where the maximum grain yield was 3.65 t ha^{-1} and 3.75 t ha^{-1} respectively, that is in both cases below or not different from the across site average for the five sites of 3.77 t ha^{-1}. The average grain yield for the other sites was 7.73 t ha^{-1}, 6.23 t ha^{-1} and 4.80 t ha^{-1} for Kiboko 2012, Harare2013b and Kiboko2013 respectively. The heritability was high at 0.62 for Harare2012 and Kiboko2012 and low at 0.20 and 0.11 for Harare2013a and 2013b respectively.

Days to mid-anthesis of the hybrids varied significantly among the hybrids in Harare2012 and 2013a, and in Kiboko2012 and 2013 at $P \leq 0.001$. There were no significant differences in Harare2013b. The heritability for days to mid-anthesis in the trial was high at more than 0.92 in all four sites. Mid-anthesis in Harare2012 was delayed with the number of days to mid-anthesis ranging from 68.5 to 84.9. The mean for the site was 72.44 d. For Kiboko2012 days to mid-anthesis ranged from 59.2 to 75.1 with the mean at 66.97. Days to mid-anthesis in Harare2013a ranged from 65.0 to 77.9 with a trial mean of 72.8. Days to mid-anthesis in Kiboko2013 ranged from 53.0 to 66.2 with an average of 61.5. The trial mean across sites was 75.8.

Anthesis-silking interval significantly differed among hybrids tested in Harare2012 and Kiboko2013 at $P \leq 0.001$, in Kiboko2012 at $P \leq 0.01$ and in Harare2013b at $P \leq 0.05$. There were no significant differences in Harare2013a. The heritability for ASI across all five sites was moderate to high, ranging from 0.27 in Harare2013b to 0.55 in Harare2013a. In Harare2012 ASI ranged from -1.70 d to 5.42 d. The average ASI for the site was 1.58 d. For Kiboko2012 ASI ranged from -0.28 d to 5.08 d with a trial mean of 2.06 d. For Harare2013a the ASI ranged from 0.07 d to 5.16 d and the trial average was 2.78 d. ASI in Kiboko2013 ranged from 1.4 d to 3.9 d with an average of 1.8. The across site trial mean was 2.14 d. Harare2012 had the widest range of ASI.

Table 5.2 Analysis of variance for low N trials across sites and two seasons

	Site	Mean	LSD (0.05)	MSe	h2	p	Min	Max	Across site mean
Grain yield (t ha^{-1})	Harare2012	2.36	0.88	0.35	0.62	***	1.21	3.65	3.77
	Kiboko2012	5.92	1.49	1.19	0.62	***	3.03	7.73	
	Harare2013a	2.66	1.00	0.53	0.20	***	1.37	3.75	
	Harare2013b	4.15	1.35	2.06	0.11	***	2.75	6.23	
	Kiboko2013	3.59	0.82	0.10	0.50	***	2.73	4.80	
Days to mid-anthesis (d)	Harare2012	77.57	2.60	4.11	0.92	***	68.52	84.92	72.44
	Kiboko2012	66.97	1.91	3.21	0.94	***	59.21	75.12	
	Harare2013a	72.77	1.95	1.03	0.94	***	65.02	77.89	
	Harare2013b	72.57		1.37	0.92	ns	64.14	79.46	
	Kiboko2013	61.50	1.70	0.30	0.94	***	53.00	66.20	
Anthesis-Silking interval (d)	Harare2012	1.58	2.32	2.10	0.53	***	-1.70	5.42	2.14
	Kiboko2012	2.06	2.22	1.93	0.38	**	-0.28	5.08	
	Harare2013a	3.36		2.85	0.55	ns	0.90	6.74	
	Harare2013b	2.78	2.67	2.15	0.27	*	0.07	5.16	
	Kiboko2013	1.8	1.8	0.0	0.49	***	-1.4	3.90	
Number of ears per plant	Harare2012	0.83	0.22	0.02	0.18	ns	0.58	0.97	0.79
	Kiboko2012	0.79	0.10	0.00	0.50	***	0.63	1.00	
	Harare2013a	0.87	0.21	0.01	.	ns	0.70	1.07	
	Harare2013b	0.88	0.18	0.01	0.28	ns	0.72	1.16	
	Kiboko2013	0.82	.	0.0	0.26	ns	0.64	0.88	
Plant height (cm)	Harare2012	152.6	23.1	251.9	0.62	*	136.7	179.3	171.0
	Kiboko2012	181.3	19.0	333.7	0.61	***	151.6	211.4	
	Harare2013a	169.4	28.1	253.8	0.20	**	120.9	200.1	
	Harare2013b	181.1	24.6	358.6	0.11	***	149.5	206.2	
	Kiboko2013	180.3	12.1	90.2	0.78	***	155.1	210.1	
Senescence	Harare2012	4.6	0.6	0.2	0.90	***	3.4	6.0	4.4
	Kiboko2012	2.4	0.6	0.3	0.40	**	1.7	3.2	
	Harare2013a	4.8	0.7	0.2	0.76	***	3.7	6.4	
	Harare2013b	4.7	1.0	0.2	0.52	***	3.7	6.4	
	Kiboko2013	2.2	.	0.0	0.70	ns	1.3	3.0	
NDVI	Harare2012	0.51	0.08	0.0	0.43	**	0.41	0.60	0.5
	Kiboko2012	-	-	-	-	-	-	-	
	Harare2013a	0.50	0.08	0.0	0.00	*	0.43	0.56	
	Harare2013b	0.51	0.10	0.0	0.00	ns	0.43	0.59	
	Kiboko2013	-	-	-	-	-	-	-	
SPAD	Harare2012	29.0	11.5	53.0	0.03	ns	17.8	37.9	-
	Kiboko2012	46.9	8.9	32.4	.	ns	39.1	58.5	
	Harare2013a	28.2	9.4	23.4	0.00	ns	20.5	41.7	
	Harare2013b	30.0	-	-	.	ns	22.7	38.2	
	Kiboko2013	35.6	34.0	38.2	.	ns	34.0	38.2	

h^2 is heritability, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$, LSD (0.05) is Least significant difference, MSe is Mean square error

The average number of ears per plant for all five sites ranged from 0.79 to 0.88. There were no significant differences in the number of ears per plant (barrenness) for Harare2012, 2013a and b, and Kiboko2013. For Kiboko2012 barrenness significantly varied at $P \leq 0.001$. Number of ears per plant varied as shown in Figure 5.2 by the number of ears harvested from two plots. Each plot was 2 x 4 m rows with a total of 34 plants. The number of ears per plant ranged from 0.63 to 1.00. The mean number of ears per plant was 0.79. Heritability for this trait across sites was 0.32 for all sites.

Figure 5.2 Number of ears from a hybrid with high tolerance (a) and one with low tolerance (b) to low N stress

Plant height means for the different sites ranged from 152.65 cm to 181.38 cm. Hybrids varied significantly in plant height at $P \leq 0.05$ in Harare2012, $P \leq 0.001$ in Kiboko2012 and Harare2013b and at $P \leq 0.01$ in Harare2013a. Harare2012 in general had the shortest plants ranging from 136.78 cm to 179.34 cm. For Kiboko2012 plant height ranged from 151.66 cm to 211.47 cm. The mean heights for the other sites were 169.4 cm, 181.1 cm and 180.3 for Harare2013a and b, and Kiboko respectively. The trial mean 171.0 cm. Heritability for plant height was 0.62, 0.61, 0.20, 0.11 and 0.78 for Harare2012, Kiboko2012, Harare2013a and b respectively.

Hybrids showed varying response to low N stress in terms of senescence. For some varieties premature lower leaf senescence set in early, before flowering. Senescence started on the lower leaves with chlorosis followed by leaf firing or drying (Figure 5.3). Senescence recorded at two, four and six weeks after 50% silking varied significantly at $P \leq 0.001$ in Harare2012 and 2013a and b, and at $P \leq 0.01$ in Kiboko2012. There was no significant variation in senescence in Kiboko2013. Senescence was lowest in Kiboko2012 ranging from 1.7 to 3.2 and a mean of 2.4. The other three sites (Harare, Zimbabwe) were within the same range of senescence.

Figure 5.3 Leaf chlorosis and senescence in a low N crop (Harare2013b)

In Harare2012, 2013a and b senescence ranged from 3.4 to 6.4 and averaging 4.6, 4.8 and 4.7 respectively. The across site mean for senescence was 4.4. Heritability was high ranging from 0.40 to 0.90. Stress level should have been higher in Harare compared to Kiboko as shown by the senescence rates that were higher in Harare compared to Kiboko.

NDVI recorded at 25- to 30 cm height was not recorded in Kiboko, significantly varied in Harare2012 and 2013a but did not in Harare2013b. Heritability was high in the Harare2012 site and low for Harare2013a and b. The across site heritability, however, was relatively high for a stress environment at 0.25. For all five sites SPAD recorded at two, four and six weeks after flowering did not vary ($P \leq 0.05$).

The hybrids were ranked based on across site average yield from the highest to the least yielding. Ten CIMMYT hybrids ranked 1 to 10 (Table 5.3). Grain yield under low N stress in the top 10 CIMMYT drought tolerant hybrids ranged from 4.23 t ha^{-1} to 4.72 t ha^{-1}, above the trial mean (3.80 t ha^{-1}). The four best yielders, CKH08032, CZH0819, CKH0749 and CZH0724, were released in 2009 and 2008. CZH03022 that was released in 2004 ranked 5th, CKH09358 (2009) ranked 6th, CZH99021 released in 2000 ranked 7th and CZH04019 released in 2005 8th, CZH03006 ranked 9th and ranking 10th was CZH0935 released in 2010. Ranking in the bottom 61 to 70 positions were eight CIMMYT drought tolerant hybrids and two commercial checks. The grain yield for the CIMMYT hybrids ranking from 61 to 70 ranged from 2.74 t ha^{-1} to 3.35 t ha^{-1}. Grain yield for checks ranged from 3.00 t ha^{-1} to 3.73 t ha^{-1}. Checks ranked at 40, 64 and 68 for checks 1, 3 and 2 respectively released in 2010, 1997 and 2003 respectively.

The days to mid-anthesis in the top 10 hybrids ranged from 66.4 to 74.2 while the last 10 hybrids ranking in positions 61 to 70 ranged from 62.2 to 73.4. For the checks the number of days to mid-anthesis ranged from 69.5 to 75.8. The top 10 yielding hybrids had a narrow ASI ranging from 1.0 d to 2.6 d. Anthesis-silking interval was longer for the hybrids ranked 61 to 70 ranging from 1.6 d to 3.9 d. The top 10 performing hybrids showed slower senescence or some degree of stay green that was better than the hybrids ranked 61 to 70. Senescence recorded at two, four and six weeks after 50% silking ranged from 2.7 to 3.4 in the top 10 and from 2.7 to 4.0 in hybrids ranked 61 to 70. NDVI recorded at 25- to 30 cm height did not significantly vary between the old and new varieties (LSD = 0.10). Root lodging was relatively lower in the top 10 performing hybrids ranging from 3.7% to 26.4% compared to 5.9% to 42.9% for the hybrids ranked in position 61 to 70. The top 10 hybrids had better standability compared to those ranked from 61 to 70. Generally the top 10 hybrids had more favourable traits described above, compared to the commercial checks and bottom 10 ranked from 61 to 70.

Table 5.3 Summary of yield and secondary traits of the 10 best and 10 least performing hybrids under low N

Name	Rank	Across Grain Yield	Harare2012 Grain Yield	Kiboko2012 Grain Yield	Kiboko2013 Grain Yield	Harare2013a Grain Yield	Harare2013b Grain Yield	Anthesis	ASI	Plant Height	Ear Position	Root lodging	Ears Plant^{-1}	Husk Cover	Ear Rots	GLS	Senescence	Grain Texture	Grain moisture	Ear Aspect	Plant Aspect	NDVI	Release
		t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}	t ha^{-1}	d	d	cm	0-1	%	#	%	%	1-5	1-10	1-5	%	1-5	1-5	#	Year
CKH08032	1	4.72	3.02	8.14	4.47	2.26	5.70	69.7	1.4	170.2	0.53	6.8	0.79	5.4	5.4	2.3	3.2	2.8	18.5	2.5	2.7	0.54	2009
CZH0819	2	4.52	2.95	7.41	3.28	3.60	5.37	67.3	1.6	174.4	0.46	18.7	0.86	7.2	4.6	2.7	2.7	2.6	17.7	2.5	3.0	0.53	2009
CKH0749	3	4.48	2.80	6.47	4.44	2.88	5.82	74.2	2.4	180.3	0.54	23.9	0.87	5.3	5.2	2.4	2.7	2.9	20.3	2.4	3.1	0.56	2008
CZH0724	4	4.43	2.55	7.22	3.88	3.75	4.75	71.1	2.1	179.8	0.52	13.0	0.81	3.3	3.0	2.5	3.4	2.8	18.1	2.4	2.6	0.58	2008
CZH03022	5	4.36	2.76	7.94	3.80	2.90	4.39	69.9	1.4	183.1	0.52	3.7	0.84	5.8	2.1	2.4	3.3	2.1	18.4	2.4	2.4	0.55	2004
CKH09358	6	4.32	3.25	7.66	3.64	2.94	4.12	70.8	2.0	174.2	0.51	17.4	0.71	3.5	4.5	2.7	3.1	2.7	18.3	2.7	2.4	0.53	2009
CZH99021	7	4.30	2.51	7.52	4.00	2.80	4.65	70.3	2.6	181.1	0.52	13.6	0.82	5.1	5.2	2.6	2.9	2.3	18.2	2.5	2.9	0.53	2000
CZH04019	8	4.29	2.39	7.46	3.65	3.30	4.66	70.4	1.0	179.1	0.52	17.4	0.81	5.9	2.0	2.2	2.9	2.2	18.2	2.3	2.9	0.50	2005
CZH03006	9	4.25	3.65	6.96	3.48	2.55	4.59	68.7	1.5	179.9	0.51	26.4	0.72	4.8	3.8	2.4	3.0	2.3	18.1	2.5	2.7	0.56	2004
CZH0935	10	4.23	2.75	6.88	4.13	3.54	3.83	66.4	1.6	166.8	0.43	6.8	0.85	6.0	6.7	2.4	3.0	1.9	17.3	2.4	2.8	0.52	2010
SC727	40	3.73	2.22	7.01	4.23	2.10	3.11	75.8	1.0	194.0	0.53	16.3	0.74	1.9	2.5	2.8	3.1	2.7	20.5	2.4	3.4	0.50	2010
CZH0837	61	3.35	2.08	6.18	3.58	2.08	2.83	67.4	2.9	174.8	0.48	13.2	0.78	10.7	7.0	2.3	3.2	2.8	18.0	2.8	2.9	0.51	2009
CZH0631	62	3.34	1.80	5.10	2.93	3.06	3.82	70.1	1.6	171.3	0.47	14.2	0.83	3.7	4.3	2.7	3.1	2.7	18.9	3.1	2.8	0.48	2007
CZH00002	63	3.28	1.28	5.99	3.29	2.56	3.30	65.9	2.6	157.2	0.46	5.9	0.86	7.1	12.1	2.5	3.7	1.4	17.2	2.7	2.2	0.49	2001
SC513	64	3.23	1.68	4.79	3.59	2.46	3.63	69.5	2.7	165.8	0.51	21.4	0.73	4.5	12.2	2.4	2.7	2.1	18.1	3.2	3.3	0.48	1997
CZH99025	65	3.22	1.80	5.68	3.23	2.15	3.24	72.6	3.9	183.8	0.50	17.0	0.75	4.6	2.1	2.4	3.1	1.9	18.6	2.6	3.3	0.48	2000
KDH3	66	3.21	1.69	5.21	3.77	2.16	.	62.5	3.1	152.9	0.43	18.9	0.79	2.1	3.2	2.4	3.0	1.8	16.2	2.7	2.6	0.50	2006
KDH4	67	3.04	1.69	5.11	3.88	1.37	3.14	62.2	3.2	153.7	0.43	20.3	0.66	7.2	2.8	2.4	4.0	1.7	16.2	2.9	3.4	0.52	2006
SC635	68	3.00	2.17	3.81	4.00	1.81	3.20	71.1	3.3	167.6	0.48	14.5	0.63	6.4	7.0	2.3	2.8	2.5	17.4	2.9	3.5	0.49	2003
CZH095	69	2.77	1.54	3.19	3.31	2.59	3.20	70.8	2.2	167.2	0.49	6.6	0.69	8.4	6.2	2.4	2.7	1.8	17.1	2.6	3.0	0.46	2010
CZH0513	70	2.74	1.28	4.92	3.07	1.67	2.75	73.4	1.9	176.8	0.50	42.9	0.76	1.6	3.0	2.8	3.0	1.5	17.1	2.8	3.1	0.48	2006
Mean		**3.80**	**2.36**	**6.23**	**3.59**	**2.66**	**4.15**	**69.7**	**2.0**	**172.9**	**0.49**	**14.6**	**0.79**	**5.4**	**4.4**	**2.4**	**3.0**	**2.3**	**17.9**	**2.6**	**2.9**	**0.50**	
LSD (0.05)		**0.67**	**0.88**	**1.56**	**0.82**	**1.0**	**1.35**	**1.2**	**1.0**	**11.6**	**10.4**	**11.6**	**0.1**	**4.4**	**3.2**	**0.6**	**0.3**	**0.3**	**12.3**	**0.3**	**0.8**	**0.07**	
h^2		**0.69**	**0.62**	**0.62**	**0.20**	**0.11**	**.**	**0.96**	**0.6**	**0.4**	**0.00**	**0.2**	**0.32**	**0.1**	**0.0**	**.**	**0.5**	**0.9**	**0.5**	**0.4**	**0.8**	**0.25**	
Minimum		**2.74**	**1.21**	**3.19**	**2.73**	**1.37**	**2.75**	**62.2**	**0.7**	**152.9**	**0.39**	**2.6**	**0.63**	**1.5**	**1.8**	**2.0**	**2.6**	**1.4**	**16.2**	**2.2**	**1.8**	**0.41**	
Maximum		**4.72**	**3.65**	**8.14**	**4.80**	**3.75**	**6.23**	**75.8**	**3.9**	**194.0**	**0.55**	**42.9**	**1.00**	**10.7**	**12.2**	**2.9**	**4.0**	**3.8**	**20.5**	**3.2**	**3.5**	**0.60**	

h^2 is heritability, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$, LSD (0.05) is Least significant difference at ($P < 0.05$)

5.3.2 Multi environment trials analysis

5.3.2.1 Genetic correlations of grain yield across sites

Genetic correlations among sites ranged from -0.15 for Kiboko2013 and Harare2013a to 0.99 for Harare2013a and b (Table 5.4). Kiboko2013 and Harare2013a also weakly correlated negatively (r = -0.08). Generally Kiboko2013 had the weakest correlations with the other sites in the trial including Kiboko2012 (0.26). Correlations among the Harare sites were all above 0.40. Harare2012 and Kiboko2012 were both grown in the same year even though in different sites.

Table 5.4 Genetic correlations of grain yield across sites under low N

	Harare2012	Kiboko2012	Kiboko2013	Harare2013a	Harare2013b
Harare2012	-				
Kiboko2012	0.42	-			
Kiboko2013	0.27	0.26	-		
Harare2013a	0.46	0.30	-0.15	-	
Harare2013b	0.44	0.26	-0.08	0.99	-

5.3.2.2 Complete linkage cluster analysis of low N sites

Harare2013a and b that were grown in the same season in Harare on the same block receiving the same treatments clustered together (Figure 5.4). Harare2013a and b. Kiboko2013 differed from the rest of the sites but its heritability (grain yield) was higher than Harare2013a and b.

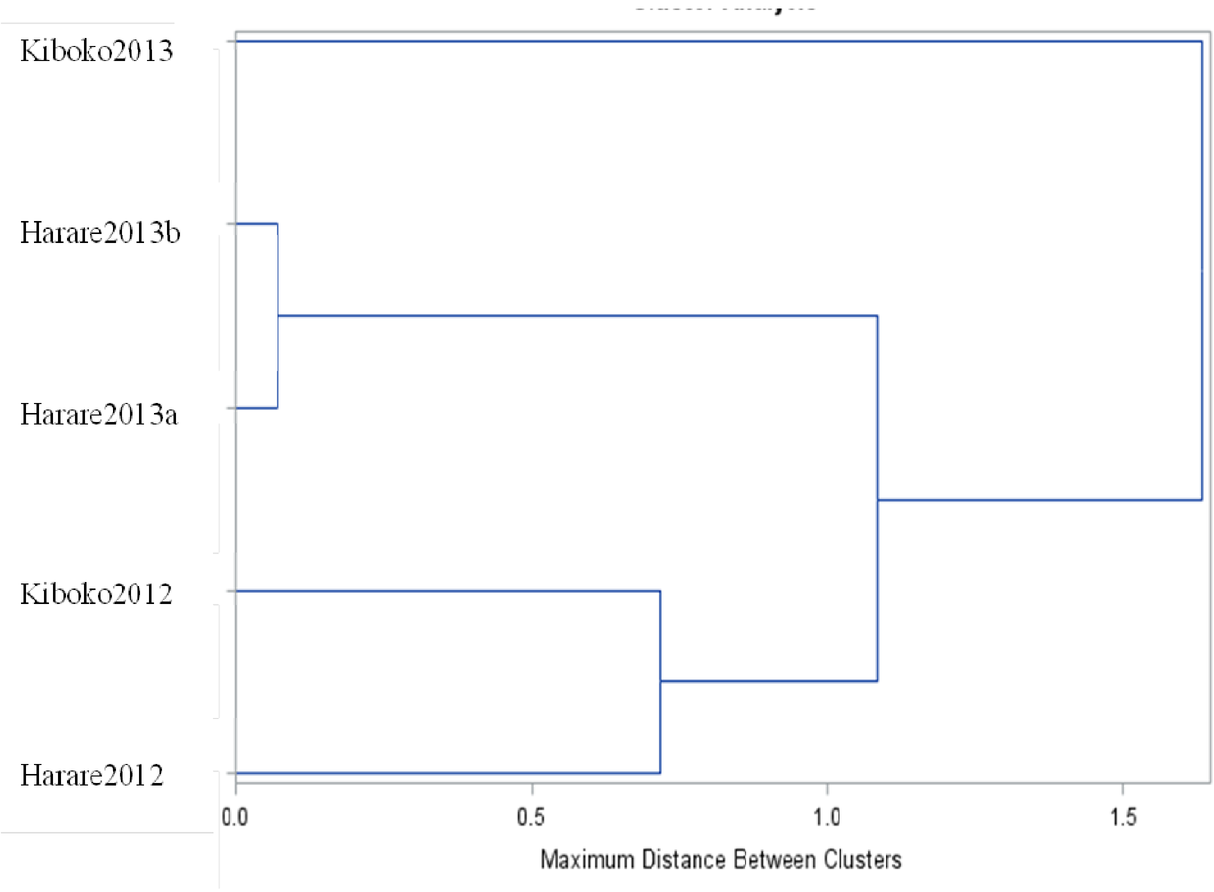
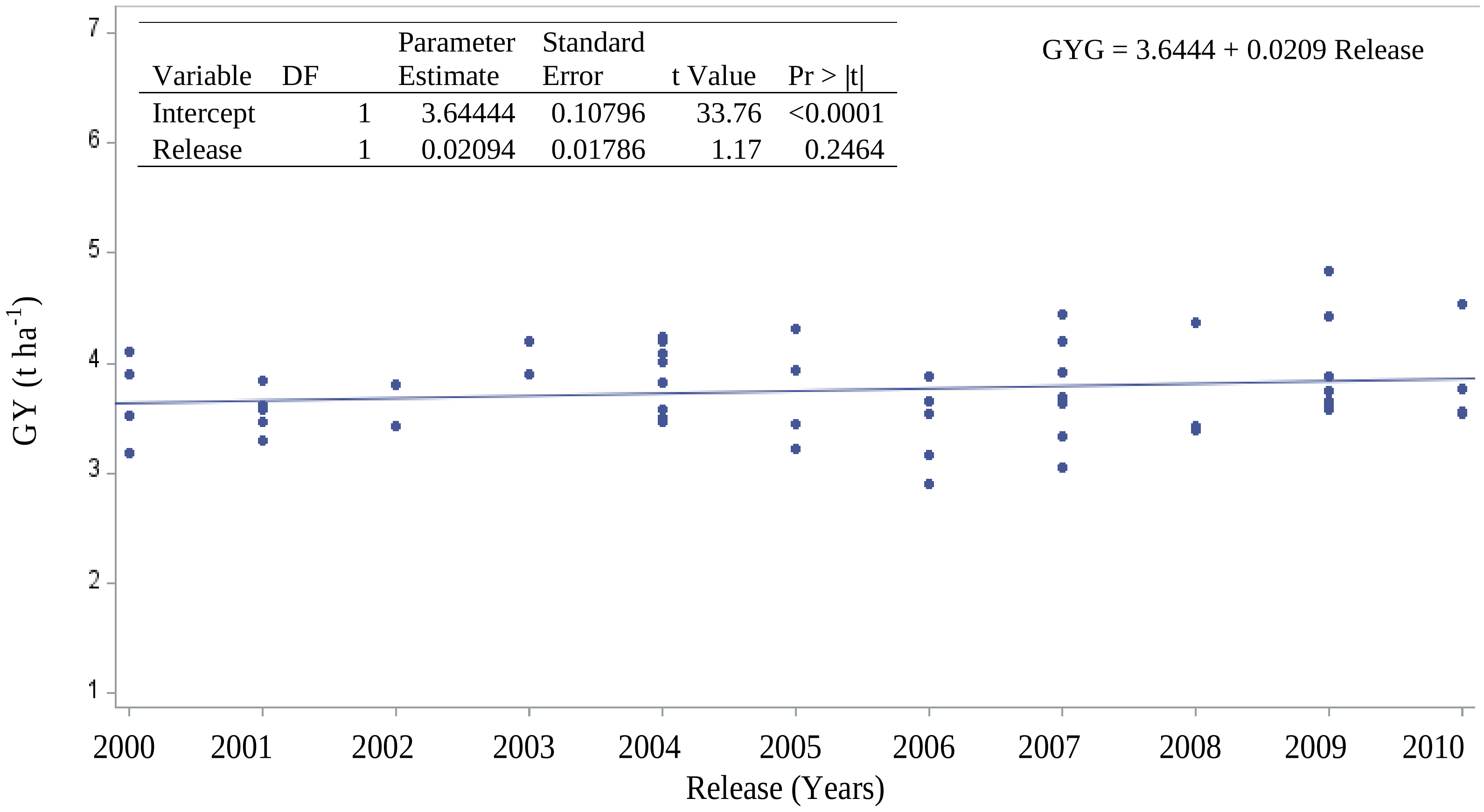


Figure 5.4 Cluster analysis of low N sites

5.3.2 Genetic gain in yield and secondary traits under low N stress

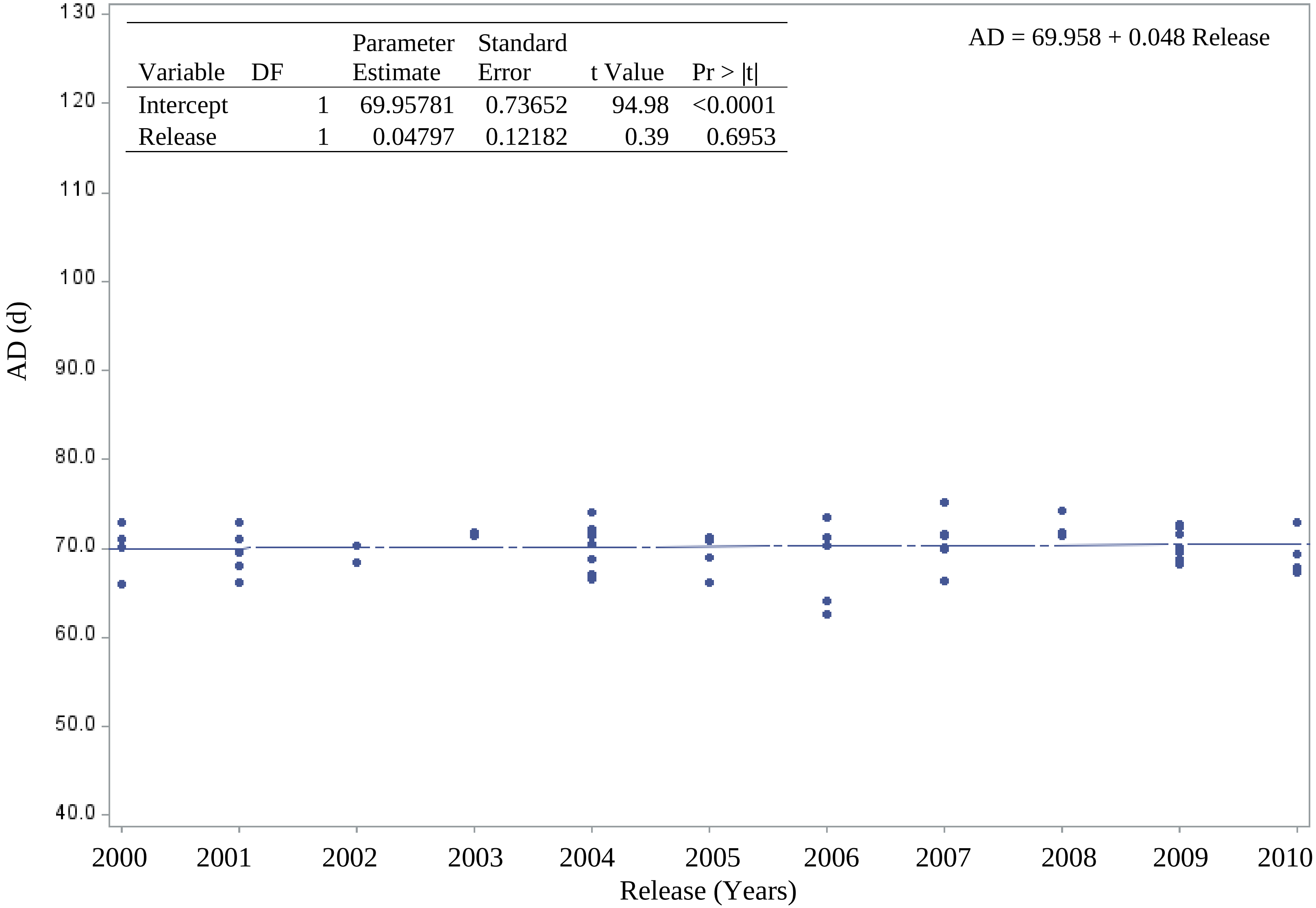
Evaluation of grain yield of the 67 CIMMYT drought tolerant hybrids released from 2000 to 2010 in this study showed positive increase under low N stress. A regression of the grain yield against the years of testing (Figure 5.5) showed a trend to increase at 20.9 kg ha^{-1} year^{-1} (0.62% year^{-1}). The new hybrids yielded relatively higher than the old hybrids. Days to mid-anthesis did not change over the 11 years (Figure 5.6). New hybrids utilised the same season length as the old hybrids. The anthesis-silking interval under low N stress decreased over time from 2000 to 2010 at a rate of 0.08 d year^{-1} (Figure 5.7) at $P \leq 0.01$. Silking in the old hybrids was delayed by low N stress more than in the new hybrids. There was no significant change in the barrenness but the number of ears per plant showed a trend to increase at a rate of 0.0008 over the 11 years (Figure 5.8). Plant height showed no significant change under low N stress (Figure 5.9). Senescence decreased at a rate of 0.02 scores year-1 (Figure 5.10) over the 11 years at $P \leq 0.05$. In this study new hybrids had shorter ASI and stayed green longer under low N stress.



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	3.64444	0.10796	33.76	<0.0001
Release	1	0.02094	0.01786	1.17	0.2464

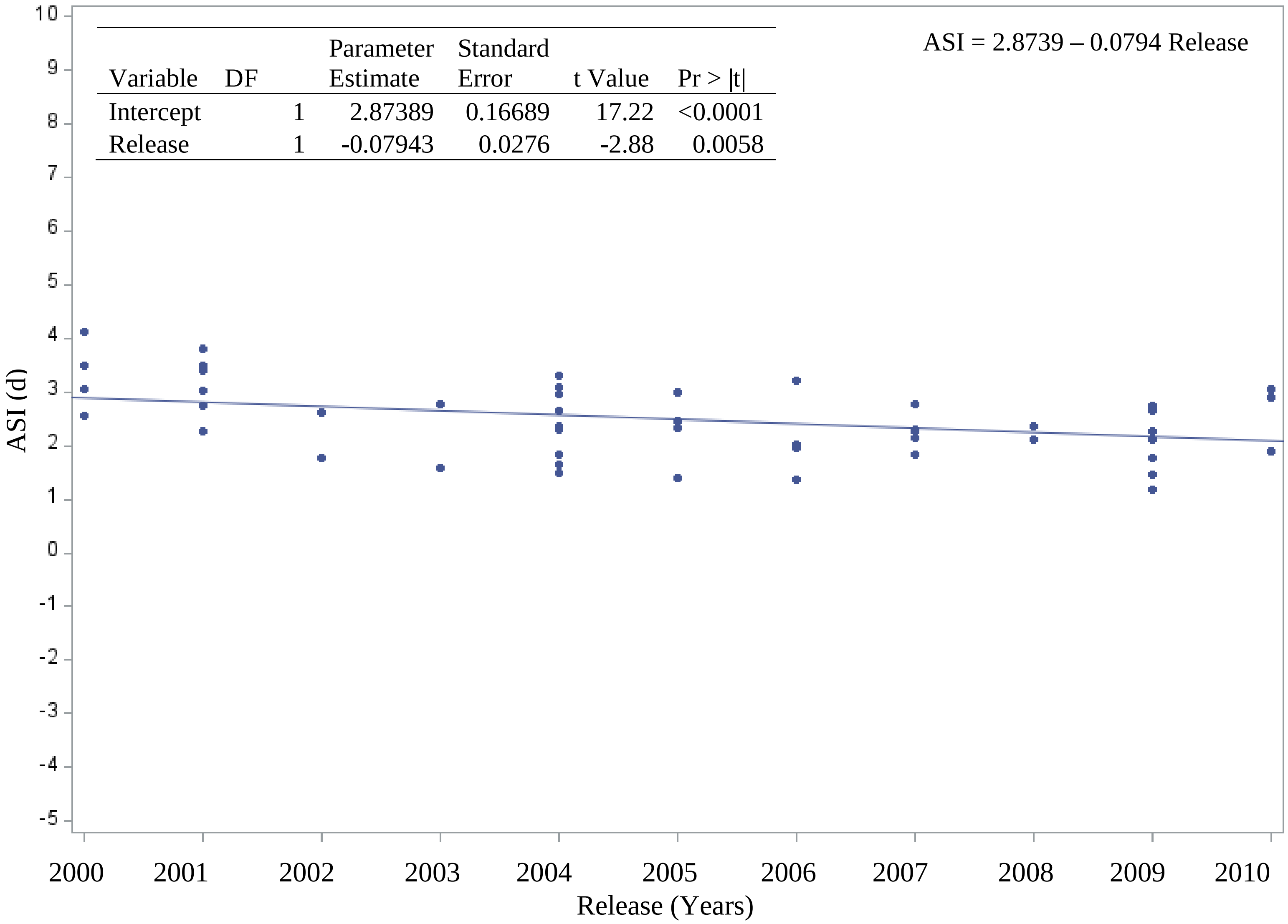
GYG = 3.6444 + 0.0209 Release

Figure 5.5 Changes in grain yield under low N in CIMMYT hybrids released from 2000 to 2010



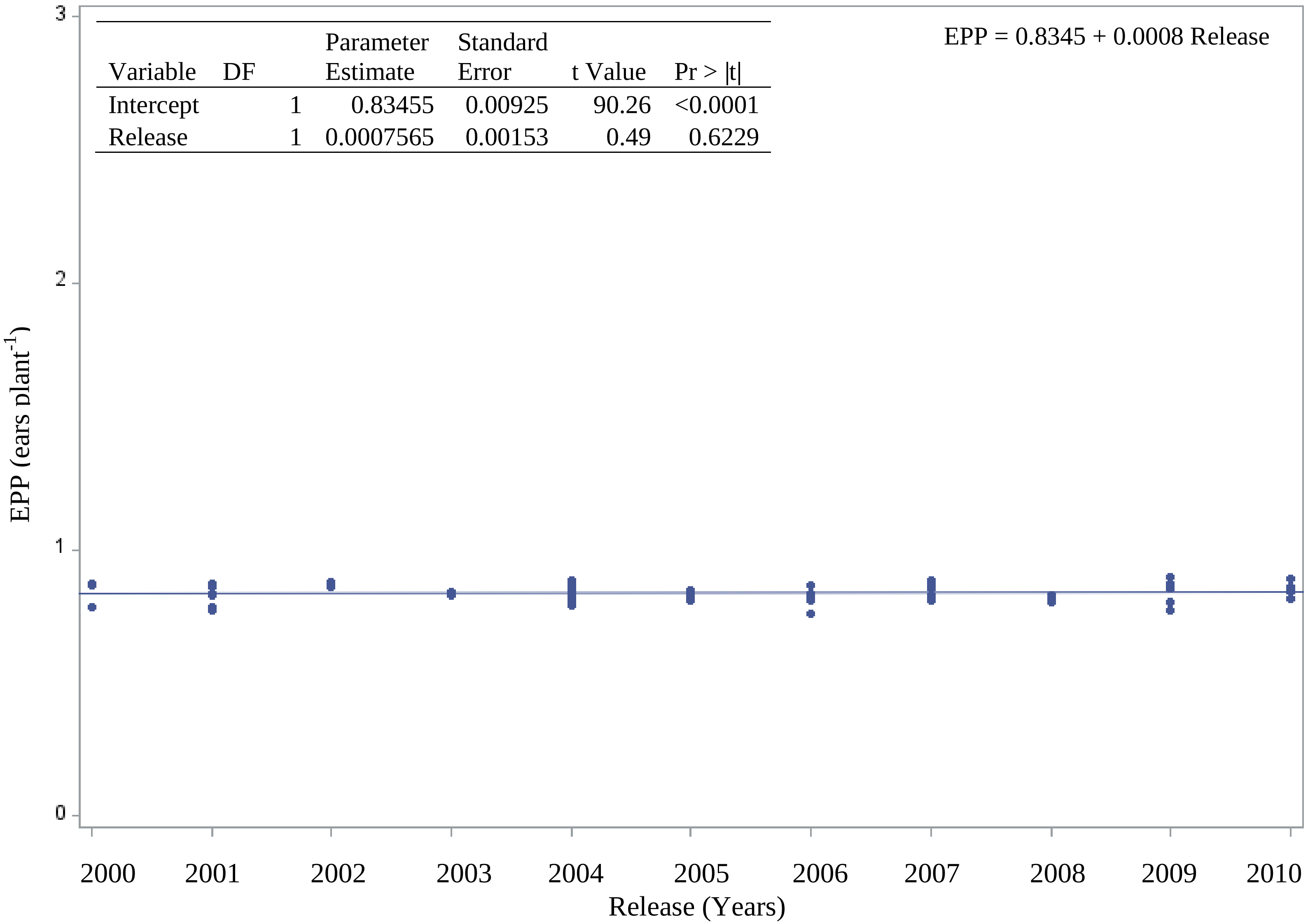
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	69.95781	0.73652	94.98	<0.0001
Release	1	0.04797	0.12182	0.39	0.6953

Figure 5.6 Changes in days to mid-anthesis under low N in CIMMYT hybrids released from 2000 to 2010



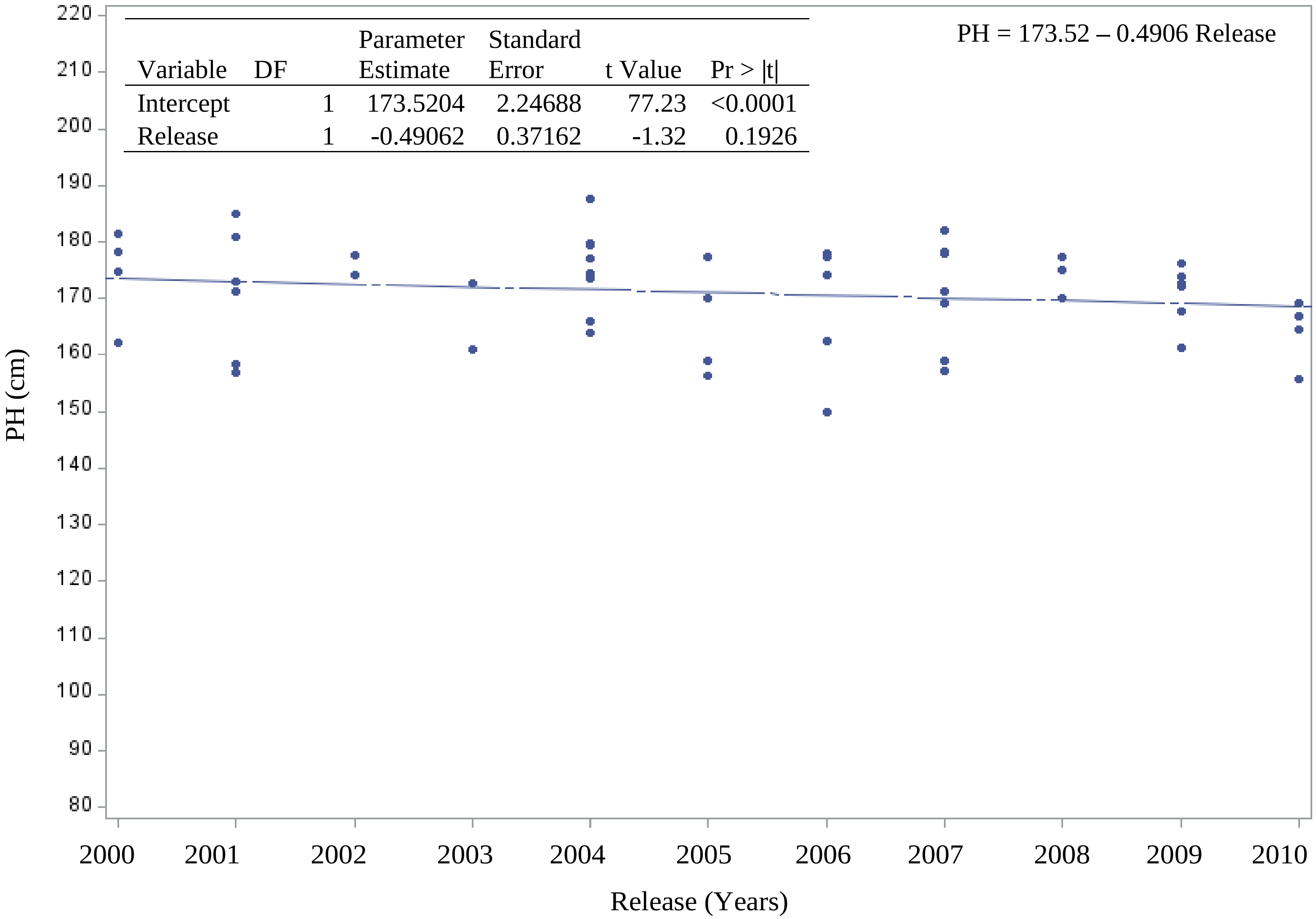
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	2.87389	0.16689	17.22	<0.0001
Release	1	-0.07943	0.0276	-2.88	0.0058

Figure 5.7 Changes in anthesis-silking interval under low N in CIMMYT hybrids released from 2000 to 2010



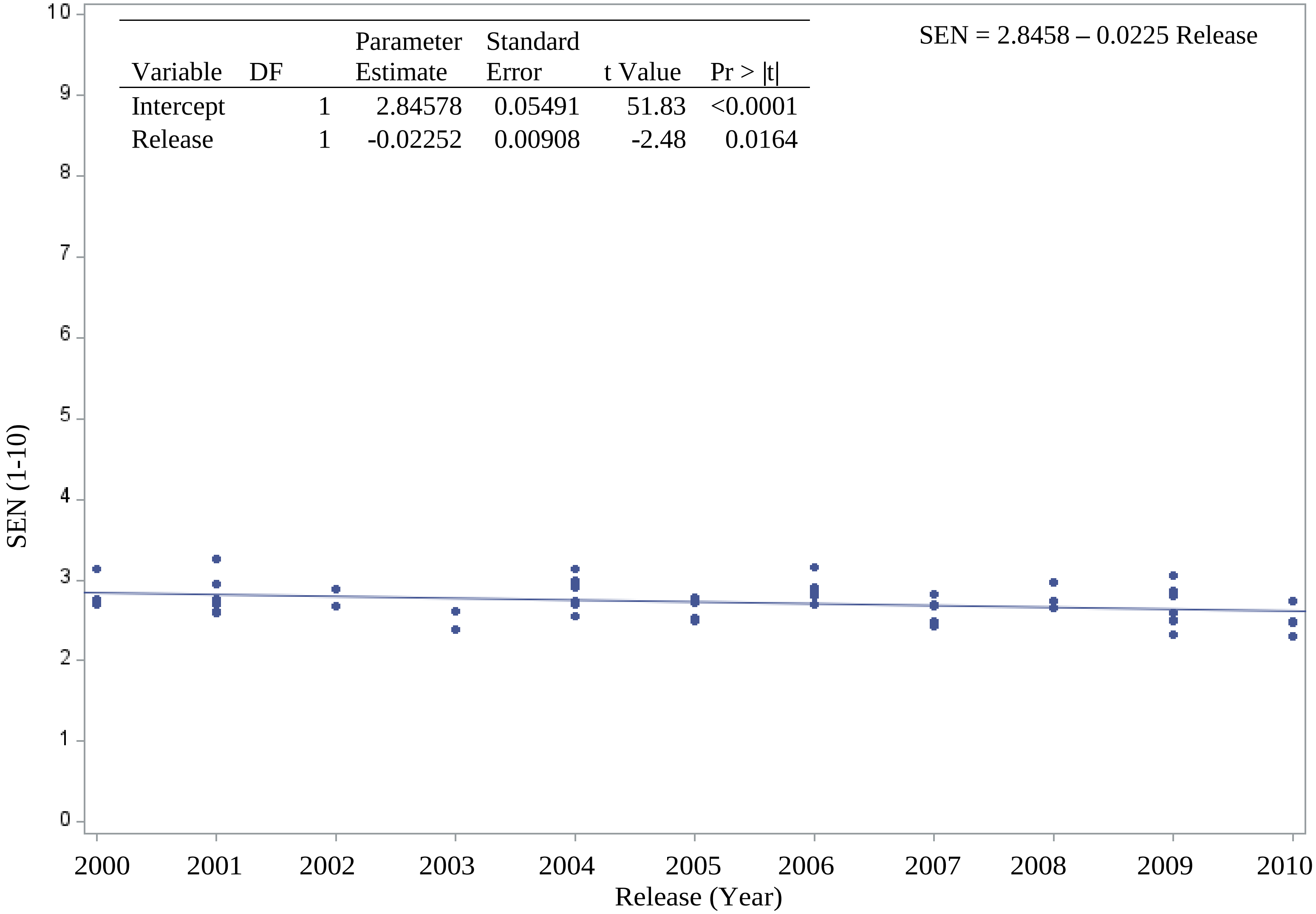
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.83455	0.00925	90.26	<0.0001
Release	1	0.0007565	0.00153	0.49	0.6229

Figure 5.8 Changes in number of ears per plant under low N in CIMMYT hybrids released from 2000 to 2010



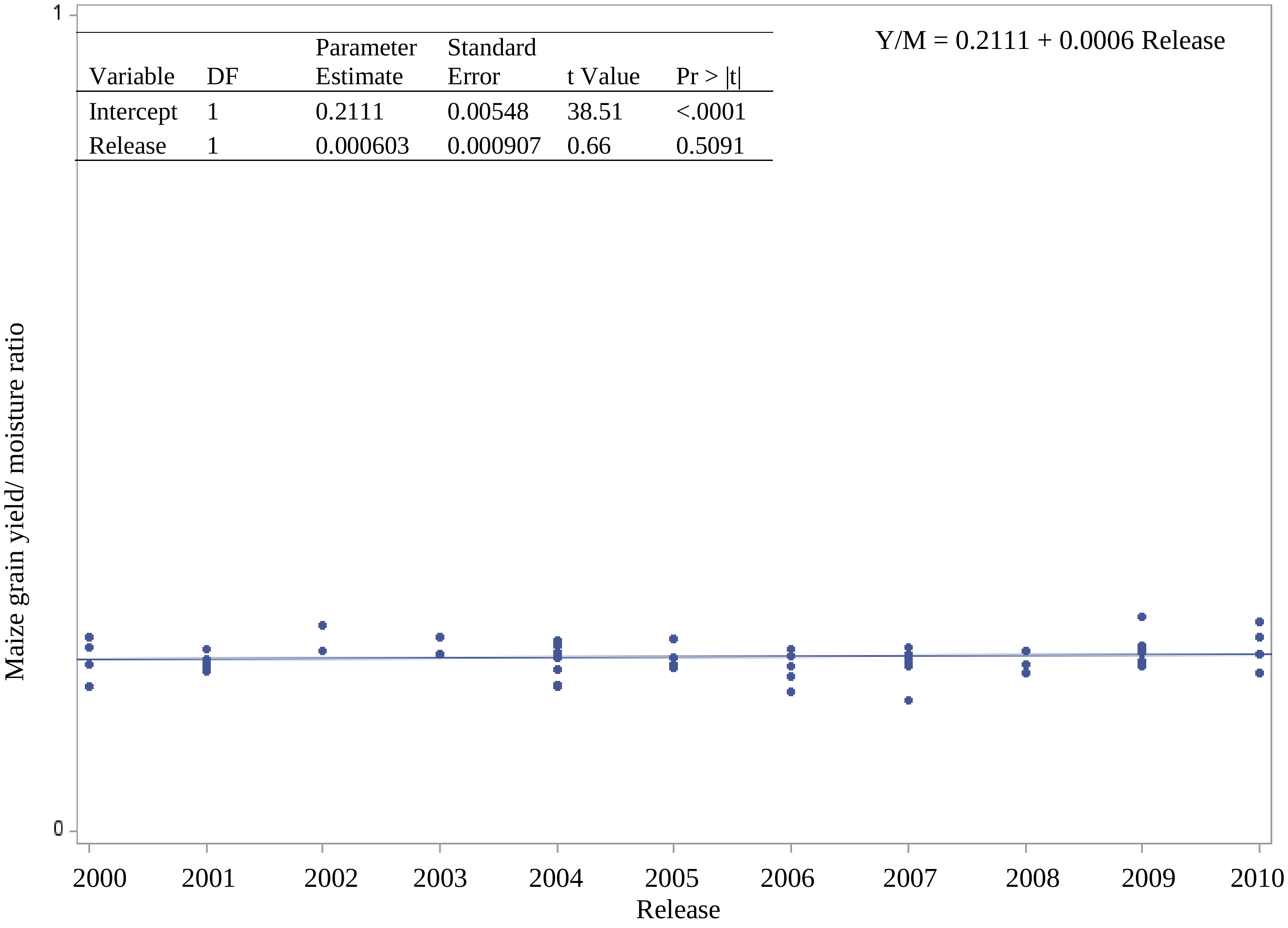
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	173.5204	2.24688	77.23	<0.0001
Release	1	-0.49062	0.37162	-1.32	0.1926

Figure 5.9 Changes in plant height under low N in CIMMYT hybrids released from 2000 to 2010



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	2.84578	0.05491	51.83	<0.0001
Release	1	-0.02252	0.00908	-2.48	0.0164

Figure 5.10 Changes in senescence under low N in CIMMYT hybrids released from 2000 to 2010



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.2111	0.00548	38.51	<.0001
Release	1	0.000603	0.000907	0.66	0.5091

Y/M = 0.2111 + 0.0006 Release

Figure 5.11 Changes in grain yield/ grain moisture ratio under low N in CIMMYT hybrids released from 2000 to 2010

5.3.4 Dissecting genetic gain in yield in CIMMYT hybrids tested from 2000 to 2010 under low N stress

There was an estimated net genetic gain in yield over the 11 years. Physiological and phenological changes contributed to the change in grain yield. Secondary traits have been used to indirectly evaluate varieties for low N tolerance (Monneveux *et al.*, 2005) and therefore there should be a relationship between secondary traits and grain yield. To help explain changes in yield in the study genetic correlation for yield against different secondary traits and among the different secondary traits that are shown in Table 5.5 were evaluated.

Table 5.5 Across site genetic correlation for grain yield and secondary traits under low N stress conditions

	GYG	AD	ASI	PH	EPP	SEN	NDVI	RL
GYG	-							
AD	-0.43^{***}	-						
ASI	-0.30^{***}	0.14^{***}	-					
PH	0.44^{***}	-0.23^{***}	-0.20^{***}	-				
EPP	-0.00^{ns}	0.07^{*}	0.00^{ns}	0.04^{ns}	-			
SEN	-0.48^{***}	0.62^{***}	0.23^{***}	-0.09^{*}	0.24^{***}	-		
NDVI	0.37^{***}	-0.82^{***}	-0.23^{***}	0.314^{***}	-0.09^{*}	-0.75^{***}	-	
RL	-0.48^{***}	0.61^{***}	0.11^{***}	-0.22^{***}	0.20^{***}	0.57^{***}	-0.60^{***}	-
Y/M	0.45^{***}	0.31^{*}	0.06^{ns}	0.32^{**}	0.15^{ns}	0.09^{ns}	-0.44^{***}	-0.99^{***}

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$

GYG is grain yield, AD is days to mid-anthesis, ASI is anthesis-silking interval, PH is plant height, EPP is number of ears per plant, SEN is leaf senescence, NDVI is normalized difference vegetation index, RL root lodging,

Grain yield showed moderate negative correlations with days to mid-anthesis, anthesis-silking interval, senescence and root lodging of -0.43, -0.30, -0.48 and -0.48 respectively (Table 5.5) and a moderate positive correlation with plant height and NDVI of 0.44 and 0.37 respectively. Under low N stress hybrids did not show a significant relationship for the number of ears per plant with grain yield, plant height and anthesis-silking interval at $P \leq 0.05$. Days to mid-anthesis had a very weak positive correlation of 0.07 with number of ears per plant at $P \leq 0.05$. Yield was higher in hybrids that flowered early, had shorter anthesis-silking interval and low senescence rates. Anthesis-silking interval was high in hybrids that flowered later ($r = 0.14$). Hybrids that flowered earlier were taller and had a slower senescence rate while the shorter hybrids were stunted, flowered later and had a faster senescence rate. The correlation of days to mid-anthesis with plant height was negative (-0.23). The correlation of days to mid-anthesis and senescence was positive at 0.62. Anthesis-silking interval was longer in the stunted hybrids (r = -0.20). Grain yield/ grain moisture content ratio positively correlated with grain yield under low N stress showed no significant relationship with barrenness.

In Kiboko, Kenya in 2012 and 2013 further analysis was done to check if grain quality changed over the 11 years. From the correlation analysis, oil and protein negatively correlated to yield ($P \leq 0.001$) at -0.40 and -0.80 respectively (Table 5.6). Starch positively correlated to grain yield (0.67) at $P \leq 0.001$. The number of kernels per hectare (KPHA) calculated using the average 200 kernel weight negatively correlated with grain yield (-0.27) at $P \leq 0.05$. The four traits did not significantly change over the 11 years. Maize grain yield/ grain moisture content ratio did not change over the eleven years but positively correlated with grain yield and the number of ears per plant.

Table 5.6 Across site genetic correlations for grain yield and number of kernels per hectare, oil, protein and starch content under low N stress conditions

Traits	GYG	OIL	PROTEIN	STARCH
GYG	-			
OIL	-0.40^{***}	-		
PROTEIN	-0.80^{***}	0.23^{ns}	-	
STARCH	0.67^{***}	-0.51^{***}	-0.75^{***}	-
KPHA	-0.27^{*}	-0.18^{ns}	-0.99^{***}	0.99^{***}

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$, GYG is grain yield
GYG is grain yield,

5.4 Discussion

An estimated grain yield increase of 20.9 kg ha^{-1} $year^{-1}$ (0.62% $year^{-1}$) under low N stress was recorded in this study. In a study conducted in 2009 to 2010, maize hybrids tested under different levels of N recorded estimated genetic gain rates of 86 kg ha^{-1} $year^{-1}$ at 252 kg N ha^{-1}, 79 kg ha^{-1} $year^{-1}$ at 67 kg N ha^{-1} and 59 kg ha^{-1} $year^{-1}$ at 0 kg N ha^{-1}. The trial followed a soybean crop (Haegele *et al*., 2013). The land used was therefore not N depleted prior to the experiment as in the case of the current study. The current study was conducted on land that had been N depleted for two to three years in Kiboko and for five and fifteen years in Harare. Soil analysis conducted in Harare indicated soil Nitrate N of 1.53 ppm at the top 0 to 30 cm to 2.58 ppm at the 60 to 90 cm depth in 2012. The Nitrate-N levels are way below the recommended 25 to 30 ppm for maize production. Given the different field histories and without the percentage gain it is difficult to compare the gains in the current study with results from the study by Haegele *et al*. (2013). In the current study yield was depressed by the deficiency of N and hybrids were not able to reach their full genetic potential as they suffered chlorosis and leaf firing from early stages of growth throughout reproduction. It is important that even under the harsh low N stress conditions, the new hybrids showed a positive yield improvement trend even though the evaluation covered a short period of 11 years. The improvement was not only in terms of yield quantity but also in terms of physiological changes that allowed production under stress conditions. The positive grain yield improvement estimates show that there is improvement in the CIMMYT breeding programme. New hybrids performed better under low N stress compared to old hybrids.

The yield trend over the 11 years showed an increase even though the change was not statistically significant. Since a separate low N programme started in 2009 and earlier on low N trials formed a small proportion of all the testing trials, it may be possible that not much focus had been given towards direct selection for low N stress tolerance. Even though currently the programme is running as a separate programme, the permanently depleted low N sites are still limited. This may have contributed towards the non-significant change in grain yield over the 11 years. More needs to be done in terms of germplasm development, direct selection and testing for low N tolerance as well as valuation of breeding progress. However, the trend could have been due to changes in some secondary traits.

Anthesis-silking interval is considered one of the most important traits in maize that affects the success of grain production. Under abiotic stress, silking is delayed (Bänziger *et al.*, 2000). This may result in poor synchronisation, poor pollination, poor grain set and poor grain yield. In the current study the new hybrids had a shorter ASI under low N stress ranging from 1.0 d to 2.6 d that increased chances of successful pollination and grain set compared to the old hybrids that ranged from 1.6 d to 3.9 d. As shown by the negative correlation (-0.30) of grain yield with ASI yield decreased significantly as ASI increased from 1 d to 4 d. The improved anthesis-silking interval in new hybrids should be a result of improved partitioning of dry matter towards the ear for ear development, grain set and grain filling (Duvick, 2005b; Tollenaar and Lee, 2006).

Maize grain yield was positively correlated with plant height ($r = 0.44$) (Table 5.5). Growth is restricted by limited N. Low N susceptible plants are stunted and may produce small or abnormal ears (Figure 5.2) or may fail to produce any ears at all. There was no significant change in plant height over the 11 years. The new hybrids, however, were relatively shorter compared to the older hybrids (Table 5.3) but not stunted. This may have possibly been a result of a change in dry matter partitioning in favour of the ear in new hybrids. Under stress, dry matter partitioning can be redirected more towards the ear (Duvick, 2005a and b; Tollenaar and Lee, 2006) for successful ear initiation, development and determination of number of kernels per row, kernel set and development. The poor performing hybrids ranked in positions 61 to 70 were shorter and stunted compared those ranked in the top 10. Breeding efforts may have improved ear development under low N stress.

Hybrids that attained higher vegetative cover (NDVI) at early stages (25 cm to 30 cm plant height) yielded better. This could be an indication of improved NUE in the form of better NUpE and/or NUtE in the better performing hybrids. There was a reduction in yield associated with increased lodging. This could be due to weak stems that result from poor plant development under low N stress. Varieties with a high incidence of lodging could have low NUE, such that stems were not strong and were susceptible to lodging and at the same time yielded less. Better yielders could have better NUE that allows development of a stronger stem as well as better grain development and filling under low N. Further investigation that include soil analysis before planting and after harvesting as well as grain and stover N to qualify or disqualify NUE as a factor affecting lodging in low N stressed maize. The new hybrids that were relatively shorter could have stronger stems and by virtue of being shorter, could have lodged relatively less compared to the taller plants or stunted plants that yielded less. Even though grain yield/ grain moisture content did not change over the 11 years, the trait positively correlated with grain yield and the number of ears per plant, indicating that the trait can be used for selection under stress as recommended by Ngure (1995).

Low N tolerant plants will either be efficient in taking up N from the soil, known as NUpE or they may be efficient in utilising the N that the plant takes up, known as NUtE (Moll *et a.l*, 1982; Bänziger *et al.*, 2000). This aspect was not evaluated in the current study but Haegele *et al*. (2013) had interesting findings showing improved NUpE.

The grain quality, oil, starch and protein content, did not change over the 11 years. This indicates that there was no indirect selection that changed grain quality over the years. The findings agree with findings by Duvick (2005a), Duvick (2005b) and Lee and Tollenaar (2007). The number of KPHA did not change over the 11 years. Other studies focused on kernel number per ear and per plant (Duvick, 2005a; Duvick, 2005b; Luque *et al*., 2006). Tollenaar (1989) reported an increase in number of kernels per unit area up to a peak after which a plateau was reached but the evaluation was under varying plant densities. In the current study under low N stress, yield was higher at lower KPHA and lower at higher KPHA. This could be due to better grain filling of the fewer kernels at lower KPHA compared to higher KPHA where grain filling could be poor and grains may not mature due to limited resources to support development up to maturity as well as premature senescence. Grain yield was higher at high starch accumulation levels. Increased KPHA may have resulted in competition for assimilates for grain filling resulting in poorly filled kernels and reduced yield. Fewer kernels per ear under stress may fill better and yield more than more poorly developed or filled kernels per ear. The findings agree with reports by Duvick et al. 2004; Duvick 2005a; Duvick 2005b; Badu-Apraku et al. 2013b. This could be due to the fact that during ear initiation under stress tolerant hybrids will determine the development of fewer kernels that will fully develop and fill up while non-tolerant hybrids will determine development of more (normal number of kernels under optimal conditions) that fail to fully develop. Other studies elsewhere under different (drought) conditions, however, reported the opposite where increasing number of kernels was associated with increased grain yield (Bolaños and Edmeades 1993; Zaidi et al. 2004; Campos et al. 2006).

Yield and secondary traits like standability and ASI were better in the new hybrids compared to the old hybrids. The new hybrids yielded better. Shorter ASI improved pollination and grain set while improved dry matter partitioning to the ear and better standability that abates premature lodging ensured complete and improved grain filling.

5.5 Conclusions

The new varieties yielded well despite the low N stress. Change in grain yield in the CIMMYT drought tolerance maize breeding programme in ESA, even though not significant, was 20.9 kg ha^{-1} year^{-1} (0.62% year^{-1}) over the 11 years from 2000 to 2010 under low N stress. There could have been improvement in low N stress tolerance but the era of evaluation (11 years) may have been too short to detect changes and also considering that a separate low N programme only started in 2009. There is need to do more work on selection and evaluation of genetic gain under low N stress. Genetic gain in yield under low N did not result in indirect selections that changed the grain quality. The oil, protein and starch composition did not significantly change over the 11 year period. The gain was associated with some phenological and physiological changes. Plant height generally showed a tendency to decrease in the new varieties that yielded better, redirecting dry matter partitioning to the ears. Lodging was lower in the high yielding varieties as they were relatively shorter and stronger, indicating an adjustment that reduced vegetative growth and channelled more dry matter towards grain development and filling, and probably improving the stem structure to make it stronger, with discontinued vegetative growth. The better yielding varieties were associated with better growth in the early stages compared to the older varieties, a sign of better NUE. The new varieties therefore should have better NUE, better dry matter partitioning that adjusts in response to stress, enabling shorter anthesis-silking interval, better pollination and grain set, better grain filling, shorter but stronger stems and reduced lodging that all contribute towards increased yields. Further studies could establish whether there was improved NUE and if the NUE is due to NUpE or NUtE or a factor of both components.

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Chapter 6

Genetic gain in biotic stress tolerance

Abstract

Diseases are one of the major constraints in maize production in most regions of the world, resulting in reduced crop production. Maize in the tropics is affected by diseases such as maize streak virus (MSV), grey leaf spot (GLS), northern leaf blight (ET) and rust. With early infections, these diseases can result in yield losses of 60 to 100%. MSV disease is a viral infection that cannot be controlled once a crop is infected. The other fungal diseases can be controlled chemically. Controlling the MSV leaf hopper vector chemically may help to control the disease. However, chemical control is expensive and unaffordable for most resource poor farmers in SSA. Host plant resistance is the best control measure that is affordable, environmentally friendly and effective for all diseases, including MSV. CIMMYT has been breeding for host plant resistance to diseases including MSV, ET and GLS. Genetic resistance or tolerance to MSV assures a farmer of a better yield. Host plant resistance or tolerance is therefore a possible intervention where MSV is a problem disease. This study focused mainly on MSV and genetic gain in MSV tolerance, although natural infestation of ET was present in all trials. Genetic gain in ET resistance was also evaluated, even though the occurrence was due to natural infestation. Three trials planted at CIMMYT, Harare for evaluation for MSV resistance, were artificially infested with MSV carrying leaf hoppers while ET was observed and recorded where incidence was high following natural infection. In the presence of MSV and ET, there were significant genetic gain estimates for yield of 141.3 kg ha^{-1} (2.2%) year^{-1} at $P < 0.01$, a decrease in ASI of 0.06 days year^{-1} ($P<0.05$), an increase in the number of ears per plant of 0.01 year^{-1} ($P < 0.01$), a decline in MSV incidence and severity of 0.03 scores year^{-1} ($P < 0.01$) on a scale of 1-5 and a decline in ET scores of 0.03 year^{-1} ($P < 0.05$) over the 11 years from 2000 to 2010. Newer CIMMYT drought tolerant hybrids yielded better, and had better MSV and ET disease stress tolerance compared to the older hybrids in this study.

6.1.1 Introduction

MSV is a major disease in maize production in SSA (Pingali and Pandey, 2001; Shiferaw *et al.*, 2011). Prevalence of MSV is high in the lowland to mid-altitude tropics (Pingali and Pandey, 2001). Losses from this disease vary from season to season (Bjarnason, 1986). The disease is widespread in Africa and can cause losses of up to 100% if infection sets in within the first three weeks after planting (Bjarnason, 1986; Fajemisin, 2001; Shepherd *et al*., 2010). MSV disease is considered economically as the most important foliar disease in maize production in SSA (Ramusi and Flett, 2012).

When a crop is infected with MSV disease, infected plants develop whitish to yellowish chlorotic streaks on the leaves. Plants can be stunted. In some cases infected plants die (Shepherd *et al*., 2010; CIMMYT, 2013). Maize grain and stover quality and quantity are reduced by MSV, especially if infection occurs within 14 days from planting (Lukuyu *et al*., 2002). Yield losses result from reduced photosynthesis due to chlorosis, poor pollination, abnormal flower and shoot development, poor or abnormal ear development and barrenness (Shepherd *et al*., 2010). Losses are worse in late planted crops compared to early plantings.

Considering the massive losses the disease can cause and the impact on the food chain, there is need for effective and affordable control measures, since chemical control of the MSV vector is not affordable for the resource poor farmer (Ramusi and Flett, 2012). Chemical control is also not always technically and economically feasible (Fajemisin, 2001). Host plant resistance is the best control measure that CIMMYT and other public and private sector researchers in SSA are working towards.

6.1.2 Objectives

This study focused on the use of host plant resistance in managing the impact of MSV and other diseases in maize production as a more affordable and effective control measure. CIMMYT developed MSV resistant hybrids that have been released and some disseminated to farmers through partners. This study aimed to evaluate if there was a net genetic gain in resistance to MSV and other diseases in the CIMMYT drought tolerant hybrids that were released from 2000 to 2010. The objectives of this study were to:

i) evaluate genetic gain in yield under MSV and other disease infestation in CIMMYT drought tolerant hybrids released from 2000 to 2010

ii) evaluate genetic gain in resistance to MSV and other disease in CIMMYT drought tolerant hybrids released from 2000 to 2010

iii) evaluate secondary traits under MSV disease stress in CIMMYT drought tolerant hybrids released from 2000 to 2010

6.2 Materials and methods

6.2.1 Germplasm

Sixty-seven hybrids listed in Appendix I and described in Chapter 3 section 3.2.1, and checks SC727, SC635 and SC513 were evaluated under MSV and ET stress.

6.2.2 Trial sites

A total of three sites (Appendix III) of the trial were planted between 2011 and 2013 in Harare. The trials were planted at the CIMMYT maize experimental station in Harare (-17°50'S, 31°2'E, 1483 masl). The soil at the station is a red clay loam with high water holding capacity.

6.2.3 Trial layout

The experiment was designed in an alpha-lattice design with two replications and five plots per incomplete block. Entries were planted in two row plots spaced at 0.75 m inter row and 0.25 m in the row with one plant per station, giving a plant density of 53 000 plants ha^{-1}.

6.2.4 Trial management

The first site, Harare2012, was planted at the CIMMYT station in Harare on the 10th of January 2012 and harvested on the 12th of August 2012. The second and third sites, Harare2013a and b, were planted on the 29th of December 2012 in a different block from the one used in the 2011/12 season. Harare2013a and b were planted in the same block next to each other. Harare2013a and b were harvested on the 13th of June 2013. All MSV trials were planted on the windward side of all the other trials and nurseries to control the spread of leaf hoppers and MSV.

All plots in Harare received 28 kg N ha^{-1}, 56 kg P ha^{-1} and 28 kg K ha^{-1} at sowing as Compound D (7 N 14 P_2O_5 7 K_2O 6.5 S) at planting. A second application of N (69 kg N ha^{-1}) was given approximately five weeks after sowing (V6 stage, Ritchie *et al.*, 1992) and a final application of N (69 kg N ha^{-1}) at flowering. For all three sites recommended plant, weed, and insect control measures were used. Trials received a total of 50 mm per irrigation cycle applied at planting and at emergence. Trials were rainfed for the rest of the season.

The 67 CIMMYT hybrids and three commercial checks were scored for MSV resistance. The experiment was inoculated with MSV at 10 to 14 days after planting using the *Cicadulina* vector. MSV was scored twice, at least four to five weeks after inoculation when symptoms were well developed and in the early reproductive stage. The presence of MSV and its severity was indicated by a score between 1 and 5 (Figure 6.1). On the 1-5 scale, clean plants with no or a few spots at the bottom of the plant are scored at 1 while plants showing symptoms from the base of the plants up to the flag leaf receive a score of 5 (adapted from Mahuku, 2010).

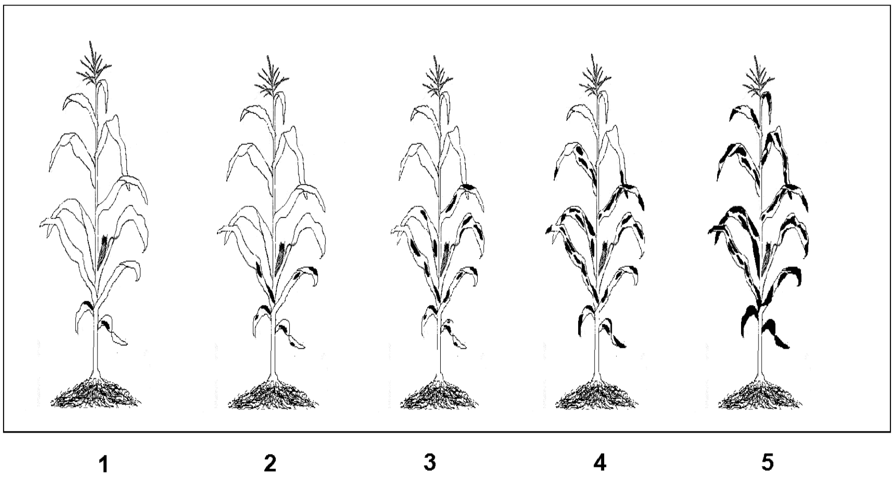


Figure 6.1 Disease score scale of 1-5 (Mahuku, 2010)

6.2.5 Other diseases

In all sites natural ET incidence was high and the disease was scored visually by the investigator. A combined score for incidence and severity on a scale of 1 to 5 was used. Scoring was done twice for ET towards flowering and during the grain filling period. GLS did not occur in these trials. The disease was not recorded.

6.2.6 Data recorded

Several traits were recorded at different development stages throughout the season up to harvesting (Table 6.1). These included days to mid-anthesis and to mid-silking which were recorded during flowering. There was no artificial inoculation for ET but this disease occurred at a significant level, and was scored on a scale of 1 to 5. MSV was scored at about four to five weeks after inoculation and during the grain filling period. Plant height (PH) from the base of the plant to the first branch of the tassel, ear height (EH), plant stand (NP), stem and root lodging (SL and RL) and husk cover (HC) were recorded within two to three weeks before harvesting. At harvesting, the number of ears harvested from each plot (NE), ear rot (ER) incidence from each plot, ear aspect (EA), ear texture (TEX), grain weight (GW) for each plot and grain moisture (MOI) were recorded. Descriptions of how the traits were evaluated are listed in Appendix IV.

Table 6.1 Traits recorded under MSV infection conditions

Secondary traits (phenology)	**Secondary traits (stress tolerance)**	**Yield components**	**Direct yield assessment**
Days to 50% anthesis	Disease scores	Plant stand	Grain weight
Days to 50% silking	Number of ears per plant / barrenness	Number of ears	Grain moisture
Plant height		Ear aspect	
Ear height		Grain texture	
Root and stem lodging		Ear rots	
		Husk cover	

6.2.7 Statistical analysis

Data was analysed as described in Chapter 3 section 3.2.6.

6.3 Results

6.3.1 Analysis of variance of hybrid performance under biotic stress

From the general analysis of variance the test hybrids varied significantly in all traits tested (Table 6.2). The heritability for the different traits in all sites was high. Grain yield among the hybrids standardised to 12.5% moisture content varied significantly within sites and across sites. Yield from the three sites averaged 6.45 t ha^{-1}. The yield ranged from 1.12 to 11.18 t ha^{-1} across the three sites with yield averages ranging from 5.21 to 7.21 t ha^{-1}. Yield levels were relatively lower in Harare2012 conducted during the 2011/12 season with a trial mean of 5.21 t ha^{-1} and a maximum yield of 7.21 t ha^{-1} compared to Harare2013a and b (2012/13 season) that yielded on average 7.21 t ha^{-1} and 6.93 t ha^{-1} respectively and a maximum of 10.51 t ha^{-1} for Harare2013a and 11.18 t ha^{-1} for Harare2013b.

The average number of days to mid-anthesis for all three sites was 73.10. The number of days to mid-anthesis for the three sites ranged from a minimum of 62.97 to a maximum of 87.20 with site averages ranging from 71.56 to 76.08.

Table 6.2 Analysis of variance of yield and secondary traits under MSV and ET stress

Trait	Units	Site	Trial mean	LSD (0.05)	MSe	h^2	P	Minimum	Maximum	Across site mean
Average yield	tha^{-1}	Harare2012	5.21	1.39	0.84	0.79	***	1.44	7.21	6.45
		Harare2013a	7.21	2.10	1.12	0.72	***	1.87	10.51	
		Harare2013b	6.93	1.62	0.75	0.92	***	1.12	11.18	
Days to 50% anthesis	d	Harare2012	76.08	2.50	2.66	0.89	***	68.91	81.32	73.10
		Harare2013a	71.70	3.71	3.49	0.91	***	62.97	86.90	
		Harare2013b	71.56	2.13	1.19	0.98	***	63.09	87.20	
Number of ears per plant		Harare2012	0.99	0.26	0.03	0.69	***	0.43	1.61	1.01
		Harare2013a	1.00	0.16	0.01	0.77	***	0.53	1.33	
		Harare2013b	1.04	0.26	0.02	0.75	***	0.54	1.52	
MSV	1-5	Harare2012	1.98	0.45	0.09	0.73	***	1.42	2.90	2.20
		Harare2013a	2.40	0.70	0.10	0.50	***	1.90	3.80	
		Harare2013b	2.50	.	0.10	0.59	ns	1.60	3.80	
ET	1-5	Harare2012	2.09	0.41	0.06	0.69	***	1.56	2.77	2.60
		Harare2013a	3.01	0.85	0.19	0.77	***	1.76	4.11	
		Harare2013b	2.66	0.84	0.19	0.75	***	1.48	3.48	

h^2 is heritability, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$ LSD (0.05) is least significant difference, MSe is Mean square error

Harare2012 had the highest average days to mid-anthesis but had the shortest duration between the earliest mid-anthesis days to the highest, ranging from 68.91 to 81.32. Harare2013a and b had the same range of average days to mid-anthesis, 71.7 and 71.56 respectively and range of minimum and maximum days recorded in the trial of 62.97 to 86.90 and 63.09 to 87.20 respectively. Pollen shedding started earlier and ended later in Harare2013a and b compared to Harare2012.

There was significant variation in number of ears per plant for all sites ($P \leq 0.001$). The across site average was 1.40 ears per plant. The number of ears per plant varied from a minimum of 0.43 to a maximum of 1.61 across all sites. The averages ranged from 0.99 to 1.04 ears per plant across the three sites. Variation was highest in Harare2012 where number of ears per plant ranged from 0.43 to 1.61. Harare2013a and b had similar averages and minimum number of ears but the maximum number of ears recorded varied significantly. For Harare2013a the average number of ears was 1.00 and the range was from 0.53 to 1.33 ears per plant. The average for Harare2013b was 1.04 and the range was 0.54 to 1.52 ears per plant.

Response of hybrids to MSV infection was significantly variable at $P \leq 0.001$ in Harare2012 and 2013a but there were no significant differences in Harare2013b, even though the heritability was high at 0.59. For MSV the average disease score for Harare2012 and 2013a was 2.20. Scores ranged from 1.42 to 3.80 across sites. The mean scores across sites ranged from 1.98 to 2.40 for the two sites. Harare2013b was not included in the mean, since MSV did not show significant variation. Disease incidence and severity was lower in Harare2012, where scores ranged from 1.42 to 2.90 with an average score of 1.98. Scores were higher in Harare2013a, ranging from 1.90 to 3.80 with an average of 2.40.

In all three sites test hybrids showed variable response to the presence of ET. Scores ranged from 1.48 to 4.11 with an across site average of 2.60. The site means ranged from 2.09 to 3.01. Harare2013a had the highest minimum, maximum and average score. Severity of ET was highest in this site. The average and maximum disease scores were lowest in Harare2012 where 2.09 and 2.77 were recorded, respectively. Harare2012 had the narrowest range of scores from 1.56 to 2.77 while Harare2013a had the widest. Scores for Harare2013b ranged from 1.48 to 3.48 with an average score of 2.66.

Of the top 10 yielding hybrids across all three sites, nine were CIMMYT hybrids (Table 6.3) and one was a commercial check, SC727. Seven of the hybrids in the top 10 yielders were released from 2007 to 2010 and only two in 2004 and one in 2001. The commercial hybrid SC727 that ranked 2nd in the trial was released in 2010. Grain yield for SC727 was 8.45 t ha^{-1}. SC635 and SC513, released in 2003 and 1997 respectively, yielded 6.05 t ha^{-1} and 3.50 t ha^{-1} respectively, that was below the trial mean of 6.41 t ha^{-1}. The yield for the top 10 ranking hybrids ranged from 7.80 t ha^{-1} to 9.11 t ha^{-1}. Three hybrids, CKH10717, CKH10723 and CZH0923, all released in 2010, ranked 5th, 7th and 9th respectively with grain yield of 8.05 t ha^{-1}, 7.99 t ha^{-1} and 7.81 t ha^{-1} respectively. CZH0713 and CKH0790, released in 2008 and 2007 respectively, ranked 1st and 3rd respectively and yielded 9.11 t ha^{-1} and 8.24 t ha^{-1} respectively. CZH0631, first tested in 2006, yielded 8.06 t ha^{-1}, and ranked 3rd. Earlier hybrids CZH03006, CZH03002 and CZH00021, first tested in 2004 for the first two and 2001 for the last, ranked 6th, 8th and 10th respectively and yielded 8.03 t ha^{-1}, 7.94 t ha^{-1} and 7.78 t ha^{-1} respectively.

The hybrids that ranked in the bottom 10 positions, from 61 to 70, included nine CIMMYT hybrids (Table 6.3). Of these, two were first tested in 2000 and 2001 while six were first tested in 2005 and 2006. The six hybrids first tested in 2005 and 2006 generally yielded less compared to some earlier and later releases (Figure 6.3) giving a depression in the yield growth curve over the 11 years. The depression in yield growth corresponded with an increase in MSV score or reduced resistance during the same period (2005 and 2006) of release (Figure 6.8). The ninth hybrid, CZH0837, ranked 68th, was first tested in 2009 and yielded 3.4 t ha^{-1}. SC513 ranked 67th. CZH04006, first tested in 2005, ranked last of the 70 entries and yielded the least (2.31 t ha^{-1}). The least yielding hybrids suffered relatively high ear rot incidence ranging from 13.9% to 23.7% compared to a trial mean of 3.5 (Table 6.3). This could have contributed significantly towards the poor performance of the check SC513 and four least performing CIMMYT hybrids from 2005, 2006 and 2009 considering the MSV scores for these entries were general less than 3.

The days to mid-anthesis ranged from 69.9 to 78.2 among the top nine CIMMYT hybrids. The number of days to mid-anthesis for hybrids ranked in positions 61 to 70 ranged from 65.0 to 84.7, that was more variable compared to the top 10 ranking hybrids. The ASI of the top nine CIMMYT hybrids ranged from -0.4 to 1.3 days and from 0.1 to 1.6 days for the checks. For hybrids ranked from 61 to 70, ASI ranged from 0.0 to 1.4 days. The trial mean for ASI was 0.3.

Table 6.3 Yield and secondary traits statistics for the top 10 and bottom 10 ranking hybrids and checks

Name	**Rank**	**Across Grain Yield**	**Harare1 Grain Yield**	**Harare2 Grain Yield**	**Harare3 Grain Yield**	**Anthesis**	**ASI**	**Plant Height**	**Ear Height**	**Ear Position**	**Lodging**	**Ears/ Plant**	**Husk Cover**	**Ear Rot**	**ET**	**Grain texture**	**Grain moisture**	**MSV**	**Ear Aspect**	**Release**
		t/ha	t/ha	t/ha	t/ha	d	d	cm	cm	0-1	%	#	%	%	1-5	1-5	%	1-5	1-5	Year
CZH0713	1	9.11	6.82	9.34	11.18	74.8	0.1	205.2	100.0	0.49	-0.1	1.36	2.3	1.2	2.3	2.8	21.3	2.3	2.0	2008
SC727	2	8.45	5.80	10.51	9.05	78.2	0.1	235.0	120.4	0.54	1.4	0.94	5.3	2.7	2.7	4.3	29.8	2.3	1.8	2010
CKH0790	3	8.24	6.31	9.12	9.29	76.5	0.9	211.2	103.7	0.52	4.7	1.02	4.6	1.0	2.1	3.0	27.9	2.0	1.8	2007
CZH0631	4	8.06	6.54	7.94	9.70	71.1	0.2	207.2	101.4	0.52	2.6	1.00	3.1	1.0	2.8	2.7	31.2	1.8	1.9	2007
CKH10717	5	8.05	6.92	7.21	10.02	75.7	-0.4	211.0	115.1	0.53	6.1	1.06	0.3	1.2	2.2	2.7	29.7	2.1	1.8	2010
CZH03006	6	8.03	5.83	9.47	8.79	70.3	1.1	207.6	99.7	0.50	5.5	0.96	4.3	3.0	2.9	2.7	22.2	1.8	1.7	2004
CKH10723	7	7.99	6.44	8.25	9.28	75.7	-0.3	214.5	109.0	0.52	7.4	1.08	4.6	0.8	2.4	2.9	22.6	2.2	2.2	2010
CZH03002	8	7.94	5.35	9.30	9.19	69.9	0.3	189.7	88.3	0.44	4.3	1.14	2.3	1.8	2.9	2.8	16.2	2.0	2.2	2004
CZH0923	9	7.81	6.09	8.76	8.57	72.8	0.6	205.1	88.0	0.47	9.7	1.16	1.3	0.8	2.5	1.7	20.7	1.7	1.7	2010
CZH00021	10	7.78	6.41	9.22	7.71	73.1	1.3	203.7	95.8	0.48	1.7	1.03	-0.1	2.3	2.3	2.2	23.0	2.2	1.8	2001
SC635	52	6.05	3.05	8.67	6.44	71.4	1.6	199.8	111.2	0.55	1.5	1.00	4.7	4.8	2.5	3.8	16.7	2.0	2.6	2003
CZH04002	61	5.04	3.40	6.76	4.96	74.5	0.6	197.6	96.1	0.47	9.5	0.99	4.7	6.1	2.8	2.1	30.7	2.4	2.2	2005
KDH3	62	4.95	3.55	5.83	5.48	66.1	0.7	191.9	75.5	0.43	10.3	0.86	2.2	1.7	3.3	2.0	17.3	2.6	2.7	2006
KDH4	63	4.62	4.31	4.64	4.90	65.0	0.1	190.7	83.9	0.44	1.9	0.91	1.5	1.9	3.2	2.1	15.8	2.4	2.4	2006
CZH99015	64	4.28	2.78	5.15	4.91	69.5	1.0	185.9	75.6	0.41	1.6	0.82	1.8	6.7	2.5	1.6	18.5	3.2	2.3	2000
CZH00002	65	3.82	2.45	5.27	3.73	69.0	1.4	180.7	69.6	0.45	4.4	0.86	3.5	7.1	2.9	1.9	16.8	2.9	3.0	2001
CZH0536	66	3.63	2.92	5.61	2.37	82.0	0.1	188.7	100.4	0.51	2.8	0.87	2.9	15.9	2.4	2.8	19.1	2.8	2.8	2006
SC513	67	3.50	1.44	4.64	4.43	72.8	1.6	189.8	95.6	0.49	9.9	0.72	4.9	21.4	2.6	3.0	15.9	2.6	3.5	1997
CZH0837	68	3.40	5.51	2.63	2.07	82.1	0.0	188.6	88.5	0.51	2.4	0.79	2.7	21.9	2.7	2.9	22.1	2.1	2.7	2009
CZH0524	69	3.40	6.52	2.24	1.43	81.6	1.1	194.3	82.1	0.45	-0.6	0.66	3.5	13.9	2.3	2.7	17.0	2.0	2.4	2006
CZH04006	70	2.31	3.94	1.87	1.12	84.7	0.5	182.8	93.7	0.51	6.6	0.78	4.1	23.7	2.4	2.7	16.6	2.1	2.9	2005
Trial mean		**6.45**	**5.21**	**7.21**	**6.93**	**73.1**	**0.3**	**199.1**	**97.7**	**0.50**	**4.8**	**1.01**	**3.2**	**3.5**	**2.6**	**2.5**	**23.3**	**2.2**	**2.2**	
LSD		**0.89**	**1.39**	**2.10**	**1.62**	**1.5**	**0.8**	**13.8**	**13.8**	**1.06**	**7.1**	**0.13**	**3.2**	**3**	**0.4**	**0.3**	**5.0**	**0.6**	**0.4**	
h^2			**0.79**	**0.72**	**0.92**	**0.85**	**0.71**	**0.72**	**0.67**	**0.42**	**0.20**	**0.69**	**0.39**	**0.75**	**0.77**	**0.91**	**0.44**	**0.67**	**0.82**	

h^2 is heritability, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$ LSD (0.05) is least significant difference, MSe is Mean square error

Number of ears per plant for the top nine CIMMYT hybrids ranged from 0.94 to 1.36 and from 0.72 to 1.00 for the commercial checks. The hybrids ranking from 61 to 70 ranged from 0.66 to 0.99. The trial mean was 1.01. Plant height was over 200 cm in eight of the top CIMMYT hybrids and SC727. Plant height for SC635 and SC513 was less than 200 cm. The trial mean was 199.1 cm. The plant height for all hybrids ranked from 61 to 70 was less than the trial mean, ranging from 180.7 cm to 197.6 cm, a possible sign of stunting.

For the CIMMYT hybrids ranked in the top 10, MSV scores ranged from 1.7 to 2.3. The MSV scores for the CIMMYT hybrids ranked in positions 61 to 70 ranged from 2.0 to 3.2. MSV scores for commercial checks ranged from 2.0 to 2.6. There was significant development of ET in the trials. ET scores among the CIMMYT hybrids ranked in the top 10 ranged from 2.1 to 2.9 and for hybrids ranked from 61 to 70 it was from 2.3 to 3.3. Scores for the commercial checks ranged from 2.5 to 2.7.

6.3.2 Multi-environment trial analysis

6.3.2.1 Genetic correlations for grain yield under MSV stress

The genetic correlations were high among all sites (Table 6.4). Harare2013a and b were planted on the same planting block during the 2012/13 season hence correlation was higher between the two sites compared to Harare2012 that was in a different planting block and season (2011/12).

Table 6.4 Genetic correlations for grain yield under MSV stress

Site	Harare2012	Harare2013a
Harare2012		
Harare2013a	0.60	
Harare2013b	0.69	0.99

6.3.2.2 Complete linkage cluster analysis under MSV stress

Harare2013a and b clustered closer with each other at a distance of <0.25 compared to Harare2012 that varied at a distance close to 1.50 (Figure 6.2). Considering the genetic correlations, cluster analysis and the average yields, all three trials were included in further analyses that included evaluation and dissection of genetic gain.

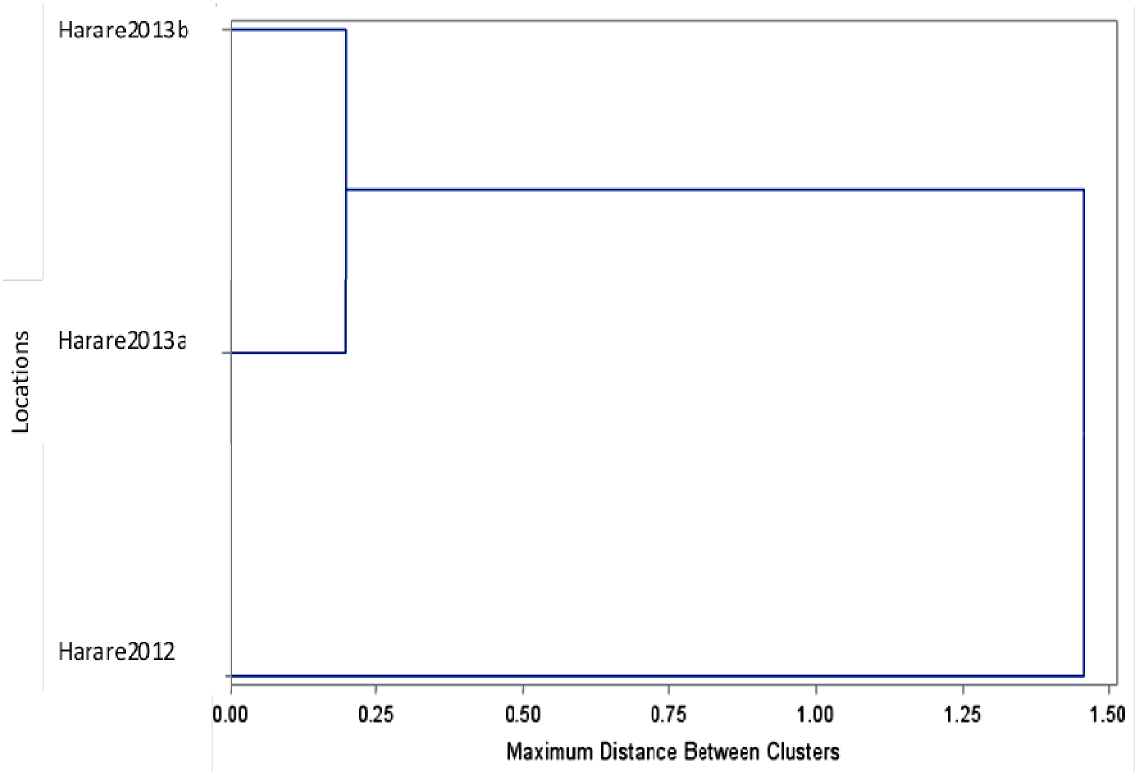


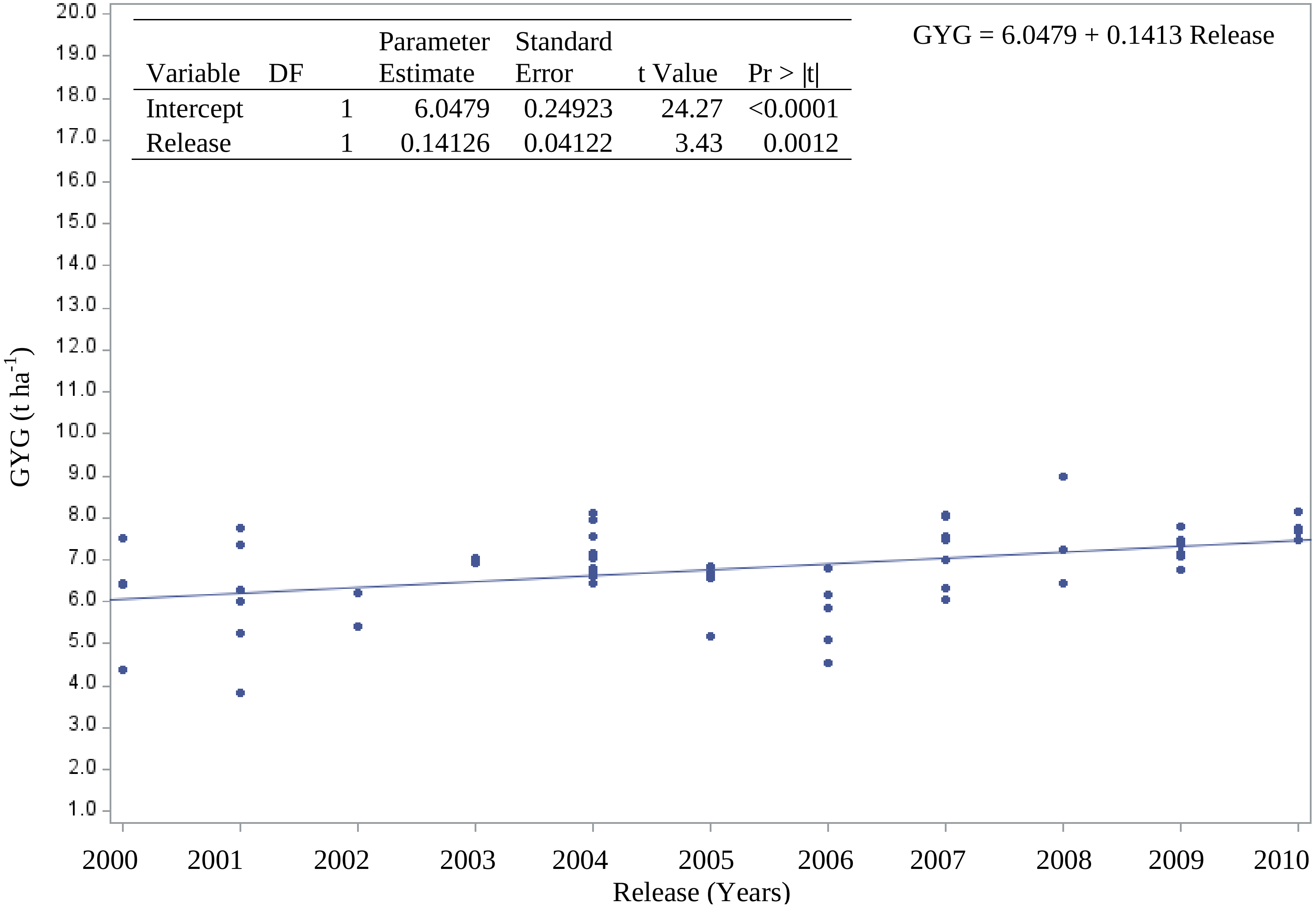
Figure 6.2 Cluster analysis for three MSV sites

6.3.3 Genetic gain in yield and secondary traits

A regression of grain yield against year of testing in regional trials showed significant and high levels of estimated genetic gain in grain yield under MSV and ET stress of 141.3 kg ha^{-1} $year^{-1}$ (2.2% $year^{-1}$) over 11 years from 2000 to 2010 at $P \leq 0.01$ (Figure 6.3). New hybrids, on average, yielded better than old hybrids. There was no significant change in the number of days to mid-anthesis ($P \leq 0.05$) even though a trend of increasing at a rate of 0.13 days $year^{-1}$ was recorded over 11 years under MSV and ET stress (Figure 6.4). New hybrids matured in the same range as the old hybrids.

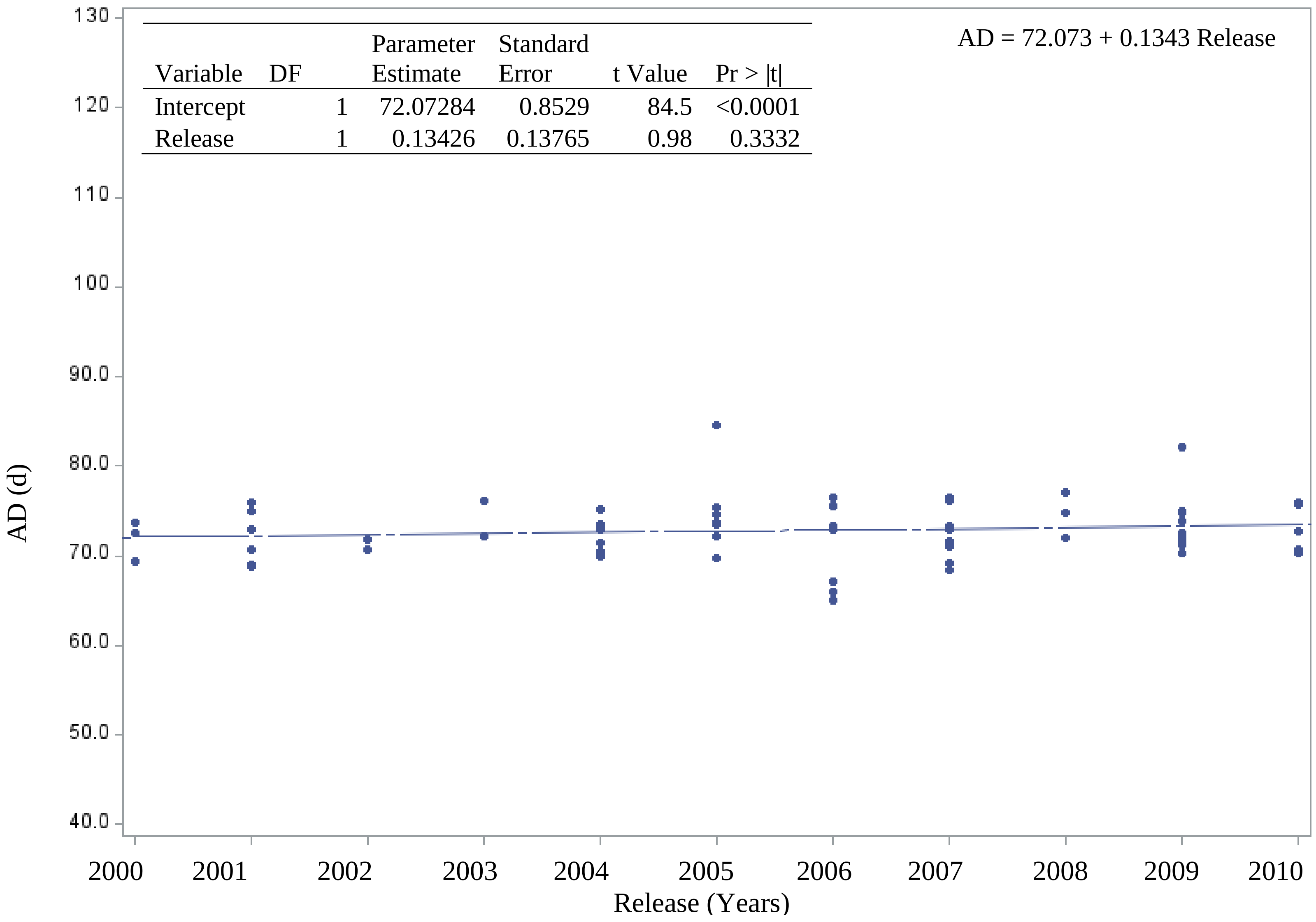
Anthesis-silking interval showed a decrease of about 0.06 days $year^{-1}$ over the 11 year period (Figure 6.5) which was significant at $P \leq 0.05$. New hybrids had shorter ASI and better synchronisation of pollen shed and silking under MSV and ET stress compared to the older hybrids. The new hybrids had reduced barrenness compared to the old hybrids. There was an increase in the number of ears per plant at a rate of 0.01 ears $plant^{-1}$ $year^{-1}$ under MSV and ET stress (Figure 6.6), significant at $P \leq 0.01$. Plant height did not significantly change at $P \leq 0.05$ (Figure 6.7).

Average disease scores decreased over the 11 years from 2000 to 2010. For MSV the average score on a 1-5 scale decreased by 0.03 $year^{-1}$ (Figure 6.8) at $P \leq 0.01$. New hybrids were more tolerant to MSV disease compared to old hybrids. Scores were relatively higher than would be expected for entries released in 2005 and 2006. Of the hybrids released in 2005 and 2006, only CZH0524 and CZH04001 had one MSV resistant donor parent which should have resulted in the sudden increase in average disease scores for the two years (Figure 6.8) and possibly the relatively low yields for the same period compared to the earlier and later years (Figure 6.3). For ET, an average decrease of 0.03 $year^{-1}$ was recorded over the period (Figure 6.9) at $P \leq 0.05$. The newer CIMMYT hybrids were more tolerant to ET disease compared to the old hybrids. The new hybrids were more resilient to both diseases and yielded better compared to the older hybrids. Grain yield/ grain moisture content ratio increased at a rate of 0.0057 $year^{-1}$ over the 11 years at $P < 0.05$ (Figure 6.10).



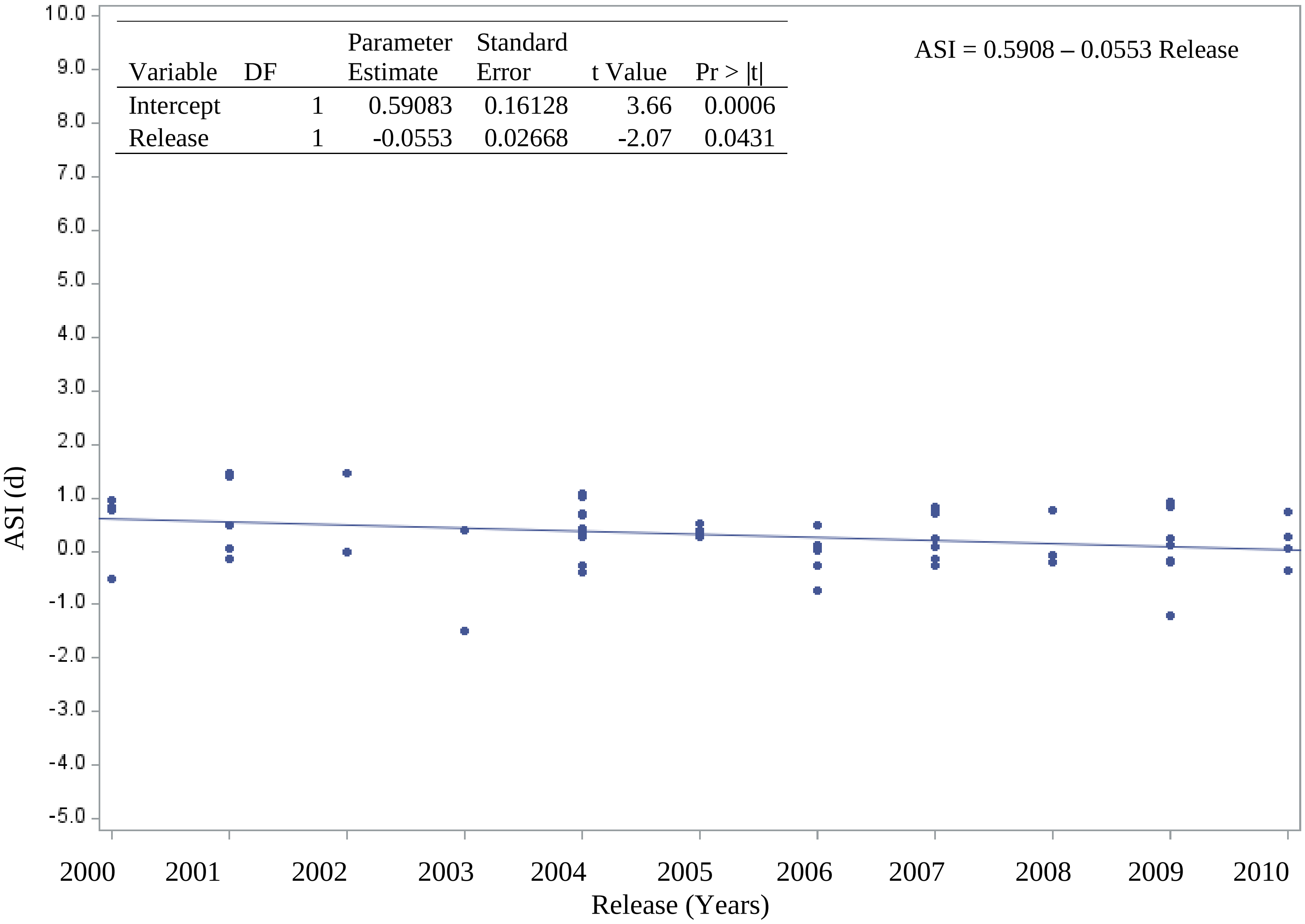
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	6.0479	0.24923	24.27	<0.0001
Release	1	0.14126	0.04122	3.43	0.0012

Figure 6.3 Changes in grain yield levels under MSV for hybrids released from 2000 to 2010



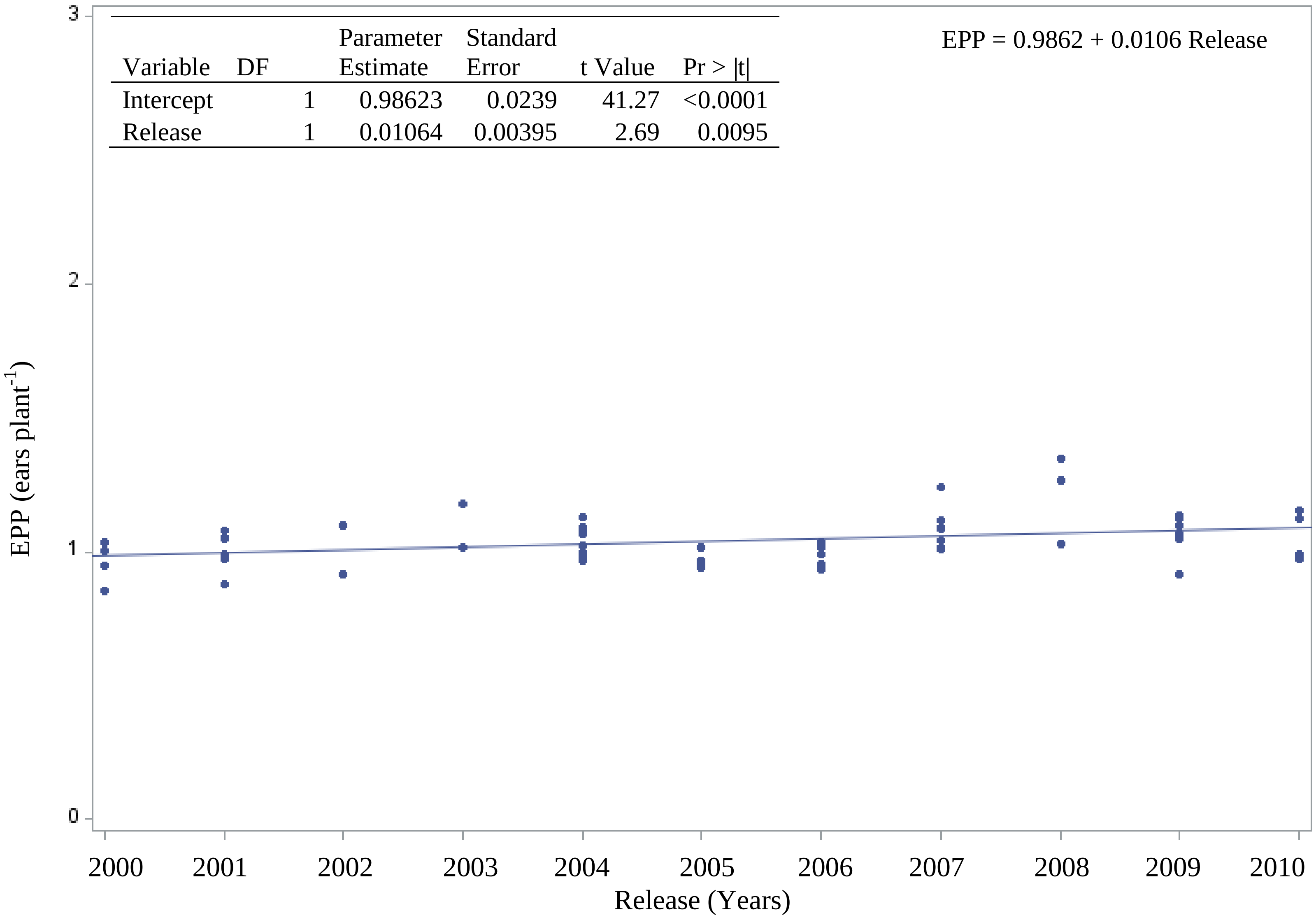
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	72.07284	0.8529	84.5	<0.0001
Release	1	0.13426	0.13765	0.98	0.3332

Figure 6.4 Changes in days to mid-anthesis under MSV for hybrids released from 2000 to 2010



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.59083	0.16128	3.66	0.0006
Release	1	-0.0553	0.02668	-2.07	0.0431

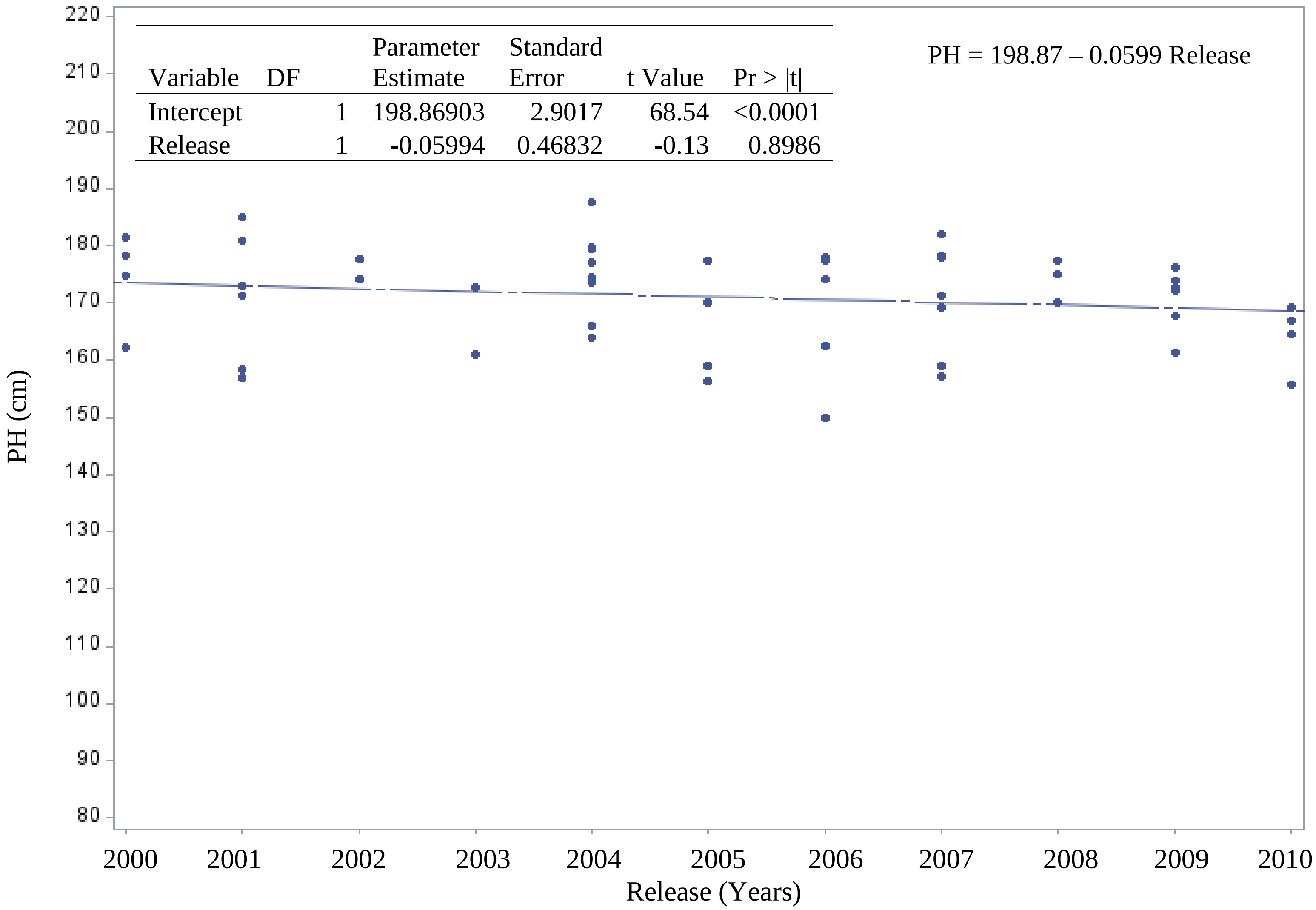
Figure 6.5 Changes in ASI under MSV for hybrids released from 2000 to 2010



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.98623	0.0239	41.27	<0.0001
Release	1	0.01064	0.00395	2.69	0.0095

EPP = 0.9862 + 0.0106 Release

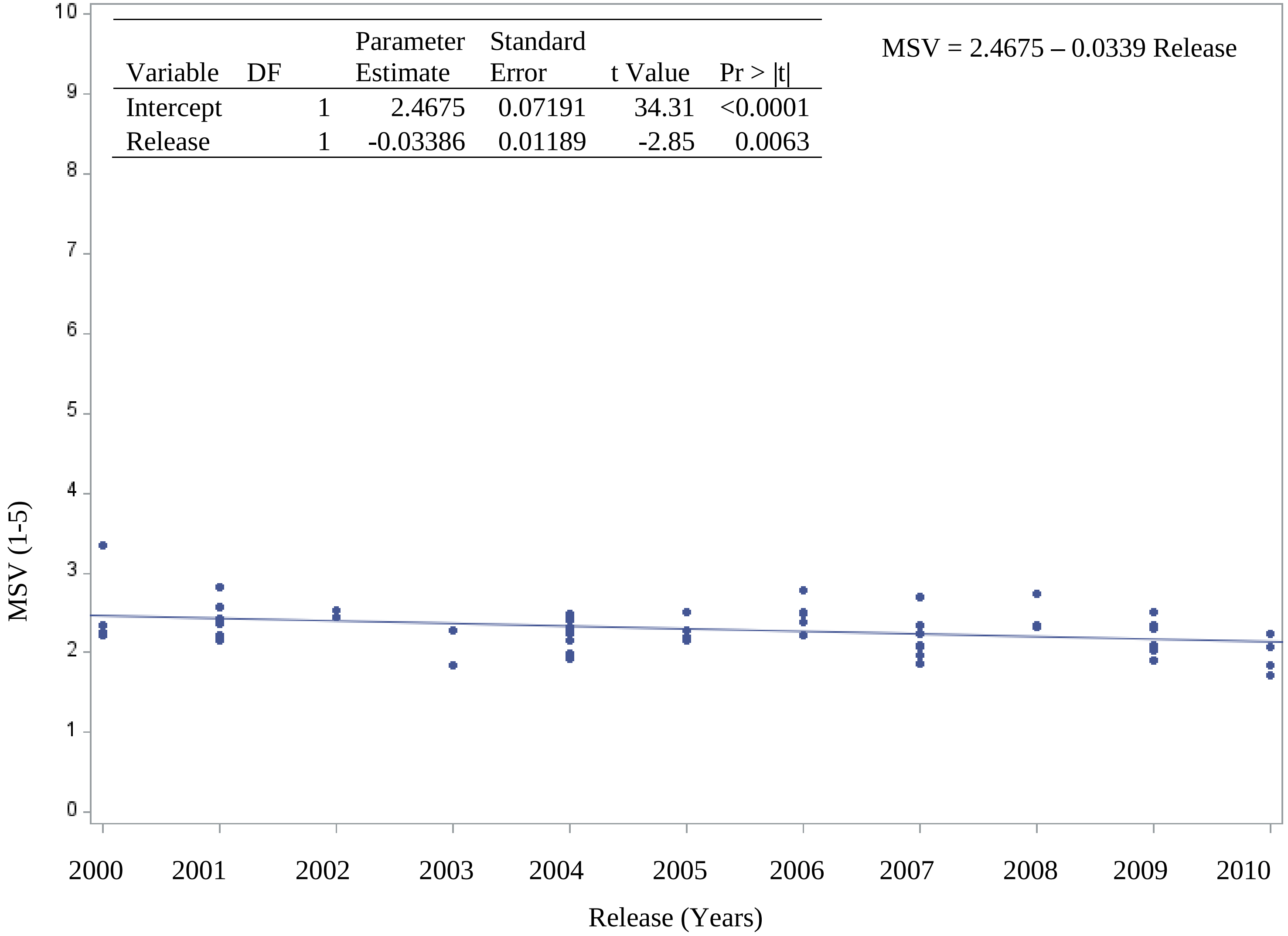
Figure 6.6 Changes in number of ears per plant under MSV for hybrids released from 2000 to 2010



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	198.86903	2.9017	68.54	<0.0001
Release	1	-0.05994	0.46832	-0.13	0.8986

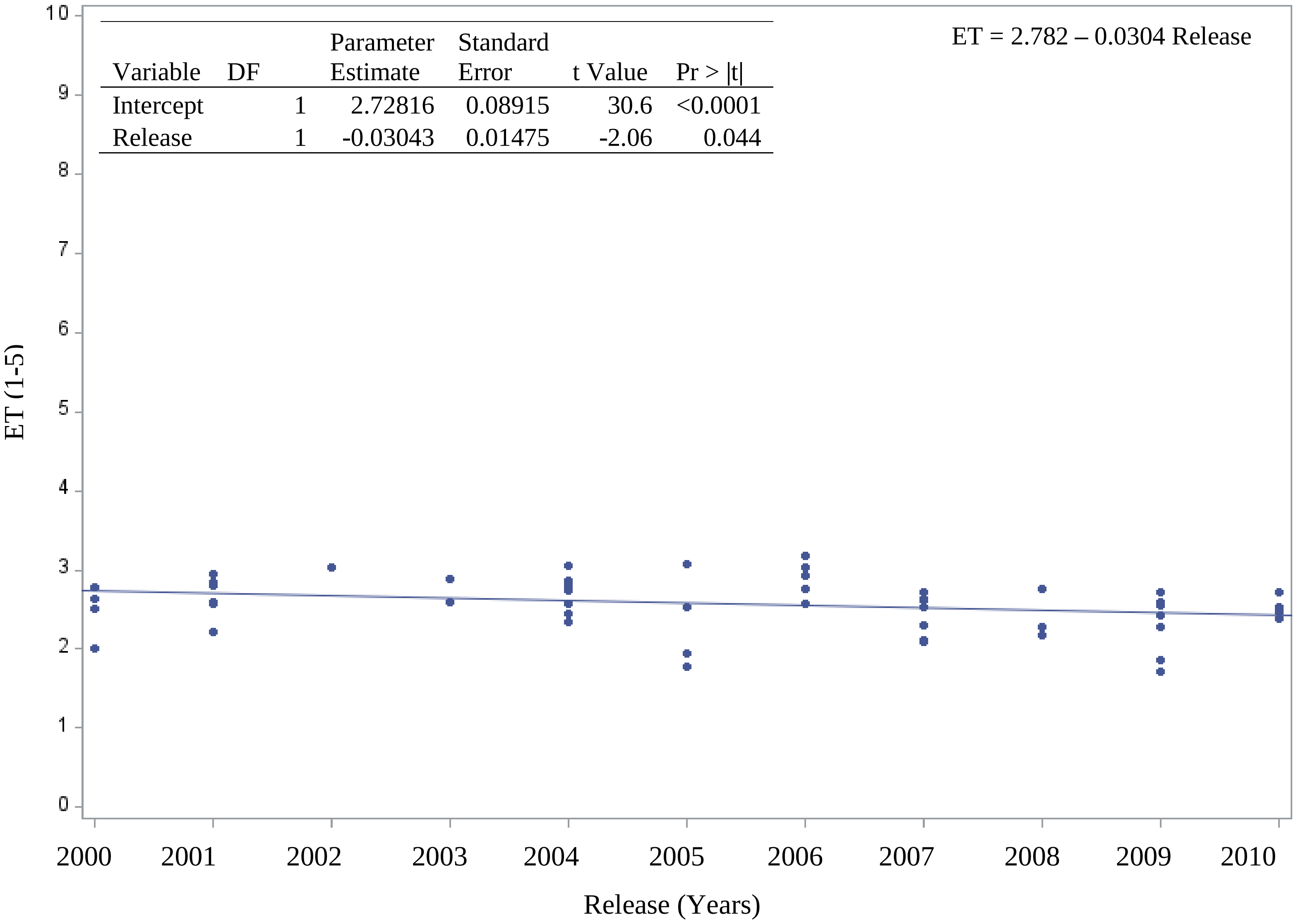
PH = 198.87 – 0.0599 Release

Figure 6.7 Changes in plant height under MSV for hybrids released from 2000 to 2010



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	2.4675	0.07191	34.31	<0.0001
Release	1	-0.03386	0.01189	-2.85	0.0063

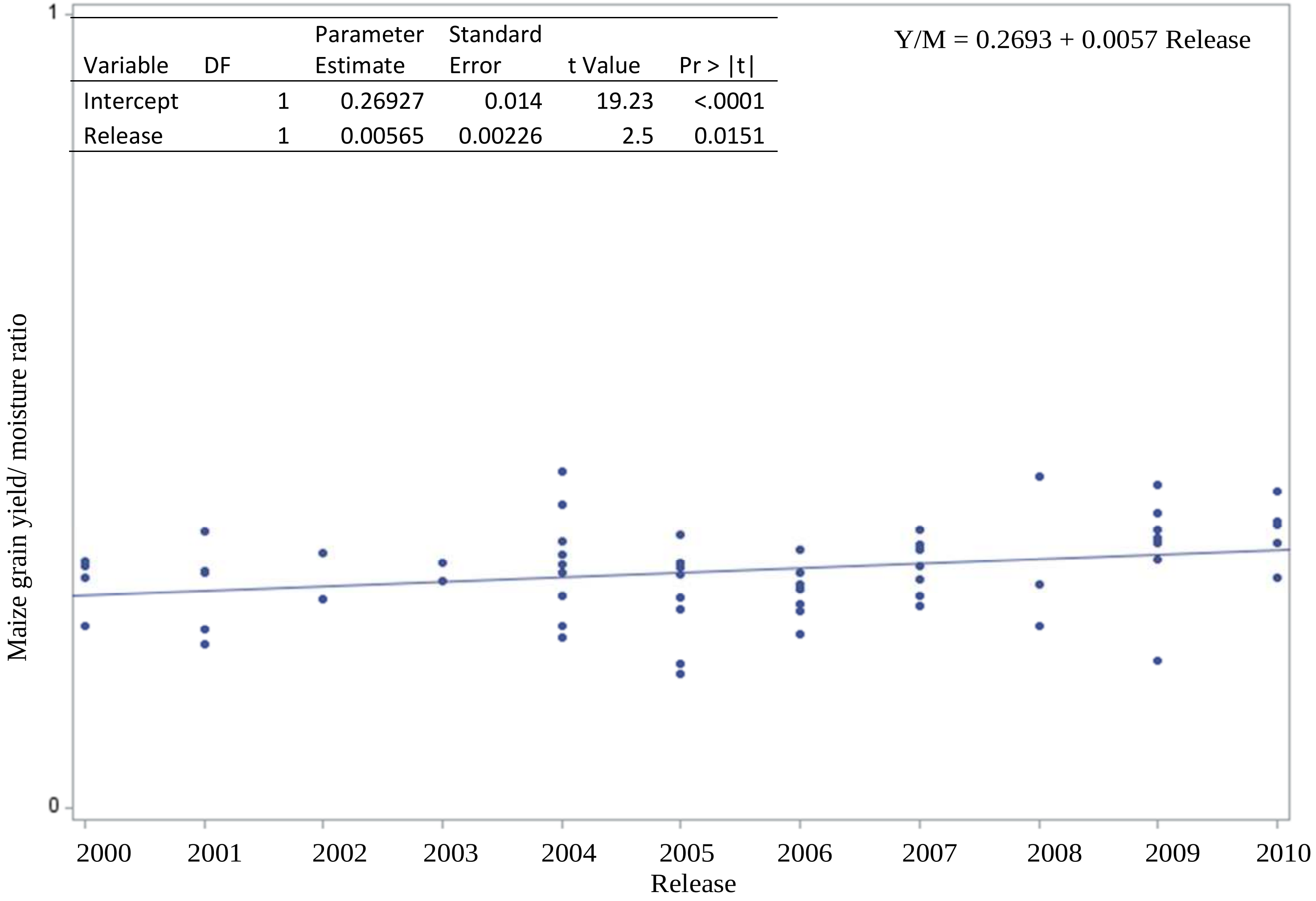
Figure 6.8 Changes in MSV resistance for hybrids released from 2000 to 2010



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	2.72816	0.08915	30.6	<0.0001
Release	1	-0.03043	0.01475	-2.06	0.044

ET = 2.782 – 0.0304 Release

Figure 6.9 Changes in resistance to ET for hybrids released from 2000 to 2010



Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.26927	0.014	19.23	<.0001
Release	1	0.00565	0.00226	2.5	0.0151

Figure 6.10 Changes in grain yield/ grain moisture ratio for hybrids released from 2000 to 2010

6.3.4 Dissecting genetic gain in yield in CIMMYT hybrids tested from 2000 to 2010 under biotic stress

There was no significant correlation between days to mid-anthesis and grain yield or barrenness (the number of ears per plant). Grain yield and the number of ears per plant decreased with increasing ASI at correlations of -0.27 and -0.43 respectively. Grain yield was lower in stunted susceptible hybrids (r = 0.65) and high in resistant hybrids where the correlations of grain yield with MSV and ET scores were -0.70 and -0.37 respectively. Reduced barrenness increased grain yield (r = 0.75). Barrenness decreased with increasing plant height (r = 0.31).

Table 6.5 Pearson's correlation for grain yield and secondary traits under biotic stress conditions

	GYG	AD	ASI	PH	EPP	MSV	ET
GYG	-						
AD	-0.06^{ns}	-					
ASI	-0.27*	-0.40***	-				
PH	0.65***	-0.35**	-0.27*	-			
EPP	0.75***	-0.05^{ns}	-0.43***	0.31*	-		
MSV	-0.70***	-0.29*	0.11^{ns}	-0.43***	-0.46*	-	
ET	-0.37**	-0.54**	0.15^{ns}	0.18^{ns}	-0.25*	0.24^{ns}	-
Y/M	0.69***	-0.43***	-0.07^{ns}	0.04^{ns}	0.66***	-0.71***	-0.39**

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, ns non significance at $P \leq 0.05$ GYG is grain yield, AD is days to mid-anthesis, ASI is anthesis-silking interval, PH is plant height, EPP is number of ears per plant, SEN is leaf senescence

MSV and ET diseases significantly reduced grain yield in susceptible hybrids at correlations of -0.70 and -0.37 respectively. MSV stunted susceptible hybrids (r = -0.43). Stunted susceptible hybrids reached mid-anthesis and mid-silking later than resistant hybrids at correlations with plant height of r = -0.35 and -0.27 respectively. Under biotic stress grain yield/ grain moisture content ratio positively correlated with grain yield and the number of ears plant^{-1}.

6.4 Discussion

Net genetic gain estimate for yield under MSV and ET disease stress was high at 141.3 kg ha^{-1} year^{-1} (2.2% year^{-1}). This was higher than the estimated gain of 109.4 kg ha^{-1} year^{-1} (1.4% year^{-1}) under optimal conditions in the same study. Earlier studies by Duvick (2005), Tollenaar (1989) and Ci *et al.* (2011) were done under optimal conditions and were not compared to the gains recorded under biotic stress in this study. Duvick (2005) highlighted that breeding for disease resistance enhances yield. For this study the hybrids were artificially inoculated with MSV using the leaf hopper vector (*Cicdulina mbila*). Innoculation was done within 14 days of planting. Artificial inoculation was used to ensure uniform coverage of the disease on the crop. The hybrids were exposed to MSV disease at an early stage of growth. Infection at this stage of growth can cause grain yield losses of up to 100% (Bjarnason, 1986; Wambugu and Wafula, 2000) in susceptible hybrids.

The susceptible hybrids suffered chlorosis resulting in reduced yields. The susceptible old hybrids suffered high chlorosis with high MSV scores and yielded lower than the new hybrids that had less chlorosis and lower MSV scores. Yield was lower in the stunted, susceptible older hybrids compared to the new hybrids. As the yield in susceptible older hybrids was reduced by MSV, the yield of MSV tolerant hybrids remained high as the hybrids maintained growth, vigour, did not suffer significant chlorosis, and grain set and filling was almost normal. Having been selected in METs, the newer hybrids had high yield potential across environments, including optimal conditions. As a result, with good MSV tolerance and all other conditions being optimal except for the MSV and ET disease, yield in the new hybrids were higher than in the old hybrids. The yield penalty in susceptible hybrids under MSV and ET disease stress resulted in the large differences in yield with the new hybrids. This reduced the trial mean yield, giving the new hybrids a significant yield advantage (high genetic gain).

Resistance to MSV disease significantly affected the performance of the hybrids under MSV disease stress. This is shown by the strong negative correlation (-0.70) between grain yield and the MSV scores (Table 6.5). At a score of 3, the plants will have high chlorosis, reduced photosynthesis, will be stunted and grain development is low. The newer hybrids had lower average MSV disease scores. There was a positive genetic gain in MSV tolerance with newer hybrids more tolerant with lower scores. The scores decreased at an estimated rate of 0.03 year^{-1} over the 11 years. The gain in MSV tolerance contributed significantly to grain yield as is shown by the correlation coefficient. The newer hybrids were more tolerant; less affected by the disease and produced more grain. Studies on genetic gain under stress conditions have so far focused mainly on abiotic stresses that include high plant density, low N and drought stress. Not much work has focused on genetic gain in biotic stresses in maize.

Under the natural infestation of ET, a decrease in grain yield with increasing disease score or reduced tolerance was observed. There was a negative correlation of -0.37 between grain yield and ET. There was a general decrease in ET score in the new hybrids compared to the old hybrids estimated at 0.03 year^{-1} that corresponded to the increase in yield. New hybrids showed better tolerance to ET, making them multiple disease tolerant.

The changes in tolerance to MSV and ET disease are associated with some phenological and physiological changes. This is because MSV and ET diseases affect the phenology and physiology of the crop through stunted growth and reduced photosynthesis or failure of chloroplast development (Rose, 1978; Shepherd *et al.*, 2010). There was no significant change in the number of days to mid-anthesis and there was no significant correlation between days to mid-anthesis and grain yield. In other studies grain yield increase was associated with increased number of days to mid-anthesis that is associated with an extended season that provided a longer grain filling period (Duvick, 2005) but there was no association observed in this current study.

The new hybrids had a shorter ASI that promoted better pollination and grain set. ASI had a negative correlation with grain yield. Under diseaese stress conditions ASI normally increases in non-stress tolerant hybrids. For this study new hybrids had shorter ASI compared to the old hybrids. They showed better tolerance to the disease stress. In studies by Bänziger *et al.* (2002), Duvick (2005) and Monneveux *et al.* (2005) improved dry matter partitioning to the ear under stress conditions enables silking before, during or soon after mid-anthesis, reducing ASI.

Closely related to ASI is barrenness or the number of ears per plant. Under stress conditions new hybrids were observed to have reduced barrenness as a result of improved grain set and grain filling attributed to better dry matter partitioning to the ear (Monneveux *et al.*, 2005; Duvick, 2005b). In this study there was a positive genetic gain in number of ears per plant (Figure 6.6) with a strong and positive correlation with yield (Table 6.5). Yield increased with increasing number of ears per plant or reduced barrenness. Barrenness decreased significantly over the 11 years. The newer hybrids had reduced barrenness compared to the older hybrids. Reduced barrenness could be a result of improved ASI, pollination, grain set and better dry matter partitioning to the ear during grain set and grain filling.

From the strong positive correlation of grain yield with plant height of 0.65, yield was higher in hybrids with normal growth compared to stunted hybrids. Considering that one of the effects of MSV disease is reduced and/or stunted growth (Shepherd *et al.*, 2010), tallness or normal height under MSV disease is an indication of improved resistance to the disease and is associated with increased yield. Susceptible hybrids were stunted as is shown by the negative correlation of plant height with MSV.

Grain yield/ grain moisture ratio positively correlated with grain yield and the number of ears per plant, and increased over the 11 years. The trait is closely linked to maize grain yield under biotic stress and can therefore be used for selection (Ngure, 1995).

As described by Duvick (2005) genetic gain is a result of physiological and phenological changes. In the current study, newer hybrids displayed physiological advantage over old hybrids. Secondary traits such as ASI and EPP have high heritability and genetic variation under stress, making them important for selection under stress. These two traits have been used in selecting for better grain yield under stress (Bolaños and Edmeades, 1996; Campos *et al*., 2006). ASI is important for grain set. The findings from this study agree with the negative correlation of grain yield with ASI that was reported by Campos *et al*. (2006).

Changes observed in this study included reduced barrenness or increased number of ears per plant, taller or normal plants that are not stunted by the disease that yielded better under MSV and ET stress with reduced ASI and improved MSV and ET tolerance. Yield was better in new hybrids compared to older hybrids.

6.5 Conclusions

There was significant positive genetic gain in MSV and ET tolerance resulting in a gain in grain yield of 141.3 kg ha^{-1} year^{-1} (2.2% year^{-1}) under MSV and ET disease stress in CIMMYT drought tolerant hybrids that were released from 2000 to 2010. This is a good indication that farmers can potentially access MSV tolerant hybrids for better production. Since the hybrids were tested and selected in METs, hybrids that have high MSV tolerance will maximise yield under optimal conditions and perform well under MSV disease stress. The hybrids also showed improved ET tolerance that is also desirable. The gain in grain yield can be attributed to improved performance under MSV and ET disease stress as a result of reduced chlorosis and stunting shown by the increased plant height (low disease scores) in new hybrids that yielded more. Plant processes such as photosynthesis have continued at high levels even though they could have been reduced by the disease infection as there was a significant presence of the disease in all entries (scores were all greater than 1). New CIMMYT drought tolerant hybrids had shorter ASI, reduced barrenness and increased grain yield/ grain moisture ratio that all positively contributed towards yield. New hybrids were therefore more MSV and ET tolerant and effectively reduced losses incurred due to MSV and ET disease stresses. With the new CIMMYT drought tolerant hybrids, costs can be saved from reduced pesticide control of leafhoppers and fungicides for controlling ET. The new CIMMYT drought tolerant hybrids have high tolerance to MSV and ET diseases and can yield significantly under MSV and ET infestations.

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Chapter 7

Evaluation of genetic diversity in the parental CIMMYT lines making up the best performing CIMMYT hybrids released from 2000 to 2010 in Eastern and Southern Africa

Abstract

Fifty-four lines from the CIMMYT Southern Africa (Zimbabwe) breeding programme were sequenced using genotyping-by-sequencing. An analysis using TASSEL and MEGA6 of the 269,693 single nucleotide polymorphism (SNPs) generated gave a genetic distance range of 0.01 to 0.40 with an average of 0.29. Frequency of distances was highest in the range from 0.2001 to 0.4000. A cluster analysis using unweighted pair group method with arithmetic means (UPGMA) did not show distinct clusters, rather 10 defined sub-clusters divided mainly by pedigree. Some of the lines did not sub-cluster as they were of different pedigree and origin. Despite the high frequency of genetic distances between 0.20 and 0.40, four lines that included CML444, CML395, CML312 and CML442, were more frequently used in the formation of the hybrids evaluated for genetic gain in this study. The rest of the lines were used at 0.48% (once) to 2.38% (five times) at most compared to 7.14% to 17.62% (19 to 37 times) for the four lines. The lines evaluated do not represent the genetic base of CIMMYT Southern Africa but are parental lines of the best performing hybrids selected from 2000 to 2010. Since the best performing lines like the ones evaluated in this study are disseminated to the farmers in the region through the public and private sector, there is need to check the frequency of use of the parental lines to avoid potential narrowing down of the genetic base of the region.

7.1 Genetic diversity in breeding systems

During the hybrid era, from 1939 to date, maize breeders achieved significant success in making continuous genetic improvement in commercial grain yield using a broad genetic base. There has been a change over the years from recurrent selection breeding to pedigree breeding that has resulted in the use of a narrow genetic base through breeding generations of lines from a biparental cross (Singh *et al.*, 2011). This reduces the genetic base. Maize improvement throughout the world has been accompanied by a narrowing of the germplasm base, as new lines and varieties have been derived from intercrosses of existing elite materials replacing landraces (Goodman, 1999; Carvalho *et al.*, 2004). However, being one of the most diverse crop species in the world with diversity manifested at both phenotypic and molecular levels (Magorokosho, 2006), there is no indication that improvement rates in maize have been adversely affected by narrowing the germplasm base (Duvick, 1990). However, narrowing the maize germplasm base that results from continued selection (Singh *et al*., 2011) in a breeding programme raises concern that there may be restrictions in breeding flexibility and slow response to new opportunities, pests, pathogens and agronomic practices in the future (Goodman, 1999). A typical example is the recent development of maize leaf necrosis (MLN) disease in Kenya and Tanzania (CIMMYT 2012; Adams *et al*., 2013; Adams *et al*., 2014). As part of the solution, exotic germplasm can be introduced in breeding programmes to counter the narrowing of germplasm bases. This has been done and is better documented in temperate areas and poorly documented in tropical breeding programmes (Goodman, 1999).

Data on genetic diversity is used in designing strategies that maximise the utility of maize genetic resources (Bracco *et al.*, 2009). Studying and understanding the molecular diversity allows preliminary classification of maize material into heterotic groups. Some studies have aimed at analysing diversity trends over time, formulating germplasm maintenance and conservation strategies, varietal identification and maintenance and relating diversity to agronomic performance (Magorokosho, 2006; Carvalho *et al*., 2004).

7.1.1 Maize diversity and maize breeding in CIMMYT ESA

Maize germplasm can be classified into three generalized geographical types, tropical, sub-tropical and temperate (Goodman, 1999). Maize is also classified according to endosperm and kernel constitution, kernel colour, kernel type and use, that is, dent, flint, floury, waxy, sweet and popcorn as well as by maturity. Maize is produced mainly for food in the tropics (Xu *et al*., 2009). The CIMMYT ESA stations in Harare and Nairobi are in the tropics. Most of the breeding material at the stations is tropical, with some occasional introductions of exotic tropical, temperate and subtropical germplasm. The breeding programme, since establishment in 1995, has been running concurrently hybrid variety development, open pollinated variety (OPV) development and pedigree line development programmes.

7.1.2 Diversity studies

In a study conducted in Argentina to assess the genetic diversity of popcorn maize landraces, 10 microsatellite markers were used. The study distinguished two main groups and an average gene diversity of 0.370 (Bracco *et al.*, 2009). In a study by Liu *et al*. (2003) to establish the genetic structure and diversity among 260 maize inbreds, lines were assayed for polymorphism at 94 microsatellite loci. The 2039 allelles identified served as raw data for estimating genetic structure and diversity. A phylogenetic tree was constructed. The study showed that tropical and subtropical material had greater gene diversity compared to temperate material. Vigouroux *et al*. (2008) analysed diversity at 462 simple sequence repeats (SSRs) spread throughout the maize genome and compared the diversity observed at these SSRs in maize to that in wild progenitor teosinte.

In a separate study on analysis of genetic diversity of 60 maize inbred lines, 20 amplified fragment length polymorphism (AFLP) marker combinations were used and 487 polymorphic fragments were detected with an average of 244 for each AFLP primer combination. Maize lines were assigned to heterotic groups based on genetic distances and origin (Li *et al*., 2004a).

A number of hybrids have been released from the CIMMYT ESA maize breeding programme over the period from 1995 to 2010. Some of the best hybrids released from 2000 to 2010 regional trials were evaluated for genetic gain from the 2011/12 to 2013 season. It is essential to check the diversity of parental lines used to make the hybrids. The aim of this study was to evaluate the genetic or molecular diversity of the parental lines constituting the 67 hybrids produced and released in ESA from 2000 to 2010.

The main objective of the study was to evaluate the genetic diversity of parental lines used to generate the 67 hybrids from the CIMMYT ESA programme from 2000 to 2010. The specific objectives were to

i) evaluate genetic diversity among the parental lines of the 67 best performing CIMMYT ESA hybrids released from 2000 to 2010

ii) evaluate the frequency of use of parental lines in the 67 best performing CIMMYT ESA hybrids released from 2000 to 2010

7.2 Methodology for molecular analysis

Fifty-four of the 73 parental lines (Table 7.1) of the hybrids in this study were sown in the greenhouse. The lines 28 to 37 and 65 to 73 (marked with ‘*’) were not available for genotyping.

Table 7.1 List of parental lines of the selected 67 hybrids from the CIMMYT ESA maize programme

Entry	Lines	Pedigree
1	CML395-B	90323(B)-1-X-1-BB
2	CML144-B	6207Q
3	CZL0617-B	MAS[206/312]-23-2-1-1-B
4	CZL0916	[[CML388/CML391]-BB-5-2/CML390]-2-1-2-1-1-B
5	DTPWC8F31-1-1-2-2-B-B	DTPWC8F31-1-1-2-2-B-B
6	CML159	P63QC2HC5-1-3-1-B-2-1-1-B-#-B
7	CML440	G16SeqC1F47-2-1-2-1-BBBBB
8	CZL03003-B	[[[K64R/G16SR]-39-1/[K64R/G16SR]-20-2]-5-1-2-B*4/CML390]-B-38-1-B-7-#-B
9	CZL03021-B	MAS[202/312]-86-1-3-1-B
10	CML312-B	S89500F2-2-2-1-1-B*5
11	CZL02015	DTPWC8F31-1-1-2-2-B
12	CZL057-B	[GQL5/[GQL5/CML202]F2-3sx]-11-1-3-1-B
13	CZL057	[GQL5/[GQL5/CML202]F2-3sx]-11-1-3-1-B
14	CZL04021-B	[GQL5/[GQL5/CML202]F2-3sx]-11-4-1-B
15	CML489-B	(CML202/LPSC3H297-2-1-1-2-2-#)-B-3-1-1-8-BB
16	CZL02014-B	([EV7992#/EVPOP43-SRBC3]#b#bsr-118//CML444)-B-1-1-3-5-B
17	CML536	[CML442/CML197//[TUXPSEQ]C1F2/P49-SR]F2-45-7-3-2-BBB]-2-1-1-2-1-BB
18	CML202-B	ZSR923-S4BULK-5-1-BBB
19	CML539	MAS[MSR/312]-117-2-2-1-B*4
20	CZL03007-B	[CML445/ZM621B]-2-1-2-3-1-B
21	CML488-B	DTPWC8F31-4-2-1-5-BBB
22	CZL04005-B	[CML198/LPSC3H144-1-2-2-2-2-#-BB]-1-4-1-1-B
23	CZL03004-B	[89[G27/TEWTSRPool]#-278-2-X-B/[COMPE2/P43SR//COMPE2]F#-20-1-1]-B-32-2-B-4-#-B
24	CZL0918	[[EV7992#/EV8449-SR]C1F2-334-1(OSU8i)-1-4-X-X-2-B/[SC/ZM605-1-2-5-2/CML395]-B-14-3-2-1-1-3-1
25	CZL095	[[KILIMA(ST94)-S5:101/CML442]-BB-2-2/CML390]-5-1-2-2-3-B
26	CZL0610-B	[(CML395/CML444)-B-4-1-3-1-B/CML444//[[TUXPSEQ]C1F2/P49-SR]F2-45-7-1-2-BBB]-2-1-2-2-B
27	CZL04006-B	ZM621A-10-1-1-1-2-B
28	*CML216	[MSR:131]-3-3-3-5-BBB
29	*CZL00025	SNSYNF2[N3/TUX-A-90]-102-1-2-2-BSR-B
30	*CML197	MSR270-2S3-5-1-BBB
31	*DRB-F2-60-1-2-B-1-1-BB	*DRB-F2-60-1-2-B-1-1-BB
32	*CZL0814	[CML312/[TUXPSEQ]C1F2/P49-SR]F2-45-3-2-1-BB//INTA-F2-192-2-1-1-1-B*4]-1-5-1-1-2-B
33	*CZL0517	[CML389/CML176]-B-29-2-2-1-4-B
34	*CKL05003	[CML202/CML395-6]-B-B-2-1-B-B*4
35	*CKL05017	[CML387/CML390]-B-1-1-4-B-B*4
36	*CKL05018	[CML387/CML390]-B-1-2-1-B*4
37	*CKL05022	[CML387/CML390]-B-1-1-5-#-B-B*4
38	CZL03011	ZM521B-66-4-1-1
39	CZL0712	[TS6C1F238-1-3-3-1-2-#-BB/[EV7992#/EV8449-SR]C1F2-334-1(OSU8i)-10-7(I)-X-X-X-2-BB-1]-1-1-2-1-1-B

Table 7.1 List of parental lines of the selected 67 hybrids from the CIMMYT ESA maize programme (Continued)

Entry	Parents	Pedigree
40	CZL0713-B	[SYN-USAB2/SYN-ELIB2]-12-1-1-1-B
41	VL05128	WWO1408
42	CZL99014-B	[COMPE2/P43-SR//COMPE2] FS#-20-1-1-B-1-B
43	CZL02012-B	ZM621A-10-1-1-3-1-B
44	CZL0720	NIP25-20-1-1-B
45	CZL0619-B	[CML312/CML445//[TUXPSEQ]C1F2/P49-SR]F2-45-3-2-1-BBB]-1-2-1-1-2-B
46	CZL076-B	[CML442/CML197//[TUXPSEQ]C1F2/P49-SR]F2-45-7-3-2-BBB]-2-1-1-1-1-B
47	CML442	M37W/ZM607#bF37sr-2-3sr-6-2-X]-8-2-X-1-BBBB
48	CZL04002-B	[Ent2:92SEW1-EarlySel-2/[DMRESR-W]EarlySel-#I-3-2-B/CML390]-B-26-1-B-1-#-1
49	CZL03018-B	[CML389/CML176]-B-29-2-2-B
50	CZL054-B	ZM523A-16-2-1-1-B
51	CML445-B	[[TUXPSEQ]C1F2/P49-SR]F2-45-7-5-1-B
52	CML443-B	[AC8342/IKENNE{1}8149SR//PL9A]C1F1-500-4-X-1-1-BB-1-BB
53	CML444-B	P43C9-1-1-1-1-1-BBBBB
54	CZL0919	00SADVEB-#-17-2-1-1-1-B
55	CZL00001-B	INTA-191-2-1-2-B
56	CML181-B	UW0417-B-2-1-1-BBBBB
57	ZEWBc1F2-216-2-2-B-1	ZEWBc1F2-216-2-2-B-1
58	CZL00003-B	DRB-F2-60-1-1-1-B
59	CZL03002-B	P100C6-61-1-4-##1-3-1-B
60	CZL0520	[MSRXG9]C1F2-205-1(OSU23i)-5-3-X-X-1-BBB-1-B
61	CZL04001-B	[P30/P45//M162W/MSR]97-323-3-1-5-B-1-#-1
62	SYN312-SR-B	MAS[MSR/312]-117-2-2-1-B*4
63	CZL00009-B	INTA-F2-192-2-1-1-1-B
64	*INTAINTB-B-41-B-1-1-B-B	*INTAINTB-B-41-B-1-1-B-B
65	*INTAINTB-B-41-B-14-1-B-B	*INTAINTB-B-41-B-14-1-B-B
66	*CKL05004	[CML202/CML395-6]-B-B-3-2-B-B*4
67	*CKL05019	[CML390/CML197]-B-B-5-1-B*4
68	*CML78	Pool32 G32C19MH32-1-#2-8 ##113-8
69	*CKL05007	[CML205/CML202]-B-2-1-1-B-B*4
70	*P100C6-200-1-1-#-#-B-B-B-B	*P100C6-200-1-1-#-#-B-B-B-B
71	*ZEWAc1F2	ZEWAc1F2
72	*ZEWBc1F2	ZEWBc1F2

*Genotypes that were not available for genotyping

Leaves were harvested at three to four weeks after planting from 10 plants from each line and bulked. Genomic DNA was extracted using the CTAB5’ (Cetyltrimethylammonium bromide) from the composite sample. A composite sample was used to ensure good representation of the line. The DNA selected was heterogeneous. DNA was amplified using the Polymerase Chain Reaction (PCR) technique. DNA was analysed using genotyping-by-sequencing (GBS).

7.2.1 Statistical analysis

Sequence data for 269,693 SNPs generated in GBS filtered using 1% minor allele frequency (MAF) before data analysis to eliminate system error as per procedure was analysed. The frequency of use of lines in hybrids was calculated. The genome summary was generated using trait analysis by association, evolution and linkage (TASSEL) (www.maizegenetics.net/tassel). Genetic distances were generated from the submitted GBS data. Frequency of genetic distances was calculated and analysed. The generated genetic distance matrix was converted to a Molecular Evolutionary Genetics Analysis version 6.0 (MEGA6) input file. Cluster analysis was done using genetic distance matrix data. The UPGMA was used for cluster analysis using the genetic distance matrix. A dendrogram was generated to show the relationship of parental lines of the best 67 CIMMYT ESA hybrids released from 2000 to 2010.

7.3 Results

7.3.1 Relative frequency of use of lines in hybrids released by CIMMYT ESA from 2000 to 2010

From an analysis of the frequency of use of lines in the CIMMYT ESA hybrids, the line that was used most was CML444 at 17.62% out of the 210 entries, that is, 37 times in the 67 hybrids (Figure 7.1). The line with the second highest frequency of use in making the 67 hybrids was CML395 at 10% (21 times), followed by CML312 at 9.05% (19 times) and CML442 at 7.14% (15 times). The rest of the lines were used not more than 2.38% (five times). Most of the lines were used once (0.48%). The single cross of CML444 and CML395 is used as a B tester and in three way and double cross hybrid formation. The same applies to the CML442 and CML312 single cross that is used as an A tester and a parent in hybrid formation. These four lines dominated in the best performing 67 CIMMYT ESA hybrids released from 2000 to 2010. Despite having a wide range of lines, some of the lines were used 37 times more than the others as in the case of CML444 while others were used only once or twice.

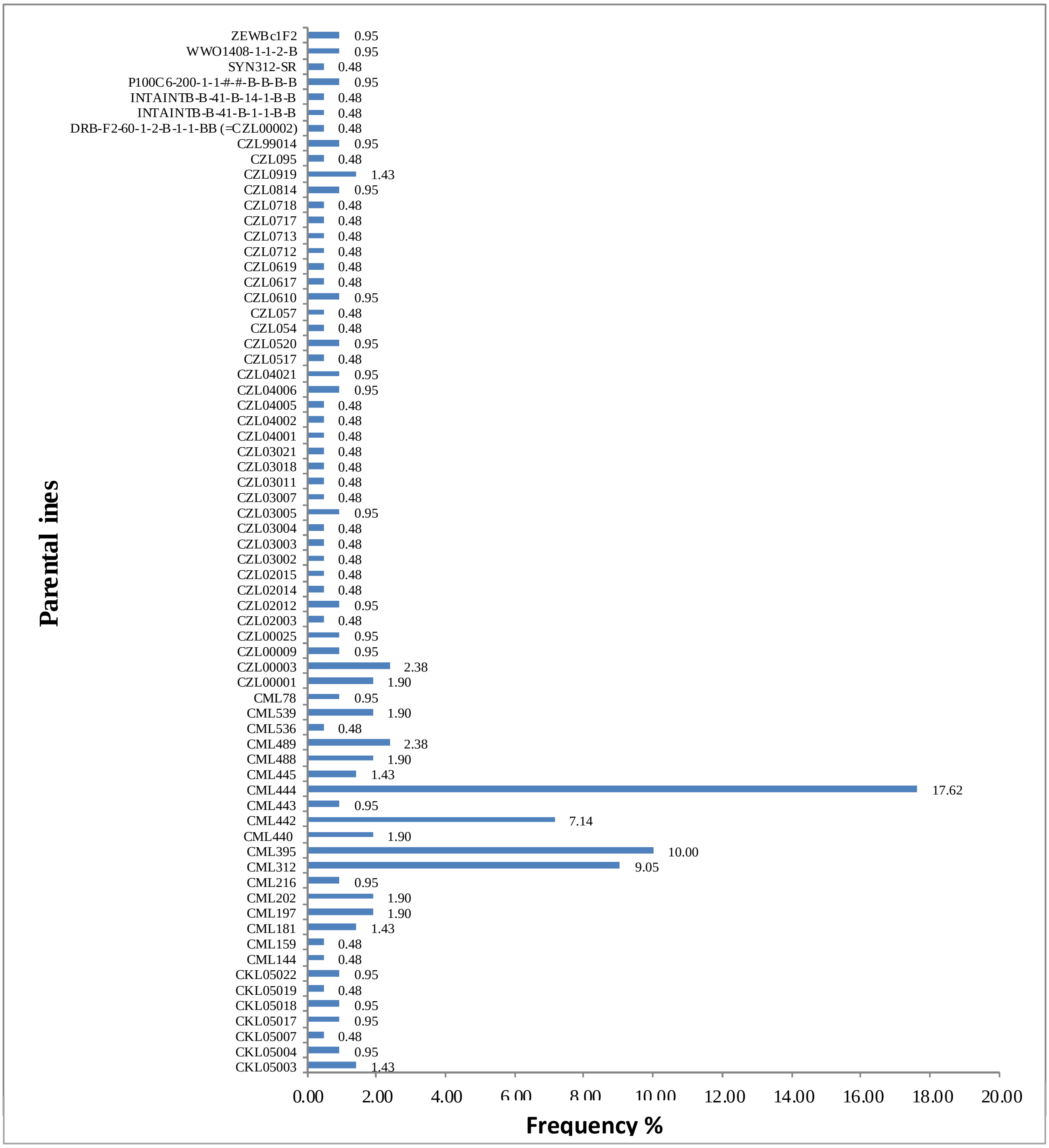


Figure 7.1 Relative frequency of use of lines in the top 67 hybrids released by CIMMYT ESA from 2000 to 2010

This may reduce diversity among the hybrids.

7.3.2 Heterogeneity test

To check the genetic purity of the parental lines, heterogeneity of the lines was assessed. Six lines that included ZEWBc1F2-216-2-2-B-1, CZL00003, CZL03002, CZL0520, CZL04001, SYN312SR-B and CZL00009 had a heterogeneity level of more than 5%, that is on the high side (Table 7.2). The rest of the lines had heterogeneity levels below 5% that is acceptable since the DNA was extracted from 10 different plants of a line that were compounded.

Table 7.2 Genetic purity/heterogeneity test of the 54 parental lines

Taxa Name	Proportion heterogeneity	Taxa Name	Proportion heterogeneity
CML395-B	0.02%	CZL03011	0.88%
CML144-B	0.06%	CZL0712	0.94%
CZL0617-B	0.07%	CZL0713-B	1.02%
CZL0916	0.08%	VL05128	1.10%
DTPWC8F31-1-1-2-2-B-B	0.09%	CZL99014-B	1.18%
CML159	0.11%	CZL02012-B	1.22%
CML440	0.12%	CZL0720	1.25%
CZL03003-B	0.12%	CZL0619-B	1.30%
CZL03021-B	0.12%	CZL076-B	1.33%
CML312-B	0.14%	CML442	1.41%
CZL02015	0.15%	CZL04002-B	1.44%
CZL057-B	0.19%	CZL03018-B	1.72%
CZL057	0.23%	CZL054-B	1.79%
CZL04021-B	0.23%	CML445-B	1.98%
CML489-B	0.32%	CML443-B	2.06%
CZL02014-B	0.33%	CML444-B	2.24%
CML536	0.34%	CZL0919	2.50%
CML202-B	0.40%	CZL00001-B	3.47%
CML539	0.49%	CML504	4.12%
CZL03007-B	0.49%	CML181-B	4.77%
CML488-B	0.52%	ZEWBc1F2-216-2-2-B-1	5.31%
CZL04005-B	0.57%	CZL00003-B	5.83%
CZL03004-B	0.62%	CZL03002-B	5.99%
CZL0918	0.62%	CZL0520	7.47%
CZL095	0.64%	CZL04001-B	9.07%
CZL0610-B	0.78%	SYN312-SR-B	10.91%
CZL04006-B	0.87%	CZL00009-B	10.98%

The DNA samples analysed were therefore heterogeneous, that, homozygous individuals of the same line that may be slightly variable at some loci. Genetic purity of the lines was generally high.

7.3.3 Genetic correlation of parental lines

The parental lines had genetic distances of 0.0056 to 0.4005 with a mean of 0.2944 as shown in Table 7.3 and Apendix VI. Genetic distance frequency from 0.000 to 0.1000 was the least at 0.5% (Figure 7.2).

Table 7.3 Genetic distances between parental lines

Minimum	0.0056
Maximum	0.4005
Mean	0.2944

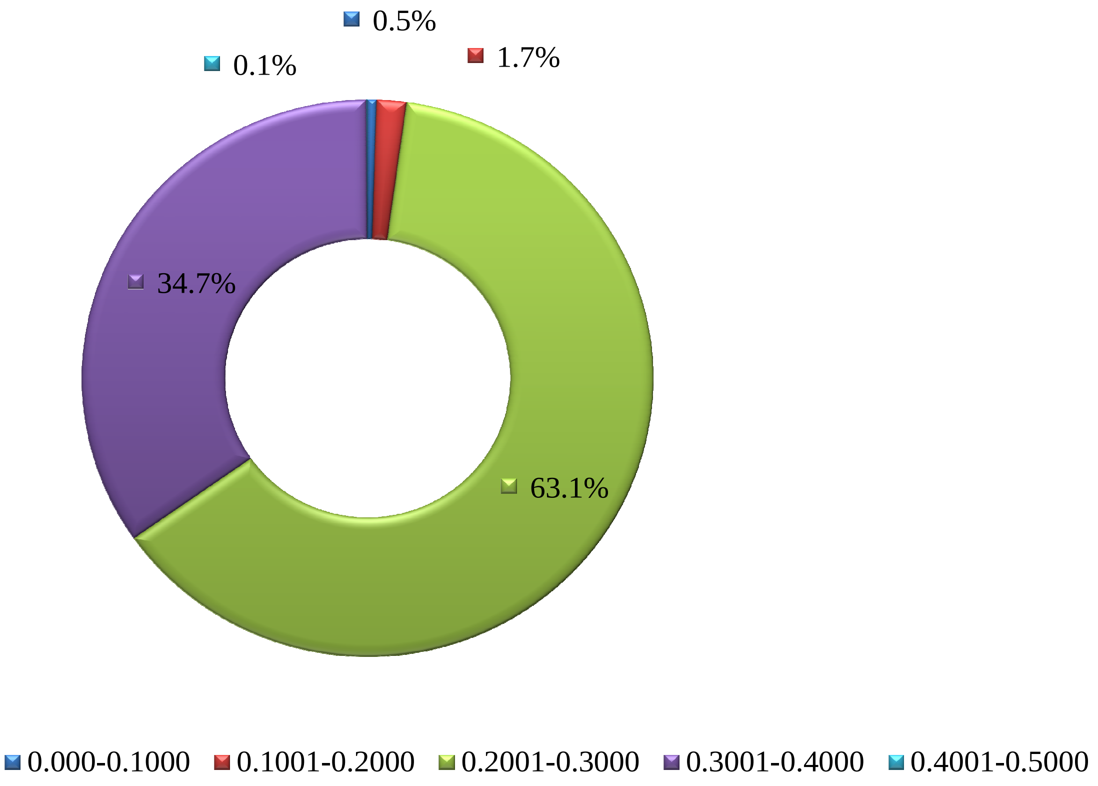


Figure 7.2 Frequency of genetic distances among parental lines of the 67 CIMMYT hybrids

This was due to the fact that the lines with genetic distances within that range were usually the same line from different sources named differently like CZL057 that is the same as CZL04021 (0.020) and CZL02015 that is DTPWC8F31-1-1-2-2-B-B (0.006). A few more lines with distances within the range of 0.1001 to 0.2000 constituted 1.7% of the total. Lines with distances within the range 0.1001 to 0.2000 were closely related to CML202 and CML489 where CML202 is a parental line of CML489 and for CML312 and CML539 where CML539 is an improved MSV resistant version of CML312 making CML312 one of the parental lines of CML539. For CZL02014 and CML444, the latter is a parent of the former, making them genetically close. CML488 (DTPWC8F31-4-2-1-5-B) is a sister line to CZL02015 that is the same as DTPWC8F31-1-1-2-2-B-B.

Above 0.2000 the lines were more diverse, based on the pedigree. The range from 0.2001 to 0.3000 had a frequency of 63.1% that was the highest and from0.3001 to 0.4000 the second highest at 34.7%. The frequency for the distances above 0.4000 was 0.1%. Genetic distance between the lines mostly ranged between 0.2001 and 0.3000 and 0.3001 to 0.4000.

7.3.4 Cluster analysis of the 54 parental lines of the 67 CIMMYT ESA hybrids released from 2000 to 2010

The lines analysed were from the CIMMYT Southern Africa maize breeding programme based in Harare, Zimbabwe. UPGMA analysis showed two main clusters, the first with nine sub-clusters and the second with the one defined sub-cluster with four of the lines and 12 other lines (Figure 7.3). There were 10 sub clusters separated mainly by pedigree and origin and some lines that did not cluster at all. Lines like CML 202, CML312, CML442 and CML445 were used as parents in the development of some of the new lines making the parental lines and developed lines genetically close.

Some of the lines shared a common parent and this gives the lines close genetic distances as was the case with most of the lines. Examples include CML312 that is a parent to CML539 with a genetic distance of 0.157, the case of CML445 that is a parental line of CZL0610 with a distance of 0.180 and CZL03021 that was developed from CML202 and CML312 has a distance of 0.130 with CML202 and 0.157 with CML312.

The first sub-cluster had CML202, CZL03021, CML489 and CZL0619. CML202 is a parental line for CZL03021 and CML489 separating at a distance of 0.130 and 0.128 respectively.

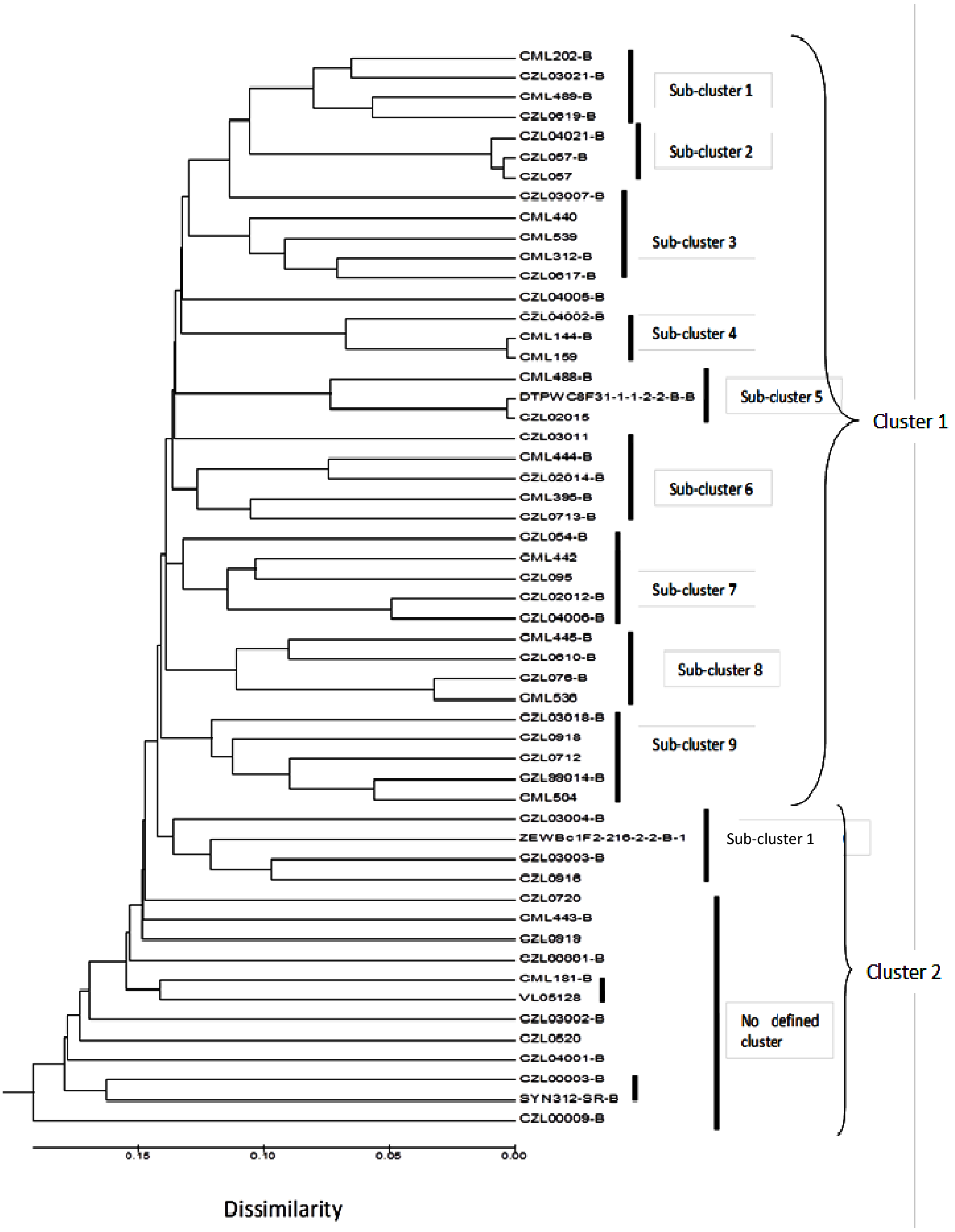


Figure 7.3 Cluster analysis using UPGMA based on genetic distances data of 54 parental lines of the 67 CIMMYT ESA hybrids

CML0619 that clustered with CML489 at a distance of 0.113 shared a parental line CML312 with CZL03021 but had a closer distance to CML489 compared to CZL03021. The second sub-cluster had the same line from three different sources, that is, CZL03007 is the same line with CZL057. CZL057-B is the same with CZL057, it was just a stock increased and bulked from CZL057. CML440 was relatively different from the rest of the lines in the third sub-cluster that included CML539, CML312 and CZL0617. CML312 is a common parent in CML539 and CZL0617. Sub-cluster 4 included CZL04002-B, CML144 and CML159. CML144 and CML159 are sister lines selected from Pop62 and Pop63 respectively with a genetic distance of 0.006. CML144 and CML159 were therefore genetically closer to each other compared to CZL04002-B that is at a distance of 0.135 with CML144, for example.

The DTPWC8F31 sister lines were in the fifth sub-cluster. CZL02015 is a code for DTPWC8F31-1-1-2-2-B. This was the same line from two different sources with a distance of 0.006. CML488 (DTPWC8F31-4-2-1-5-B) is a sister line to CZL02015 or DTPWC8F31-1-1-2-2-B. The two sister lines were genetically close at 0.146. In the sixth sub-cluster CML444 is a parental line of CZL02014. CML444 and CZL02014 clustered together at a distance of 0.149 while CML395 and CZL0713 also clustered together but at a longer distance of 0.210.

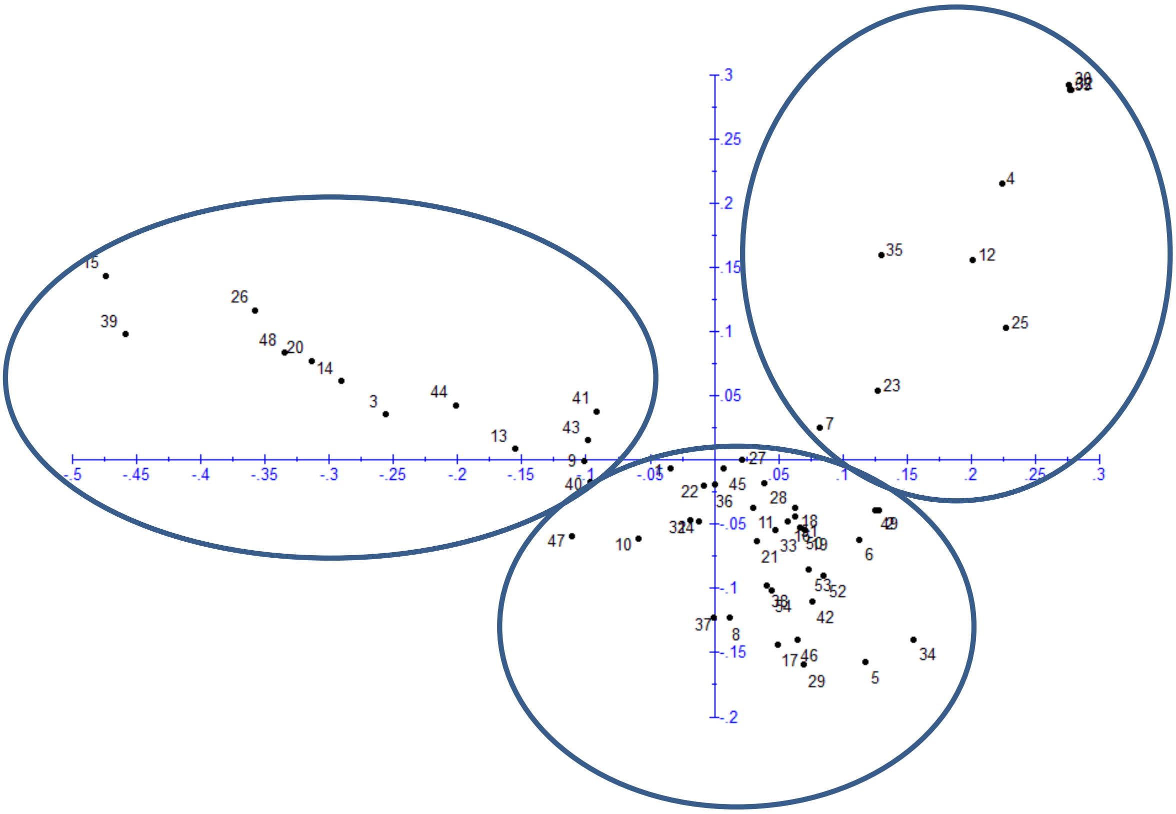
The next sub-cluster (7) was made up of lines that had a population background including CZL054, a selection from ZM523A, CML442, CZL095 with a CML442 component, and sister lines CZL02012-B and CZL04006-B (0.098) that are selections from ZM621A.

CML445 was used as a parental line in CZL0610, CZL076-B and CML536 with a distance of 0.064 between CZL076-B and CML536. The four lines clustered together (sub-cluster 8). CML445 clustered more closely with CZL0610 and CZL076-B with CML536. In sub-cluster 9 CZL03018-B, CZL0918 and CZL0712 all had a common parent CML389 in their pedigree. CZL99014-B was a different source of CML504 and the two lines had the same genetic distance. The four lines were all derived or selected from population sources.

In the first sub-cluster of the second cluster CZL0916 and CZL03003-B that had a common parent CML390. All the lines in this cluster were derived from populations. The remaining lines in the second cluster that included CZL0720, CML443-B, CZL0919, CZL00001-B, CML181 that closely clustered with a sister line VL05128 (0.28), CZL03002-B, CZL0520, CZL04001-B, CZL00003-B that clustered with SYN312-SR-B and CZL00009 did not form defined clusters. These lines had different pedigrees except CML181 and VL05128 that are sister lines. The lines are probably of different geographical origins. CML181 and VL05128 are originally from South Africa.

7.3.5 Principal component analysis

The first three principal components (PCs) from principal coordinate analysis explained 36.7% of the total SNP variation among samples. A plot of PC1 (21.6%) and PC2 (8.8%) formed 3 major groups (Figure 7.4). Sub-clusters 1 and 2 of the first cluster in the UPGMA analysis formed one group. The lines in this group shared CML202 and CML312 as common parents for the different lines. Sub-clusters 3 to 9 formed the other group with most of the lines in this group developed from populations. The second cluster in UPGMA formed the third group. Lines grouped based on pedigrees or parentage. Groups were composed of sister lines and lines that shared a common parent in most cases as highlighted in sections 7.3.3 and 7.3.4 of this chapter. Most of the lines were developed in the CIMMYT southern Africa maize breeding programme making the most of the lines related as sister lines or by sharing common parentage. The lines were selected for adaptation to both local abiotic and biotic environmental conditions. The lines therefore share a reasonable proportion of traits in common but some differences could be established through cluster analysis that showed two major groups and PCoA that produced three groups.



1	CML444	12	CML489	23	CZL03007	34	CZL0617	45	CZL03011
2	CML144	13	CZL00001	24	CZL03018	35	CZL0619	46	CML536
3	CML181	14	CZL00003	25	CZL03021	36	CZL0713	47	CML504
4	CML202	15	CZL00009	26	CZL04001	37	CZL076	48	CZL0520
5	CML206	16	CML539	27	CZL04002	38	CZL99014	49	CML159
6	CML312	17	CZL02012	28	CZL04005	39	SYN312-SR	50	CZL02015
7	CML395	18	CZL02014	29	CZL04006	40	VL05128	51	CZL0918
8	CML442	19	DTPWC8F31-1-1-2-2	30	CZL042021	41	CZL0919	52	CZL0916
9	CML443	20	CZL03002	31	CZL054	42	CZL0712	53	CML440
10	CML445	21	CZL03003	32	CZL057	43	CZL0720	54	CZL095
11	CML488	22	CZL03004	33	CZL0610	44	ZEWBc1F2-216-2-2-1	55	CZL057

Axis	**Eigenvalue**	**Inertia%**
1	0.0291	21.63
2	0.0119	8.84
3	0.00843	6.27
4	0.00742	5.51
5	0.00699	5.19

Figure 7.4 PCoA of 55 lines from the CIMMYT Southern Africa research programme used to make the 67 hybrids evaluated for genetic gain

7.4 Discussion

The 55 parental lines assessed in this study were all from the CIMMYT Southern Africa (Zimbabwe) breeding programme. The station has a mid-altitude tropical environment. All lines used in this programme are improved for high yield and adaptation to the mid-altitude environment. Lines were developed using the pedigree breeding programme by crossing donor lines to established lines with good performance in terms of yield, adaptation, biotic and abiotic stresses. Lines like CML202, CML312, CML442 and CML445 are some of the lines that have been commonly used as parents in line development. The diversity of the lines therefore might have been restricted by the pedigree breeding method and adaptation of material to the mid-altitude tropical environments. Two major clusters were noted in the UPGMA analysis and three in the PCoA with most of the variation explained by the first three PCs.

Genetic distances were all less than 0.4005 for all the lines evaluated. Average diversity or genetic distance recorded was 0.2944 that is relatively low compared to findings from other studies. A study in China evaluating 29 hybrids showed that one major cluster with most of the hybrids showed 82.5% similarity (Wang *et al.*, 2011) that is equivalent to a genetic distance of about 0.350. Legesse *et al.* (2007) reported an average diversity of 59% (0.590) in an evaluation of lines from CIMMYT Zimbabwe and Ethiopia using SSR markers. In an evaluation of popcorn lines in China using SSR markers genetic distances ranged from 0.125 to 0.730, averaging 0.477 (Li *et al.*, 2004b). Studies that reported higher diversity evaluated lines from different sources as in the study by Legesse *et al.* (2007) and Li *et al.* (2004b). Different genotyping methods were used in all these studies have contributed to the diifferences among the different studies.

This study analysed lines and hybrids developed in a specific environment for adaptation to specific biotic and abiotic conditions and to meet specified grain quality. The focus on quality was mainly on flint to semi-flint or semi-dent for mainly flour and other products like samp (dehusked maize grain served as part of the main course of a meal or as a snack). Biotic factors commonly selected for included MSV, GLS and ET resistance while abiotic factors included low N and drought stress tolerance. Selection pressure for defined traits can result in the narrowing down of the genetic base of a breeding programme. This may not be immediately noted since according to Duvick (1990), maize is so diverse such that narrowing down of germplasm has not been noted to seriously affect performance or breeding efficiency. Challenges of a narrow germplasm base come in when there are new disease or pest outbreaks, like more recently the development of MLN disease in East Africa. If most hybrids share common parents they may succumb to such outbreaks.

Even though frequency of genetic distance may be high between 0.2001 and 0.4000, a few lines were used in most of the hybrids including CML444, CML395, CML312 and CML442 giving the hybrids a narrow genetic base. Some of the lines were developed from these four lines. The genetic base of the 67 hybrids released in the CIMMYT ESA programme from 2000 to 2010, some of which have been disseminated to farmers throughout ESA through collaborators could be narrow in terms of line use in hybrid formation and genetic distance among the lines used. There is need to ensure that through selection of the best performing varieties the genetic base is not narrowed down.

7.5 Conclusions

Diversity was low at genetic distances < 0.4005 and with a few lines being used up to 37 times more than most of the lines listed. This evaluation focused on a subset of all the hybrids selected as it evaluated the best performers from each year. The best performers are usually passed onto the farmer through collaborators. The sample of parental lines analysed do not represent the entire germplasm of CIMMYT Southern Africa or CIMMYT ESA and these findings should not be generalised for the whole programme. However, because these best performing hybrids are disseminated to farmers in the region, implications of disease or pest outbreaks can be devastating if the hybrids have common susceptible parents. The results give an indication of the danger of eventually narrowing down the germplasm base and the need to maintain a large or wide genetic base for the whole programme.

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Chapter 8

General discussion and conclusions

Discussion

To evaluate genetic gain, the study was designed in such a way that all entries selected from the different years were grown and evaluated in one trial planted on the same day in defined environments. With entries planted side by side, in the same environment and same season, receiving the same agronomic and other treatments like irrigation or disease inoculation, the differences observed among entries can be attributed to genetic differences. Better performance can therefore be attributed to genetic gain.

The study showed positive genetic gain under different biotic and abiotic conditions. Genetic gain in yield under optimal conditions was 109.4 kg ha^{-1} year^{-1} (1.4% year^{-1}) for the period from 2000 to 2010. This was higher than the gains under random and managed drought stress at 22.7 kg ha^{-1} year^{-1} (0.85% year^{-1}) and 32.5 kg ha^{-1} year^{-1} (0.85 year^{-1}) respectively, as well as the change under low N stress at 20.9 kg ha^{-1} year^{-1} (0.62% year^{-1}) over the same period from 2000 to 2010. Genetic gain in yield under MSV and ET stress was higher than gains under optimal conditions at 141.3 kg ha^{-1} year^{-1} (2.2% year^{-1}). The gains were higher under MSV compared to optimal conditions, probably because when the crop was exposed to MSV and ET diseases, all other conditions were maintained at optimal levels such that if a hybrid was tolerant it would develop and produce optimally. At the same time, in susceptible hybrids, yield was significantly reduced. The reduction in yield in susceptible varieties reduced the trial mean and this must have given the resistant hybrids a yield advantage, hence a genetic gain that was higher than under optimal conditions. The gain in yield under optimal conditions is comparable to other studies, including the classical studies in the US by Duvick *et al.* (2004) that reported gains of 77 kg ha^{-1} year^{-1} and 115 kg ha^{-1} year^{-1} (Duvick, 2005); 60 kg ha^{-1} year^{-1} in China (Wang *et al*., 2011) and 62- to 81 kg ha^{-1} year^{-1} (Li *et al.*, 2011).

The change in grain yield was associated with phenological and physiological changes as reported in earlier studies (Duvick, 2005a; Lee and Tollenaar, 2007). Days to mid-anthesis generally showed a trend to increase over the 11 years at a rate of 0.06 d year^{-1} under optimal conditions, 0.05 d year^{-1} and 0.095d year^{-1} under random and managed drought stress respectively, 0.048 d year^{-1} under low N and 0.134 d year^{-1} under MSV stress but did not change significantly. The new hybrids used the same season length as old hybrids but yielded better. The moisture content in the high yielding varieties was higher at harvesting compared to the low yielding varieties. The higher moisture content is a possible indication that the new, better yielding varieties had an extended grain filling period that improves final grain yield and maybe a quick dry down there after as described by Duvick 2005a and Duvick 2005b. Badu-Apraku *et al.* (2013) reported an increase in number of days to mid-anthesis in a study conducted in West Africa. No particular trend or changes were reported in studies by Duvick (2005a; 2005b) and Tollenaar and Lee (2011). For this study no change was recorded. Ngugi *et al.* (2013) reported better yield under severe drought stress in varieties that had short ASI and a longer season compared to the low yielding varieties that had a longer ASI and matured faster.

ASI is strongly correlated or linked with grain yield (Edmeades *et al.*, 1999; Monneveux *et al.*, 2006; Ngugi *et al.*, 2013). Significant decrease in ASI was recorded over the 11 years across all other environments except under random drought stress. A decrease of-0.08 d year^{-1}, -0.16 d year^{-1}, -0.79 d year^{-1} and -0.055 d year^{-1} under optimal conditions, managed drought stress, low N and MSV stress respectively was recorded. Shorter ASI was directly selected for as one of the favourable secondary traits used to indirectly select for yield under stress environments as reported by (Bolanos and Edmeades 1996; Bänziger et al. 2000; Betrán et al. 2003; Araus et al. 2012; Ziyomo and Bernardo 2013). Shorter ASI ensures better pollination, better grain set and reduced barrenness. The decrease in ASI was associated with a yield increase across all environments. The correlation of grain yield with ASI was negative under optimal conditions, random drought, managed drought, low N and MSV stress at -0.25, -0.32, -0.23, -0.30 and -0.27 respectively. These findings agree with reports by other authors (Betrán *et al.*, 2003a; 2003b; Zaidi *et al.*, 2004).

The number of ears per plant showed a trend of increasing across all environments at 0.004 ears plant^{-1} year^{-1} under optimal and managed drought stress, 0.001 ears plant^{-1} year^{-1}, 0.0008 ears plant^{-1} year^{-1} and 0.011 ears plant^{-1} year^{-1} under random drought, low N and MSV stresses respectively but significantly changed under managed drought and MSV stress. Number of ears per plant is a secondary trait that has been directly selected for indirect selection for grain yield (Bänziger *et al.*, 2000; Betrán *et al.*, 2003) When regressed against the number of ears per plant, yield generally increased with increased number of ears per plant across all environments except low N. In separate studies yield was reported to increase as the number of ears per plant increased (Betrán *et al.*, 2003b; Duvick, 2005a; Lee and Tollenaar, 2007).

There was a general trend of decrease in plant height under optimal (-0.16 cm year^{-1}), random drought stress (-0.39 cm year^{-1}), low N (-0.49 cm year^{-1}) and MSV (-0.06 cm year^{-1}). Under managed drought, plant height was relatively higher in the new hybrids (0.119 cm year^{-1}. There was no significant change in plant height across all environments over the 11 years. Other studies reported a decrease in plant height that could have resulted from a possible improvement in dry matter partitioning to the ear for ear development, grain set and grain filling, especially under stress conditions (Bolaños and Edmeades, 1993a; Duvick, 2005a).

Senescence was generally lower in the new hybrids. Over the 11 years evaluated, senescence decreased significantly under low N conditions at a rate of -0.028 year^{-1}. The new varieties probably had improved nitrogen uptake efficiency or better nitrogen utilisation efficiency compared to the old hybrids under low N stress, and better nitrogen uptake efficiency or better nitrogen utilization efficiency that should have resulted in reduced senescence. Reduced senescence or improved stay green improves stress tolerance in maize and grain yield (Bolaños and Edmeades, 1993b; Betrán *et al*., 2003b; Duvick, 2005b; Campos *et al*., 2006). Under random and managed drought stress senescence showed a trend of decreasing at a rate of 0.012 year^{-1} and 0.011 year^{-1} respectively but the change was not significant. It is expected that improved hybrids under stress would have lower senescence rates but there was no significant change under drought. The new hybrids may have a longer grain filling period and quick dry-down under drought stress as explained by Duvick (2005a) and Tollenaar and Lee (2011).

New hybrids significantly had better resistance to MSV and ET compared to old hybrids that improved at -0.034 year^{-1} and 0.030 year^{-1}, respectively. Grain yield reduction is less when hybrids are disease resistant. Yield of the new hybrids that had high resistance or tolerance to the diseases was higher than in the old hybrids that were more susceptible. Duvick (2005a) states that not much had been reported on the effect of improved disease resistance on grain yield. A lot of literature has covered much on the biology, effect and potential control of the pathogens but not much has been reported on yield improvements in tolerant or resistant varieties.

Genetic gains in yield have been recorded in SSA as reported in West Africa (Badu-Apraku *et al.*, 2013) and in East and Southern Africa in the current study but yields still remain at about 2 t ha^{-1}. Small holder farmers should have been benefitting from these gains but not all are benefitting. The yield average is still low. The use of inherently poor soils, low fertilizer use and low hybrid turnover contribute towards this low productivity, among other factors. The genetic potential of the new hybrids if used is not maximised due to limitations in resources. Stress tolerant hybrids if disseminated to farmers effectively should alleviate production restrictions and improve productivity. The stress tolerant hybrids developed and under development should also be screened under heat as an additional stress to prepare climate ready material since the world, particularly SSA is faced by more drought and heat stress as forecasted in climate change studies.

Besides the failure to maximise on the genetic potential of the already improved hybrids, commercial hybrids stay in the system for a long time, for example, the third check in this current study was released in 1997 and has been on the market for almost 17 years although better varieties have been developed. As seed houses maintain the old hybrids on the market, the new, better performing varieties will not reach the intended target and will not benefit the farmers. There is need to further develop strategies for more effective dissemination to get the products to the farmers in time so that they can benefit from these recorded genetic gains in yield and stress tolerance.

The diversity of the parental lines used in the formation of the hybrids evaluated in this study was relatively low with an average distance of 0.2944, ranging from 0.0056 to 0.4005. It should be noted that GBS covers the whole genome including rare alleles while other methods like SSR cover selected polymorphic markers and not the full genome such that higher diversity may realised when using the other methods compared to GBS. For this study the distances or diversity among the lines was low probably because they were from the same breeding programme and from the same station, bred and selected for adaptation to the local environment and for tolerance or resistance to the same stresses. The lines sub-clustered mainly by pedigree and by origin for a few entries such as CML181 and VL05128. Some of the lines did not fall in a cluster, having different pedigrees from all the other lines. Even though distances were as high as 0.4005, some lines were used more than the others such that frequency ranged from 0.48 to 2.38 for 50 out of the 54 lines and the remaining four that included CML444, CML395, CML312 and CML442 were used at frequencies of 17.62%, 10.00%, 9.05% and 7.14% respectively. This means that most of the hybrids shared common parents. The four lines as well as CML202 and CML489 were parental lines in some of the lines evaluated, contributing significant composition in such lines. It should be noted that this sample of lines does not represent the whole breeding programme but just the parental lines of the best yielding hybrids released by CIMMYT ESA from 2000 to 2010 that were evaluated in this study. However, considering the best performing hybrids are disseminated to farmers throughout ESA through the public and private sector, caution should be taken to ensure the diversity of maize in the region is not gradually reduced.

Conclusions

The new hybrids yielded better than the old hybrids across all environments that is, for optimal, low N, MSV and random and managed drought stresses, demonstrating positive genetic gain in yield and tolerance to low N, biotic (MSV and ET) stress and drought stress. Yield gains of 109.4 kg ha^{-1} $year^{-1}$ (1.4% $year^{-1}$), 20.9 kg ha^{-1} $year^{-1}$ (0.62% $year^{-1}$), 141.3 kg ha^{-1} $year^{-1}$ (2.2% $year^{-1}$), 23.7 kg ha^{-1} $year^{-1}$ (0.85% $year^{-1}$) and 32.5 kg ha^{-1} $year^{-1}$ (0.85% $year^{-1}$) respectively, were recorded. The gain in yield was associated with, generally, shorter ASI that promoted better pollination and grain set, increased number of ears per plant or reduced barrenness, slower senescence or improved stay green and improved disease resistance. There was no significant change in days to mid anthesis and plant height. The diversity of the parental lines analysed was relatively low at an average genetic distance of 0.2944. A few lines were used more frequently in both line development and hybrid formation. Considering that the best performing hybrids have been or are being disseminated, farmers are and will benefit from the genetic gain realised, but it is important to look into how long the hybrids are taking to get to the farmer, to check if the materials are getting to the farmer as well as the economic benefit analysis. It is also important to review the use of key lines in future breeding to avoid the possibility of eventually narrowing down the genetic base in the ESA region and a possibility of a disease or pest outbreak such as the more recent case of the recent MLN disease outbreak in East Africa. There is need to maintain the genetic gains, balance the diversity and to ensure the improved hybrids get to the farmers on time so that they benefit from the improvements.

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Summary

Maize is a staple food crop in ESA, making it a priority in terms of production. Farmers face challenges in production when maize crops fail in the face of drought, limited or no access to fertiliser or lack of capital to purchase the fertiliser and when there are disease outbreaks. CIMMYT embarked on a drought breeding programme in 1975 that spread to Southern Africa in the 1990s. 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.

A study to evaluate 67 hybrid best yielders selected from an 11 year period covering 2000 to 2010 for genetic gain in grain yield, random and managed drought stress tolerance, low N tolerance and disease (MSV and ET) resistance was conducted from the 2011/12 main season to the 2013 off season. Parental lines of the 67 hybrids were evaluated for genetic diversity. Sixty-seven CIMMYT ESA hybrids released from 2000 to 2010, checked by three commercial hybrids, were evaluated for genetic gain in grain yield and secondary traits in a multi-site alpha-lattice designed experiment over two seasons. The hybrids were evaluated across 14 optimal sites in Zimbabwe, Zambia, Malawi, Kenya and Uganda; seven random drought sites in Chiredzi and Kadoma (Zimbabwe) and Potchefstroom in South Africa; four managed drought sites in Chiredzi, Zimbabwe and Kiboko, Kenya; five low N sites in Harare, Zimbabwe and Kiboko, Kenya and three MSV sites all in Harare, Zimbabwe. Significant gain in grain yield and stress tolerance was realised in the CIMMYT ESA maize breeding programme under optimal and stress conditions.

Genetic gain in grain yield of 109.4 kg ha^{-1} year^{-1} (1.4% year^{-1}), 20.9 kg ha^{-1} year^{-1} (0.62% year^{-1}), 141.3 kg ha^{-1} year^{-1} (2.2% year^{-1}), 22.7 kg ha^{-1} year^{-1} (0.85% year^{-1}) and 32.5 kg ha^{-1} year^{-1} (0.85% year^{-1}) were realised under optimal, low N, MSV, random drought and managed drought stress respectively. This gain in grain yield was associated with phenological and physiological changes. The season length showed a tendency to increase across all environments at rates ranging from 0.048 d year^{-1} to 0.134 d year^{-1} but did not significantly change. The season length of the new hybrids was still within the length of the local checks, meaning that the new hybrids still fit in the normal season.

The new hybrids had shorter ASI under all environments that insured better pollination and grain set. An increase in the number of ears per plant was recorded across all environments and this positively contributes towards increased grain yield and reduced barrenness. New hybrids were shorter in all environments except managed drought stress. A decrease in plant height indicate improved dry matter partitioning to the ear for ear development, silking, pollination, grain set and grain development and filling. Under random and -managed drought, low N and biotic stress newer hybrids were taller showing normal growth under stress while the old hybrids were stunted. Senescence was lower in new hybrids across all environments except managed drought. There was no change in the number of days to mid-anthesis. MSV and ET resistance or tolerance was higher in the new hybrids that had low disease scores and yielded better than the old hybrid. Overall the genetic gain in yield, drought stress tolerance, low N stress tolerance and biotic (MSV and ET) tolerance over the 11 years from 2000 to 2010 was high.

The diversity of the parental lines was relatively low with genetic distances ranging from 0.006 to 0.4005 with most distances ranging from 0.2000 to 0.4000. Considering the 54 parental lines were from the CIMMYT Southern Africa breeding programme only, and that the lines are a sub-sample of the breeding programme being parents of a sample (only 67) of the produced CIMMYT hybrids, the diversity was narrow because the lines are from the same geographical origin selected in a defined system for adaptation to the local environment and needs. A few lines (four) out of the 54 were used more frequently in most of the hybrids. The same lines were parental lines for some of the other 50 lines. Despite the wide range of lines, using a few lines frequently in formation of most of the hybrids can potentially narrow down the diversity of hybrids in the region if the hybrids as the best performers, are disseminated to farmers. This will pose a risk in the event of outbreaks of new pests and disease.

The CIMMYT ESA maize breeding programme for drought tolerance realised significant gains in yield and stress tolerance providing farmers with better yielding varieties that are more resilient to stresses.

Opsomming

Mielies is ‘n stapelvoedsel in ESA, wat dit ‘n prioriteit maak in terme van produksie. Kleinboere staar baie uitdagings in die gesig as daar misoeste is as gevolg van droogte, beperkte of geen kunsmis of ‘n gebrek aan kapitaal om kunsmis te koop of wanneer daar siekte uitbrake is. CIMMYT het in 1975 begin met ‘n teelprogram vir droogtetoleransie, wat uitgebrei is na Suidelike Afrika in die 1990s. Sedert 1997 het CIMMYT ESA gefokus op die produksie van droogte tolerante variëteite, maar seleksie vir hoë opbrengs onder optimale toestande en lae N stremming toleransie, sowel as MSV, GLS and ET siekte weerstand is ingesluit.

‘n Studie van genetiese vooruitgang in die beste 67 basters van ‘n 11 jaar periode van 2000 tot 2010 in graanopbrengs onder toevallige en beheerde droogtetoestande, lae N toleransie en siekte (MSV en ET) weerstand is gedoen in die 2011/12 hoofseisoen tot die 2013 af seisoen. Die ouerlyne van die 67 basters is geëvalueer vir genetiese diversiteit. Sewe en sestig CIMMYT ESA basters vrygestel van 2000 tot 2010, met drie kommersiële kontroles, is geëvalueer vir genetiese vooruitgang in graanopbrengs en sekondêre eienskappe in ‘n multi-lokaliteit alfa-reghoek ontwerp oor twee seisoene. Die basters is oor 14 optimale lokaliteite in Zimbabwe, Zambië, Malawi, Kenia en Uganda; sewe toevallige droogte lokaliteite in Chiredzi en Kadoma (Zimbabwe) en Potchefstroom in Suid Afrika, vier beheerde droogtelokaliteite in Chiredzi, Zimbabwe en Kiboko, Kenia; vyf lae N lokaliteite in Harare, Zimbabwe en Kiboko, Kenia en drie MSV lokaliteite in Harare, Zimbabwe. Betekenisvolle toename in opbrengs en stremmingstoleransie is gerealiseer in die CIMMYT ESA mielie teelprogram onder optimale en stremming toestande.

Genetiese verbetering in graanopbrengs van 109.4 kg ha^{-1} jaar^{-1} (1.4% jaar^{-1}), 20.9 kg ha^{-1} jaar^{-1} (0.62% jaar^{-1}), 141.3 kg ha^{-1} jaar^{-1} (2.2% year^{-1}), 22.7 kg ha^{-1} jaar^{-1} (0.85% jaar^{-1}) en 32.5 kg ha^{-1} jaar^{-1} (0.85% jaar^{-1}) is gerealiseer onder optimale, lae N, MSV, toevallige en beheerde droogtetoestande onderskeidelik. Hierdie vooruitgang in opbrengs is geassosieër met fenologiese en fisiologiese veranderings. Die lengte van die groeiseisoen het toegeneem oor al die omgewings teen tempos’s wat van 0.006 d jaar^{-1} tot 0.134 d jaar^{-1} maar het nie betekenisvol verander het nie. Die verlengde seisoen was binne die groeiseisoen van die plaaslike kontroles, dus pas dit nog in die normale groeiseisoen in. ‘n Langer groeiseisoen verleng die graanvul

periode wat opbrengs van die basters verbeter.

Die nuwe basters het korter ASI onder alle omgewings gehad wat beter bestuiwing en graanset veroorsaak het. ‘n Toename in die getal koppe per plant is gesien in alle omgewings en dit dra positief by tot verhoogde graanopbrengs en verminderde onvrugbaarheid. Nuwe basters was korter in alle omgewings behalwe in beheerde droogtetoestande. ‘n Afname in planthoogte wys op verbeterde droëmateriaal toedeling na die koppe wat verbetering veroorsaak in kopontwikkeling, stuifmeeldraad ontwikkeling, bestuiwing, graanset en graanvul en graanontwikkeling. Die nuwe basters was langer onder beheerde droogte, met normale groei onder stremming, terwyl die ou basters verdwerg was. Blaarvrekte was minder in nuwe basters oor alle omgewings behalwe onder beheerde droogte. Onder beheerde droogte het die basters die verlengde seisoen gebruik, wat die graanvul periode verleng het, maar hulle het ‘n vinnige afdroog aan die einde van die seisoen gehad. MSV en ET weerstand en toleransie was hoër in nuwe basters, en hulle het laer siektetellings gehad, met hoër opbrengs as ou basters. Oor die algemeen was die genetiese vooruitgang in opbrengs, droogte toleransie, lae N stremmings toleransie en biotiese (MSV en ET) toleransie oor die 11 jaar van 2000 tot 2010 hoog.

Die diversiteit in die ouerlyne was relatief laag en het gewissel van 0.006 tot 0.4005 met meeste afstande wat gewissel het van 0.2000 tot 0.4000. Omdat die 54 ouerlyne net van die CIMMYT Suidelike Afrika teelprogram kom, was die diversiteit min omdat die lyne uit dieselfde geografiese oorsprong geselekteer is en aangepas is vir die plaaslike omgewing en behoeftes. ‘n Paar lyne (vier) van die 54 is meer gereeld gebruik in meeste van die basters. Dieselfde lyne was ouerlyne vir sommige van die ander 50 basters. Ten spyte van die wye reeks lyne beskikbaar kan die herhaaldelike gebruik van ‘n paar lyne die diversiteit van die basters verminder in die gebied as hierdie basters as die beste produseerders, versprei word aan die boere. Dit veroorsaak ‘n risiko as daar nuwe siektes en insekte uitbreek.

Die CIMMYT ESA mielie teelprogram vir droogte toleransie het betekenisvolle vooruitgang in opbrengs en stremmingstoleransie gemaak, en boere voorsien van basters wat beter opbrengs het met beter toleransie vir stremming.

Appendices

Appendix I Hybrids tested in the study

Entry	Programme	Hybrid	Pedigree	Release
1	CIMMYT-Z	CZH99026	CML202/CML216//SNSYNF2[N3/TUX-A-90]-102-1-2-2-BSR-BBBB-1-B	2000
2	CIMMYT-Z	CZH99025	CML202/CML395//CML312/CML197	2000
3	CIMMYT-Z	CZH99021	CML202/CML395//CML312	2000
4	CIMMYT-Z	CZH99015	CML395/CML312//CML440	2000
5	CIMMYT-Z	CZH0724	CML312/CML442//CZL0713	2008
6	CIMMYT-Z	CZH0524	CML395/CZL0520//CZL00009	2006
7	CIMMYT-Z	CZH0713	CML489/CML444//CZL0617	2008
8	CIMMYT-Z	CZH0631	CML444/CML395//CZL0619	2007
9	CIMMYT-Z	CZH0615	CZL00003/CML488//CZL03014	2007
10	CIMMYT-Z	CZH0613	CML312/CML440//CZL0610	2007
11	CIMMYT-Z	CZH0610	CML312/CML444//CML445/CML488	2007
12	CIMMYT-Z	CZH056	CML312/CML444//CML489	2006
13	CIMMYT-Z	CZH055	CML312/CML444//CZL04006	2006
14	CIMMYT-Z	CZH0533	CML444/CZL99014//CZL00001/CML312	2006
15	CIMMYT-Z	CZH0524	CZL0520/CML395//CZL00009	2006
16	CIMMYT-Z	CZH0513	CML144/CML159//CZL057	2006
17	CIMMYT-Z	CZH0511	CML444/CML445//CZL054	2005
18	CIMMYT-Z	CZH04034	CML181/WWO1408-1-1-2-B//CZL042021	2005
19	CIMMYT-Z	CZH04032	CML181/WWO1408-1-1-2-B//CZL03018	2005
20	CIMMYT-Z	CZH04019	CML395/CML444//CZL04006	2005
21	CIMMYT-Z	CZH04002	CML312/CML442//CZL04002	2005
22	CIMMYT-Z	CZH04001	CML395/CML444//CZL04001	2005
23	CIMMYT-Z	CZH03042	CML444/CML395//CZL03021	2004
24	CIMMYT-Z	CZH03031	CML444/CML395//SYN312-SR	2004
25	CIMMYT-Z	CZH03028	CML444/CML395//CML312	2004
26	CIMMYT-Z	CZH03022	CML312/CML444//CML488	2004
27	CIMMYT-Z	CZH03021	CML197/CML444//CZL03007	2004
28	CIMMYT-Z	CZH03013	CML395/CML444//CZL02012	2004
29	CIMMYT-Z	CZH03006	CML395/CML444//CZL03005	2004
30	CIMMYT-Z	CZH03005	CML395/CML444//CZL03004	2004
31	CIMMYT-Z	CZH03004	CML312/CML442//CZL03003	2004
32	CIMMYT-Z	CZH03002	CML312/CML442//CZL03002	2004
33	CIMMYT-Z	CZH02020	CZL02014/CML197//CZL02015	2003
34	CIMMYT-Z	CZH02018	CML197/CML444//CZL02012	2003
35	CIMMYT-Z	CZH01008	CML444/CML443//CZL00003	2002
36	CIMMYT-Z	CZH01006	CML312/CML444//CZL00001	2002
37	CIMMYT-Z	CZH00027	CZL00025/CML312//CML444/CML395	2001
38	CIMMYT-Z	CZH00026	CML395/CML444//CML442	2001
39	CIMMYT-Z	CZH00021	CML395/CML216//CZL99014	2001

Appendix I Hybrids tested in the study (continued)

Entry	**Programme**	**Hybrid**	**Pedigree**	**Release**
40	CIMMYT-Z	CZH00013	CML395/CML312//CZL00001	2001
41	CIMMYT-Z	CZH00011	CML395/CML440//CZL00001	2001
42	CIMMYT-Z	CZH00002	DRB-F2-60-1-2-B-1-1-BB/CML395//CML440	2001
43	CIMMYT-Z	CZH0836	CML489/CML444//CZL0814	2009
47	CIMMYT-Z	CZH0935	CZL0919/CZL03011//CML539	2010
48	CIMMYT-Z	CZH0928	CZL0919/CZL0712//CML539	2010
49	CIMMYT-Z	CZH0923	CML489/CML444//CZL0916	2010
50	CIMMYT-Z	CZH095	CML489/CML444//CZL095	2010
51	CIMMYT-Z	CZH0735	[SC/CML204//FR812]-X-30-2-3-2-1-B/ZEWBc1F2-216-2-2-B-2-B//[Ent320:92SEW2-77/[DMRESR-W]EarlySel-#I-2-4-B/CML386]-B-11-3-B-2-#-B/[ENT52:92SEW1-2/[DMRESR-W]EARLYSEL-#L-2-1-B/CML386]-B-22-1-B-4-#-B	2005
52	CIMMYT-Z	CZH0536	[GQL5/[GQL5/CML202]F2-3sx]-11-4-1-1-BB/[CML389/CML176]-B-29-2-2-1-4-BBB//CML181	2006
53	CIMMYT-Z	CZH04006	[CML198/LPSC3H144-1-2-2-2-2-#-BB]-1-4-1-1-B/CML445//CML312	2005
54	CIMMYT-K	CKH0749	CML442/CML444//CKL05003	2007
55	CIMMYT-K	CKH08048	CML442/CML444//CKL05017	2009
56	CIMMYT-K	CKH08049	CML442/CML444//CKL05018	2009
57	CIMMYT-K	CKH08051	CML442/CML444//CKL05022	2008
58	CIMMYT-K	CKH08032	CML442/CML444//INTA/INTB-B-41-B-1-1-B-B	2009
59	CIMMYT-K	CKH08105	CML442/CML444//INTA/INTB-B-41-B-14-1-B-B	2009
60	CIMMYT-K	CKH0790	CML442/CML444//CKL05003/CKL05007	2007
61	CIMMYT-K	CKH10717	CML442/CML444//CKL05003/CKL05017	2010
62	CIMMYT-K	CKH09358	CML442/CML444//CKL05004/CKL05018	2009
63	CIMMYT-K	CKH10723	CML442/CML444//CKL05004/CKL05022	2010
64	CIMMYT-K	CKH0713	CML202/CML395//CKL05019	2007
65	CIMMYT-K	KDH3	ZEWAc1F2//P100C6-200-1-1-#-#-B-B-B-B/CML78	2006
66	CIMMYT-K	KDH4	ZEWBc1F2//P100C6-200-1-1-#-#-B-B-B-B/CML78	2006
67	CIMMYT-Z	CZH0616	[CML144/SNSYNF2[N3/TUX-A-90]-102-1-2-2-BSR-B*4]-B-4-3-B	2007
68	SEEDCO	SC727	SC727	2010
69	SEEDCO	SC635	SC635	2003
70	SEEDCO	SC513	SC513	1997

Appendix II CIMMYT hybrids tested by year of first testing or release

Year of first testing or release	Number of entries
2000	4
2001	6
2002	2
2003	2
2004	11
2005	7
2006	9
2007	8
2008	3
2009	9
2010	6

Appendix III Experimental sites summary

Trial	Site#	TrialName	Location	Country	Environment	PlantingDate	Season	GPS coordinates	Collaborator
GGHYB12	18	GGHYB1218	Harare2012	Zimbabwe	Low Nitrogen	20-Dec-11	Main	(-17^{o}50'S, +31^{o}2'E, 1483 masl	CIMMYT
GGHYB12	19	GGHYB1219	Kiboko2012	Kenya	Low Nitrogen	1-May-12	Main	2° 12' 52"N, 37° 43' 26$^{”}$E	Dr B Das
GGHYB12	37	GGHYB1237	Kiboko2013	Kenya	Low Nitrogen	19-Mar-13	Main	2° 12' 52"N, 37° 43' 26$^{”}$E	Dr B Das
GGHYB13	36	GGHYB1236	Embu2013	Kenya	Low Nitrogen		Main		Dr B Das
GGHYB12	43	GGHYB1243	Harare2013a	Zimbabwe	Low Nitrogen	6-Dec-12	Main	(-17^{o}50'S, +31^{o}2'E, 1483 masl	CIMMYT
GGHYB12	44	GGHYB1244	Harare2013b	Zimbabwe	Low Nitrogen	6-Dec-12	Main	(-17^{o}50'S, +31^{o}2'E, 1483 masl	CIMMYT
GGHYB12	1	GGHYB121	Chiredzi2012	Zimbabwe	Random drought	31-Dec-11	Main	21° 0' 58"S, 31° 34' 17E"	S Gokoma
GGHYB12	40	GGHYB1240	Chiredzi2013a	Zimbabwe	Random drought	4-Jan-13	Main	21° 0' 58"S, 31° 34' 17E"	S Gokoma
GGHYB12	46	GGHYB1246	Chiredzi2013b	Zimbabwe	Random drought	4-Jan-13	Main	21° 0' 58"S, 31° 34' 17E"	S Gokoma
GGHYB12	17	GGHYB1217	Kadoma2012	Zimbabwe	Random drought	30-Dec-11	Main	(-17^{o}19'19.4", +29^{o}50'35.0"	G Mabuyaye
GGHYB12	27	GGHYB1227	Potchefstroom	South Africa	Random drought	9-Jan-12	Main	(-26.73607 S; 27.07553E 1349 masl	Dr K. Mashingaidze
GGHYB12	32	GGHYB1232	Kadoma2013a	Zimbabwe	Random drought	21-Dec-12	Main	(-17o19'19.4", +29o50'35.0"	G Mabuyaye
GGHYB12	38	GGHYB1238	Kadoma2013b	Zimbabwe	Random drought	21-Dec-12	Main	(-17o19'19.4", +29o50'35.0"	G Mabuyaye
GGHYB12	2	GGHYB122	Chiredzi2012	Zimbabwe	Managed drought	15-Jul-12	Off	21° 0' 58"S, 31° 34' 17E"	S Gokoma
GGHYB12	6	GGHYB126	Kiboko2012	Zimbabwe	Managed drought	15-Jul-12	Off	2° 12' 52"N, 37° 43' 26$^{”}$E	Dr B Das
GGHYB12	47	GGHYB1247	Chiredzi2013a	Zimbabwe	Managed drought	15-May-13	Off	21° 0' 58"S, 31° 34' 17E"	S Gokoma
GGHYB12	48	GGHYB1248	Chiredzi2013b	Zimbabwe	Managed drought	15-May-13	Off	21° 0' 58"S, 31° 34' 17E"	S Gokoma
GGHYB12	3	GGHYB123	Bulindi2012a	Uganda	Optimal	20-Apr-12	Main		Dr G Asea
GGHYB12	4	GGHYB124	Harare2012a	Zimbabwe	Optimal	17-Dec-11	Main	(-17^{o}50'S, +31^{o}2'E, 1483 masl	CIMMYT
GGHYB12	10	GGHYB1210	Kakamega2012	Kenya	Optimal	18-Apr-12	Main		Dr D Makumbi
GGHYB12	11	GGHYB1211	Chiredzi2012	Zimbabwe	Optimal	16-Jun-12	Off	21° 0' 58"S, 31° 34' 17E"	CIMMYT
GGHYB12	12	GGHYB1212	Serere2012	Uganda	Optimal	24-Apr-12	Main		Dr G Asea
GGHYB12	13	GGHYB1213	*Bulindi2012b	Uganda	*Stress*	20-Apr-12	Main		Dr G Asea
GGHYB12	15	GGHYB1215	ART Farm Harare201	Zimbabwe	Optimal	4-Nov-12	Main	(-17^{o}42'9.1", +31^{o}3'48.4", 1556 masl	L Mutemeri
GGHYB12	21	GGHYB1221	Chitedze2012	Malawi	Optimal	12-Nov-12	Main	(-13° 58' 51", +33° 38' 13"	C Mwale

*Bulindi was planned and planted as a rainfed optimal site but was affected by drought

Appendix III Experimental sites summary (continued)

Trial	Site#	TrialName	Location	Country	Environment	PlantingDate	Season	GPS coordinates	Collaborator
GGHYB12	24	GGHYB1224	Nanga2012	Zambia	Optimal	20-Nov-11	Main	(-15° 49' 21.35"S, +27° 46' 35.48"E	N Bbebe
GGHYB12	25	GGHYB1225	Golden Valley2012	Zambia	Optimal	23-Dec-11	Main	(-14°10'6.72"S, +28° 22' 12.48"E, 1170 m	K Mwansa
GGHYB12	29	GGHYB1229	Harare2012b	Zimbabwe	Optimal	21-Dec-11	Main	(-17^{o}50'S, +31^{o}2'E, 1483 masl	CIMMYT
GGHYB12	30	GGHYB1230	Harare2012c	Zimbabwe	Optimal	17-Dec-11	Main	(-17^{o}50'S, +31^{o}2'E, 1483 masl	CIMMYT
GGHYB12	33	GGHYB1233	ART Farm Harare201	Zimbabwe	Optimal	16-Nov-12	Main	(-17^{o}42'9.1", +31^{o}3'48.4", 1556 masl	L Mutemeri
GGHYB12	39	GGHYB1239	Harare2013	Zimbabwe	Optimal	17-Nov-12	Main	(-17^{o}50'S, +31^{o}2'E, 1483 masl	CIMMYT
GGHYB12	14	GGHYB1214	Harare2012	Zimbabwe	MSV	10-Jan-12	Main	(-17^{o}50'S, +31^{o}2'E, 1483 masl	CIMMYT
GGHYB12	34	GGHYB1234	Harare2013a	Zimbabwe	MSV	29-Dec-12	Main	(-17^{o}50'S, +31^{o}2'E, 1483 masl	CIMMYT
GGHYB12	35	GGHYB1235	Harare2013b	Zimbabwe	MSV	29-Dec-12	Main	(-17^{o}50'S, +31^{o}2'E, 1483 masl	CIMMYT

Appendix IV Protocol for recording traits

No	Traits	Description	Unit/Precision
1	Plant height	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. Enter the data individually and use the computer to calculate the average.	Measure: 1 cm Average: 01 cm
2	Ear height	Measured as height between the base of a plant to the insertion of the top ear of the same plant; observations taken of 6 alternating plants in the plot. Enter the data individually and use the computer to calculate the average.	Measure: 1 cm Average: 01 cm
3	Anthesis date	Measured as number of days after planting when 50% of the plants shed pollen. This is determined on a daily basis by counting the number of plants with at least one anther extruded divided by the total number of plants in the plot.	1 day
4	Silking date	Measured as number of days after planting when 50% of the plants silk. This is determined on a daily basis by counting the number of plants with at least one silk extruded divided by the total number of plants in the plot.	1 day
5	Anthesis-silking interval	Calculated as Silking date minus Anthesis date	1 day
6	Senescence	Scored using a scale from 0 to 10, dividing the percentage of estimated total leaf area that is dead by 10: 1 = 10% dead leaf area;2 = 20% dead leaf area;3 = 30% dead leaf area;4 = 40% dead leaf area;5 = 50% dead leaf area;6 = 60% dead leaf area;7 = 70% dead leaf area;8 = 80% dead leaf area;9 = 90% dead leaf area;10= 100% dead leaf area; leaf senescence is scored on 3 occasions (at 2, 4 and 6 weeks after female flowering).	%
7	Grain weight, grain moisture, and grain yield	Shelled grain from all ears in a plot is weighed and grain moisture measured. Grain yield per plot is calculated, adjusted to 12.5% grain moisture and converted to tons per hectare.	1 gram
8	Ear number per plant	Number of ears per plant Counted as number of ears with at least one fully developed grain divided by the number of harvested plants.	01
9	Normalized Difference Vegetation Index (NDVI)	Measured with a portable field spectroradiometer (GreenSeeker). Recorded at 60 cm above the plants on at 25 cm height	NDVI Index

Appendix IV Protocol for recording traits (continued)

10	Hundred kernel weight	Three samples of 100 kernels randomly selected from the total kernels and their weight measured. Enter the data individually and use the computer to calculate the average.	01gram
11	Chlorophyll content (SPAD)	Measured with a portable chlorophyll meter (SPAD meter). Take the measure from six alternating plants. Take the measures in the middle between leaf tip and leaf base, and in between leaf rib and between leaf rim/edge; enter the data individually and use the computer to calculate the average.	SPAD units
12	Protein, starch and oil content	Measured with a near infra red (NIR) grain analyser.	
13	Lodging	Physical count of number of plants broken at along the stem or at the base.	1
14	Ear aspect		1-5
15	Ear texture	Reference: Appendix V.	1-5
16	Ear rots	Proportion of total rotten proportion of total ears per plot.	0.5

Appendix V Scale for scoring grain texture used in this study

1. corné *flint*

2. corné à corné-denté *flint-like*

3. corné-denté *intermediate*

4. corné-denté à denté *dent-like*

5. denté *dent*

Appendix VI Genetic distances among parental lines of the 67 hybrids

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
		CML44	CML14	CML18	CML18	CML20	CML20	CML31	CML39	CML39	CML44	CML44	CML44	CML48	CML48	CML50	CML50	CZL000	CZL000	CZL000	CZL000	MAS[M	CZL020	CZL020	DTPWC	CZL030	CZL030	CZL030	CZL030	CZL030	CZL030	CZL040	CZL040	CZL040
1	CML444-B																																	
2	CML144-B	0.277																																
3	CML181-B	0.343	0.319																															
4	CML181den	0.332	0.306	0.153																														
5	CML202-B	0.286	0.267	0.327	0.316																													
6	CML206-B	0.282	0.260	0.319	0.308	0.268																												
7	CML312-B	0.284	0.258	0.324	0.313	0.272	0.261																											
8	CML390-B	0.288	0.263	0.320	0.309	0.269	0.257	0.268																										
9	CML395-B	0.288	0.268	0.321	0.309	0.229	0.268	0.277	0.270																									
10	CML442	0.300	0.280	0.300	0.280	0.284	0.188	0.284	0.278	0.278																								
11	CML443-B	0.310	0.290	0.320	0.309	0.291	0.294	0.292	0.287	0.287	0.266																							
12	CML445-B	0.306	0.285	0.329	0.322	0.292	0.285	0.291	0.287	0.291	0.299	0.309																						
13	CML488-B	0.288	0.262	0.327	0.317	0.278	0.272	0.271	0.272	0.278	0.290	0.300	0.290																					
14	CML489-B	0.270	0.261	0.325	0.315	0.128	0.265	0.263	0.264	0.250	0.278	0.288	0.290	0.272																				
15	CML505-B	0.288	0.276	0.328	0.322	0.280	0.278	0.280	0.274	0.281	0.293	0.299	0.299	0.285	0.273																			
16	CML509-B	0.293	0.267	0.322	0.310	0.277	0.268	0.273	0.149	0.277	0.283	0.293	0.291	0.275	0.273	0.284																		
17	CZL00001-B	0.321	0.301	0.343	0.334	0.306	0.300	0.300	0.298	0.298	0.316	0.318	0.316	0.309	0.301	0.314	0.302																	
18	CZL00003-B	0.340	0.327	0.351	0.344	0.330	0.322	0.332	0.331	0.333	0.321	0.339	0.340	0.335	0.330	0.338	0.329	0.346																
19	CZL00006-B	0.056	0.254	0.326	0.315	0.264	0.258	0.261	0.265	0.265	0.280	0.291	0.287	0.265	0.247	0.270	0.270	0.303	0.329															
20	CZL00009-B	0.386	0.381	0.396	0.395	0.376	0.380	0.386	0.382	0.378	0.388	0.386	0.391	0.388	0.375	0.384	0.389	0.385	0.391	0.385														
21	CML539	0.293	0.264	0.324	0.313	0.273	0.266	0.157	0.271	0.264	0.286	0.287	0.293	0.278	0.266	0.281	0.279	0.297	0.332	0.270	0.383													
22	CZL02012-B	0.297	0.273	0.320	0.305	0.277	0.209	0.274	0.266	0.276	0.220	0.293	0.294	0.284	0.278	0.288	0.273	0.306	0.325	0.275	0.382	0.276												
23	CZL02014-B	0.149	0.258	0.328	0.316	0.268	0.260	0.265	0.264	0.271	0.286	0.296	0.289	0.271	0.259	0.214	0.273	0.304	0.332	0.115	0.383	0.272	0.274											
24	DTPWC8F31	0.288	0.263	0.326	0.315	0.272	0.267	0.267	0.269	0.273	0.287	0.292	0.286	0.146	0.267	0.282	0.272	0.304	0.334	0.265	0.386	0.272	0.280	0.268										
25	CZL03002-B	0.342	0.329	0.366	0.358	0.339	0.333	0.335	0.334	0.338	0.346	0.346	0.340	0.340	0.334	0.342	0.337	0.350	0.366	0.330	0.394	0.338	0.340	0.333	0.335									
26	CZL03003-B	0.292	0.266	0.322	0.309	0.276	0.267	0.272	0.149	0.275	0.282	0.293	0.290	0.273	0.271	0.284	0.013	0.301	0.328	0.269	0.390	0.278	0.272	0.272	0.270	0.336								
27	CZL03004-B	0.296	0.271	0.328	0.313	0.274	0.273	0.276	0.277	0.275	0.288	0.297	0.298	0.281	0.271	0.280	0.213	0.304	0.332	0.273	0.388	0.279	0.277	0.275	0.279	0.343	0.212							
28	CZL03007-B	0.288	0.266	0.322	0.315	0.187	0.265	0.269	0.267	0.258	0.284	0.298	0.291	0.276	0.220	0.282	0.274	0.298	0.329	0.265	0.384	0.276	0.274	0.271	0.274	0.338	0.273	0.280						
29	CZL03018-B	0.292	0.271	0.335	0.326	0.287	0.275	0.273	0.278	0.284	0.294	0.308	0.298	0.289	0.281	0.289	0.285	0.311	0.340	0.274	0.385	0.281	0.287	0.276	0.282	0.344	0.283	0.294	0.256					
30	CZL03021-B	0.280	0.260	0.323	0.311	0.130	0.260	0.150	0.265	0.245	0.281	0.286	0.287	0.273	0.176	0.275	0.271	0.299	0.327	0.257	0.375	0.205	0.276	0.262	0.266	0.334	0.270	0.267	0.237	0.281				
31	CZL04001-B	0.363	0.272	0.379	0.377	0.357	0.361	0.363	0.363	0.332	0.368	0.372	0.372	0.359	0.353	0.363	0.368	0.370	0.378	0.357	0.382	0.359	0.366	0.360	0.362	0.382	0.367	0.368	0.364	0.362	0.357			
32	CZL04002-B	0.292	0.135	0.331	0.322	0.280	0.277	0.278	0.280	0.256	0.295	0.303	0.301	0.281	0.275	0.292	0.286	0.313	0.337	0.272	0.384	0.284	0.289	0.277	0.280	0.339	0.284	0.290	0.281	0.285	0.278	0.275		
33	CZL04005-B	0.263	0.262	0.322	0.314	0.268	0.261	0.262	0.270	0.271	0.278	0.291	0.289	0.271	0.255	0.278	0.274	0.304	0.331	0.251	0.380	0.269	0.276	0.264	0.272	0.333	0.273	0.276	0.269	0.282	0.261	0.356	0.277	
34	CZL04006-B	0.296	0.268	0.314	0.302	0.276	0.190	0.273	0.263	0.273	0.202	0.290	0.293	0.284	0.275	0.285	0.269	0.301	0.325	0.274	0.381	0.275	0.098	0.272	0.278	0.337	0.268	0.277	0.271	0.284	0.273	0.366	0.286	0.272
35	CZL04008-B	0.295	0.263	0.320	0.308	0.266	0.263	0.272	0.264	0.267	0.280	0.292	0.288	0.275	0.270	0.285	0.273	0.308	0.332	0.271	0.365	0.275	0.281	0.273	0.271	0.338	0.272	0.278	0.269	0.286	0.265	0.366	0.277	0.271
36	CZL04009-B	0.318	0.298	0.346	0.341	0.296	0.303	0.307	0.304	0.289	0.318	0.318	0.316	0.311	0.295	0.307	0.309	0.322	0.351	0.301	0.361	0.307	0.311	0.303	0.307	0.351	0.308	0.310	0.304	0.320	0.296	0.365	0.306	0.306
37	CZL042021-	0.282	0.252	0.324	0.313	0.179	0.263	0.265	0.266	0.250	0.280	0.291	0.291	0.270	0.212	0.275	0.272	0.304	0.331	0.259	0.382	0.273	0.274	0.262	0.265	0.334	0.271	0.274	0.234	0.278	0.207	0.354	0.268	0.263
38	CZL052-B	0.302	0.284	0.333	0.322	0.294	0.284	0.291	0.287	0.296	0.302	0.310	0.310	0.218	0.288	0.304	0.292	0.320	0.340	0.287	0.387	0.296	0.294	0.289	0.251	0.348	0.291	0.304	0.285	0.291	0.289	0.366	0.299	0.286
39	CZL054-B	0.297	0.276	0.331	0.320	0.280	0.270	0.281	0.277	0.278	0.294	0.301	0.295	0.285	0.280	0.284	0.282	0.309	0.336	0.276	0.381	0.283	0.232	0.278	0.284	0.341	0.281	0.279	0.281	0.291	0.278	0.358	0.294	0.283
40	CZL057-B	0.282	0.248	0.323	0.313	0.187	0.262	0.263	0.266	0.252	0.278	0.292	0.290	0.267	0.217	0.275	0.273	0.307	0.330	0.259	0.383	0.273	0.273	0.262	0.265	0.334	0.271	0.275	0.235	0.277	0.215	0.351	0.264	0.262
41	CZL0610-B	0.233	0.270	0.326	0.315	0.264	0.272	0.278	0.278	0.241	0.289	0.293	0.180	0.277	0.261	0.285	0.279	0.305	0.335	0.210	0.382	0.277	0.286	0.237	0.276	0.334	0.278	0.281	0.274	0.287	0.259	0.360	0.284	0.275
42	CZL0617-B	0.279	0.257	0.319	0.306	0.263	0.123	0.141	0.262	0.269	0.228	0.291	0.284	0.269	0.260	0.277	0.269	0.297	0.327	0.254	0.383	0.209	0.230	0.258	0.265	0.331	0.268	0.269	0.261	0.271	0.201	0.361	0.275	0.258
43	CZL0619-B	0.294	0.277	0.331	0.319	0.139	0.270	0.277	0.277	0.254	0.279	0.294	0.300	0.284	0.113	0.290	0.283	0.311	0.330	0.274	0.376	0.277	0.283	0.279	0.276	0.339	0.281	0.283	0.242	0.296	0.198	0.359	0.289	0.276
44	CZL0713-B	0.220	0.273	0.335	0.324	0.269	0.273	0.282	0.282	0.210	0.295	0.300	0.297	0.284	0.266	0.294	0.285	0.306	0.340	0.190	0.380	0.284	0.286	0.231	0.284	0.343	0.283	0.286	0.281	0.296	0.271	0.349	0.288	0.276
45	CZL076-B	0.303	0.277	0.326	0.314	0.287	0.263	0.282	0.281	0.282	0.249	0.297	0.228	0.288	0.284	0.296	0.285	0.309	0.332	0.281	0.388	0.285	0.273	0.287	0.285	0.343	0.284	0.290	0.285	0.291	0.280	0.368	0.294	0.283
46	CZL077-B	0.297	0.277	0.331	0.321	0.263	0.281	0.289	0.282	0.188	0.298	0.303	0.301	0.287	0.270	0.285	0.255	0.310	0.340	0.276	0.385	0.288	0.285	0.279	0.282	0.345	0.252	0.246	0.278	0.290	0.268	0.357	0.289	0.284
47	CZL99013-B	0.323	0.302	0.325	0.312	0.305	0.299	0.309	0.303	0.308	0.312	0.317	0.314	0.308	0.304	0.313	0.301	0.325	0.343	0.303	0.386	0.311	0.302	0.306	0.308	0.352	0.301	0.304	0.267	0.318	0.305	0.375	0.319	0.306
48	CZL99014-B	0.291	0.270	0.329	0.320	0.282	0.264	0.271	0.272	0.281	0.287	0.304	0.295	0.284	0.274	0.291	0.279	0.302	0.337	0.270	0.380	0.282	0.281	0.272	0.280	0.337	0.279	0.288	0.230	0.216	0.275	0.362	0.287	0.278
49	SYN312-SR-	0.388	0.388	0.391	0.390	0.385	0.341	0.336	0.391	0.394	0.375	0.395	0.391	0.394	0.386	0.389	0.392	0.390	0.326	0.387	0.395	0.349	0.371	0.388	0.393	0.398	0.391	0.384	0.386	0.386	0.351	0.396	0.390	0.386
50	VL05128	0.316	0.284	0.283	0.267	0.295	0.287	0.292	0.284	0.289	0.283	0.298	0.305	0.294	0.294	0.298	0.285	0.315	0.332	0.292	0.396	0.292	0.288	0.293	0.290	0.352	0.284	0.291	0.287	0.304	0.291	0.379	0.300	0.291
51	CZL0919	0.315	0.291	0.335	0.325	0.280	0.291	0.296	0.290	0.289	0.296	0.298	0.309	0.303	0.286	0.293	0.298	0.320	0.339	0.294	0.383	0.291	0.301	0.279	0.297	0.351	0.297	0.296	0.284	0.311	0.280	0.367	0.306	0.298
52	CZL0712	0.291	0.265	0.324	0.313	0.273	0.246	0.268	0.267	0.271	0.276	0.293	0.291	0.279	0.265	0.278	0.269	0.306	0.332	0.268	0.378	0.273	0.244	0.267	0.275	0.336	0.270	0.277	0.245	0.246	0.268	0.357	0.283	0.269
53	CZL0720	0.314	0.288	0.334	0.325	0.282	0.290	0.288	0.287	0.288	0.302	0.310	0.308	0.294	0.288	0.299	0.288	0.321	0.327	0.291	0.394	0.292	0.298	0.293	0.288	0.348	0.287	0.287	0.289	0.304	0.284	0.375	0.304	0.289
54	ZEWBc1F2-	0.315	0.319	0.357	0.348	0.296	0.317	0.323	0.183	0.323	0.332	0.332	0.335	0.326	0.300	0.324	0.261	0.339	0.358	0.302	0.382	0.323	0.320	0.309	0.323	0.360	0.260	0.328	0.313	0.327	0.304	0.373	0.329	0.322
55	CZL03011	0.289	0.260	0.330	0.317	0.272	0.275	0.273	0.271	0.267	0.293	0.294	0.294	0.273	0.267	0.286	0.277	0.309	0.331	0.266	0.381	0.272	0.282	0.272	0.271	0.335	0.275	0.286	0.277	0.283	0.268	0.350	0.279	0.275
56	CML536	0.293	0.265	0.319	0.307	0.276	0.248	0.271	0.267	0.269	0.241	0.285	0.209	0.278	0.272	0.285	0.273	0.301	0.326	0.270	0.385	0.276	0.263	0.276	0.275	0.337	0.272	0.279	0.273	0.282	0.270	0.364	0.282	0.271
57	CML504	0.307	0.301	0.349	0.343	0.313	0.294	0.301	0.303	0.313	0.312	0.330	0.315	0.313	0.303	0.318	0.309	0.326	0.352	0.290	0.382	0.310	0.309	0.295	0.311	0.352	0.309	0.319	0.266	0.250	0.305	0.370	0.315	0.306
58	CZL0520	0.358	0.346	0.376	0.369	0.343	0.344	0.350	0.347	0.339	0.356	0.350	0.355	0.287	0.341	0.353	0.352	0.364	0.370	0.349	0.390	0.339	0.344	0.351	0.322	0.373	0.351	0.355	0.348	0.354	0.343	0.385	0.350	0.344
59	CML159	0.278	0.006	0.319	0.307	0.267	0.261	0.258	0.263	0.269	0.280	0.290	0.286	0.262	0.261	0.277	0.267	0.302	0.327	0.255	0.381	0.264	0.273	0.259	0.264	0.329	0.266	0.270	0.266	0.271	0.260	0.273	0.134	0.263
60	CZL02015	0.289	0.264	0.326	0.315	0.272	0.268	0.268	0.269	0.273	0.288	0.293	0.287	0.147	0.268	0.282	0.272	0.305	0.335	0.266	0.386	0.273	0.280	0.268	0.006	0.335	0.271	0.280	0.275	0.283	0.267	0.362	0.280	0.273
61	CML505	0.286	0.275	0.329	0.321	0.278	0.275	0.280	0.273	0.281	0.290	0.300	0.298	0.286	0.273	0.044	0.284	0.314	0.337	0.268	0.383	0.281	0.287	0.211	0.281	0.343	0.283	0.280	0.281	0.286	0.273	0.364	0.289	0.277
62	CML509	0.400	0.396	0.399	0.399	0.396	0.396	0.400	0.346	0.401	0.398	0.396	0.398	0.401	0.396	0.394	0.292	0.394	0.395	0.399	0.395	0.396	0.392	0.397	0.403	0.400	0.295	0.372	0.397	0.397	0.395	0.394	0.399	0.398
63	CZL0918	0.289	0.269	0.326	0.316	0.264	0.266	0.273	0.269	0.212	0.286	0.293	0.293	0.277	0.263	0.282	0.279	0.299	0.329	0.264	0.384	0.277	0.282	0.270	0.272	0.339	0.276	0.280	0.244	0.251	0.263	0.355	0.274	0.275
64	CZL0916	0.284	0.262	0.321	0.310	0.268	0.259	0.264	0.081	0.272	0.278	0.287	0.286	0.275	0.261	0.274	0.193	0.293	0.328	0.262	0.381	0.271	0.265	0.259	0.271	0.331	0.193	0.275	0.249	0.244	0.262	0.361	0.278	0.267
65	CML440	0.292	0.261	0.322	0.312	0.274	0.259	0.179	0.266	0.274	0.282	0.292	0.250	0.269	0.268	0.282	0.269	0.280	0.330	0.268	0.386	0.225	0.271	0.269	0.270	0.336	0.268	0.276	0.270	0.282	0.233	0.361	0.280	0.269
66	CZL095	0.300	0.269	0.314	0.295	0.276	0.221	0.278	0.150	0.273	0.206	0.288	0.293	0.281	0.272	0.282	0.219	0.305	0.330	0.277	0.380	0.281	0.251	0.277	0.278	0.341	0.218	0.281	0.275	0.285	0.272	0.367	0.285	0.276
67	CZL057	0.283	0.248	0.323	0.312	0.188	0.262	0.263	0.266	0.251	0.278	0.292	0.291	0.268	0.218	0.275	0.273	0.307	0.330	0.259	0.383	0.273	0.274	0.262	0.265	0.335	0.271	0.275	0.235	0.277	0.214	0.351	0.264	0.263

Appendix VI Genetic distances among parental lines of the 67 hybrids continued

		34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67
		CZL040	CZL040	CZL040	CZL042	CZL052	CZL054	CZL057	CZL061	CZL061	CZL061	CZL071	CZL076	CZL077	CZL990	CZL990	SYN312	VL0512	CZL091	CZL071	CZL072	ZEWBc	CZL030	CML53	CML50	CZL052	CML15	CZL020	CML50	CML50	CZL091	CZL091	CML44	CZL095	CZL057
1	CML444-B																																		
2	CML144-B																																		
3	CML181-B																																		
4	CML181dent-B																																		
5	CML202-B																																		
6	CML206-B																																		
7	CML312-B																																		
8	CML390-B																																		
9	CML395-B																																		
10	CML442																																		
11	CML443-B																																		
12	CML445-B																																		
13	CML488-B																																		
14	CML489-B																																		
15	CML505-B																																		
16	CML509-B																																		
17	CZL00001-B																																		
18	CZL00003-B																																		
19	CZL00006-B																																		
20	CZL00009-B																																		
21	CML539																																		
22	CZL02012-B																																		
23	CZL02014-B																																		
24	DTPWC8F31-1-1-2-2-B-B																																		
25	CZL03002-B																																		
26	CZL03003-B																																		
27	CZL03004-B																																		
28	CZL03007-B																																		
29	CZL03018-B																																		
30	CZL03021-B																																		
31	CZL04001-B																																		
32	CZL04002-B																																		
33	CZL04005-B																																		
34	CZL04006-B																																		
35	CZL04008-B	0.276																																	
36	CZL04009-B	0.312	0.292																																
37	CZL042021-	0.273	0.267	0.299																															
38	CZL052-B	0.294	0.294	0.325	0.287																														
39	CZL054-B	0.246	0.285	0.310	0.278	0.303																													
40	CZL057-B	0.273	0.265	0.299	0.018	0.287	0.278																												
41	CZL0610-B	0.284	0.275	0.291	0.271	0.296	0.284	0.271																											
42	CZL0617-B	0.214	0.264	0.303	0.260	0.284	0.270	0.259	0.272																										
43	CZL0619-B	0.278	0.280	0.302	0.230	0.298	0.290	0.233	0.275	0.268																									
44	CZL0713-B	0.282	0.283	0.306	0.280	0.300	0.286	0.281	0.242	0.276	0.279																								
45	CZL076-B	0.267	0.280	0.315	0.284	0.303	0.289	0.282	0.236	0.267	0.291	0.278																							
46	CZL077-B	0.285	0.285	0.302	0.268	0.307	0.283	0.269	0.268	0.280	0.282	0.262	0.293																						
47	CZL99013-B	0.299	0.306	0.332	0.304	0.314	0.304	0.305	0.305	0.301	0.310	0.310	0.313	0.307																					
48	CZL99014-B	0.277	0.280	0.315	0.276	0.281	0.289	0.275	0.284	0.265	0.283	0.290	0.284	0.294	0.303																				
49	SYN312-SR-	0.368	0.391	0.393	0.386	0.388	0.385	0.387	0.392	0.296	0.385	0.396	0.387	0.395	0.394	0.388																			
50	VL05128	0.284	0.285	0.321	0.289	0.308	0.298	0.288	0.297	0.287	0.302	0.299	0.289	0.300	0.297	0.298	0.395																		
51	CZL0919	0.299	0.292	0.316	0.281	0.314	0.301	0.283	0.290	0.291	0.298	0.301	0.303	0.300	0.319	0.305	0.388	0.307																	
52	CZL0712	0.244	0.275	0.306	0.270	0.279	0.243	0.269	0.276	0.259	0.275	0.279	0.282	0.282	0.298	0.156	0.384	0.290	0.294																
53	CZL0720	0.295	0.291	0.322	0.290	0.316	0.302	0.289	0.291	0.288	0.292	0.302	0.303	0.298	0.319	0.298	0.401	0.292	0.311	0.294															
54	ZEWBc1F2-	0.319	0.319	0.341	0.321	0.333	0.327	0.321	0.326	0.317	0.300	0.321	0.333	0.332	0.344	0.324	0.391	0.338	0.335	0.319	0.331														
55	CZL03011	0.279	0.278	0.302	0.268	0.294	0.281	0.268	0.276	0.272	0.277	0.278	0.289	0.284	0.310	0.278	0.388	0.294	0.290	0.268	0.290	0.324													
56	CML536	0.259	0.269	0.303	0.272	0.295	0.279	0.270	0.216	0.254	0.279	0.265	0.064	0.283	0.303	0.276	0.386	0.281	0.292	0.270	0.293	0.325	0.277												
57	CML504	0.307	0.312	0.333	0.308	0.309	0.314	0.307	0.308	0.296	0.312	0.312	0.302	0.321	0.332	0.112	0.389	0.327	0.330	0.202	0.325	0.340	0.307	0.294											
58	CZL0520	0.343	0.352	0.358	0.347	0.338	0.351	0.346	0.346	0.344	0.344	0.353	0.353	0.359	0.362	0.354	0.388	0.358	0.349	0.346	0.359	0.366	0.303	0.348	0.364										
59	CML159	0.268	0.264	0.299	0.253	0.284	0.276	0.249	0.271	0.258	0.278	0.274	0.277	0.278	0.302	0.271	0.388	0.285	0.291	0.266	0.288	0.319	0.261	0.265	0.301	0.345									
60	CZL02015	0.279	0.271	0.308	0.265	0.252	0.284	0.265	0.277	0.266	0.277	0.285	0.286	0.283	0.308	0.282	0.393	0.290	0.298	0.276	0.288	0.323	0.272	0.276	0.312	0.323	0.264								
61	CML505	0.282	0.284	0.308	0.274	0.304	0.279	0.274	0.285	0.274	0.290	0.293	0.295	0.285	0.315	0.289	0.388	0.299	0.291	0.278	0.298	0.323	0.284	0.283	0.317	0.354	0.277	0.281							
62	CML509	0.391	0.400	0.396	0.396	0.394	0.392	0.397	0.398	0.397	0.396	0.400	0.401	0.388	0.397	0.398	0.396	0.403	0.373	0.390	0.405	0.362	0.396	0.399	0.397	0.396	0.396	0.403	0.393						
63	CZL0918	0.277	0.275	0.299	0.265	0.292	0.286	0.263	0.266	0.265	0.276	0.271	0.282	0.263	0.307	0.205	0.389	0.292	0.279	0.224	0.283	0.324	0.276	0.273	0.246	0.349	0.270	0.273	0.281	0.398					
64	CZL0916	0.262	0.262	0.304	0.265	0.274	0.275	0.265	0.277	0.259	0.273	0.281	0.279	0.282	0.300	0.225	0.389	0.283	0.289	0.234	0.287	0.224	0.272	0.266	0.262	0.349	0.263	0.271	0.273	0.364	0.242				
65	CML440	0.267	0.269	0.298	0.272	0.292	0.276	0.270	0.246	0.230	0.281	0.275	0.267	0.282	0.305	0.272	0.375	0.284	0.293	0.259	0.288	0.322	0.273	0.249	0.303	0.344	0.262	0.271	0.280	0.400	0.270	0.266			
66	CZL095	0.240	0.267	0.310	0.270	0.294	0.283	0.270	0.284	0.239	0.279	0.288	0.280	0.286	0.303	0.281	0.382	0.282	0.294	0.265	0.293	0.271	0.279	0.265	0.310	0.349	0.270	0.278	0.283	0.376	0.278	0.198	0.271		
67	CZL057	0.273	0.266	0.298	0.020	0.287	0.279	0.009	0.271	0.259	0.233	0.281	0.282	0.269	0.305	0.275	0.387	0.288	0.283	0.269	0.290	0.321	0.268	0.271	0.307	0.346	0.249	0.266	0.274	0.396	0.263	0.265	0.270	0.270	

Appendix VII. Weather data

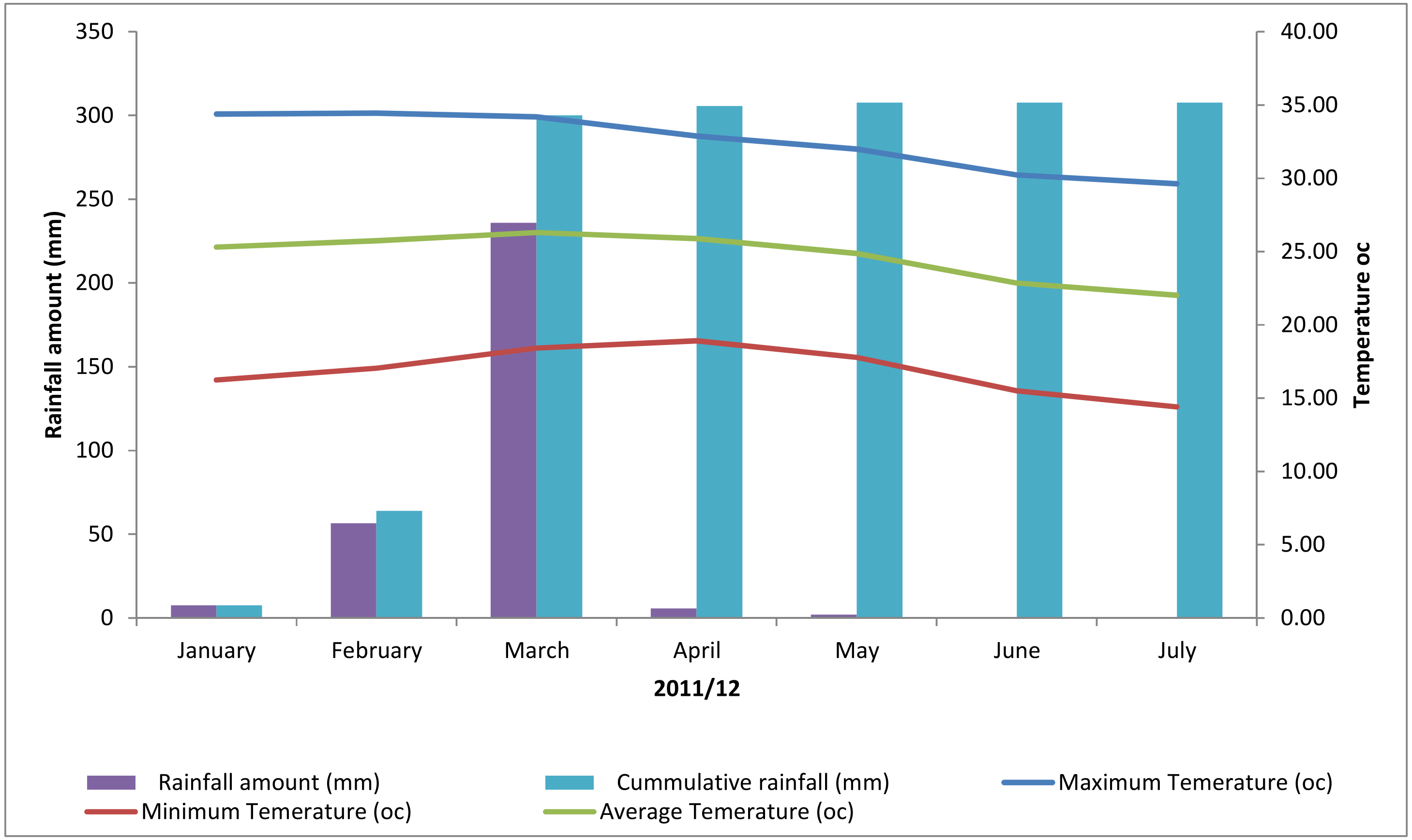


Figure A1 ART Farm, Zimbabwe 2011/12 weather data

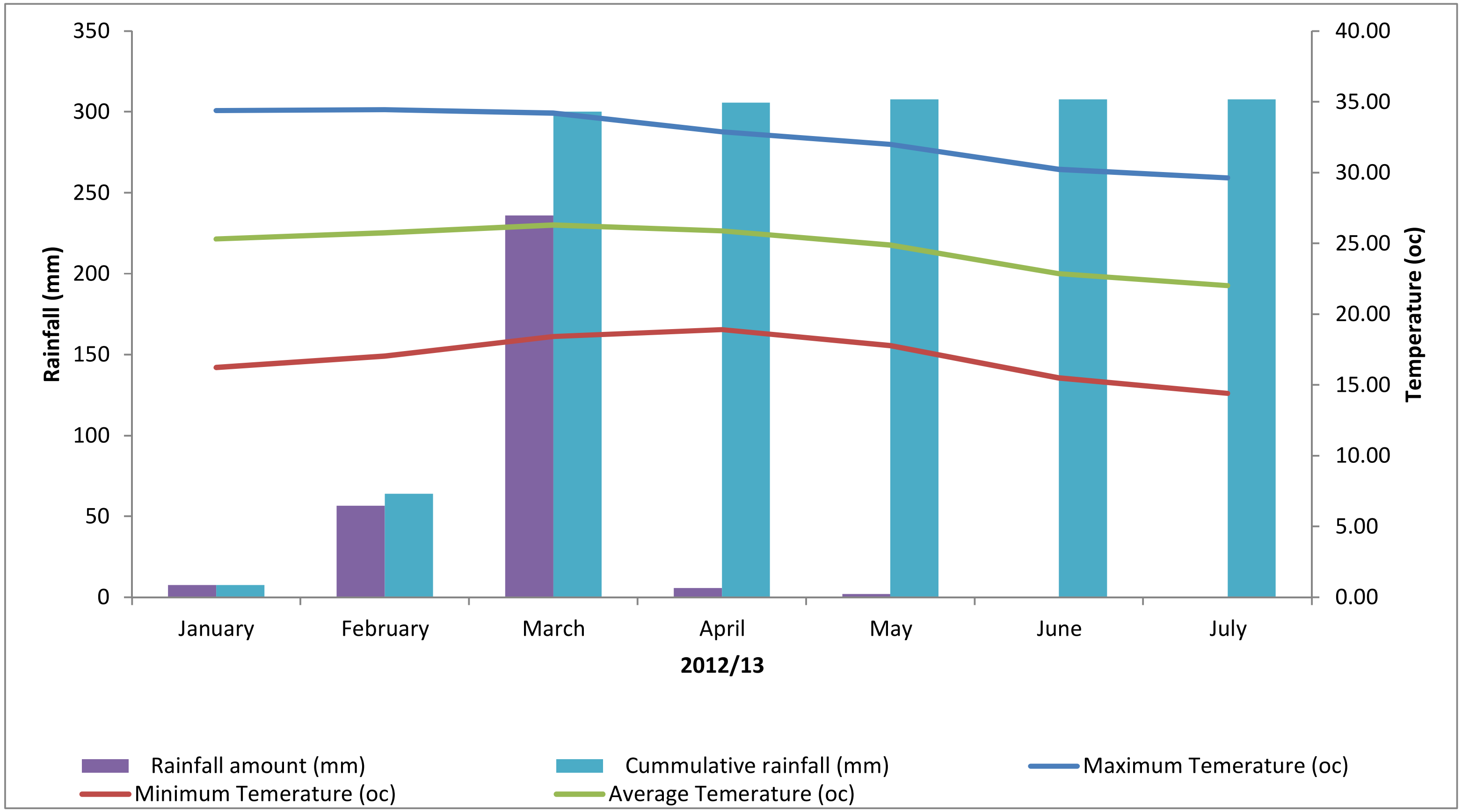


Figure A2 ART Farm, Zimbabwe 2012/13 weather data

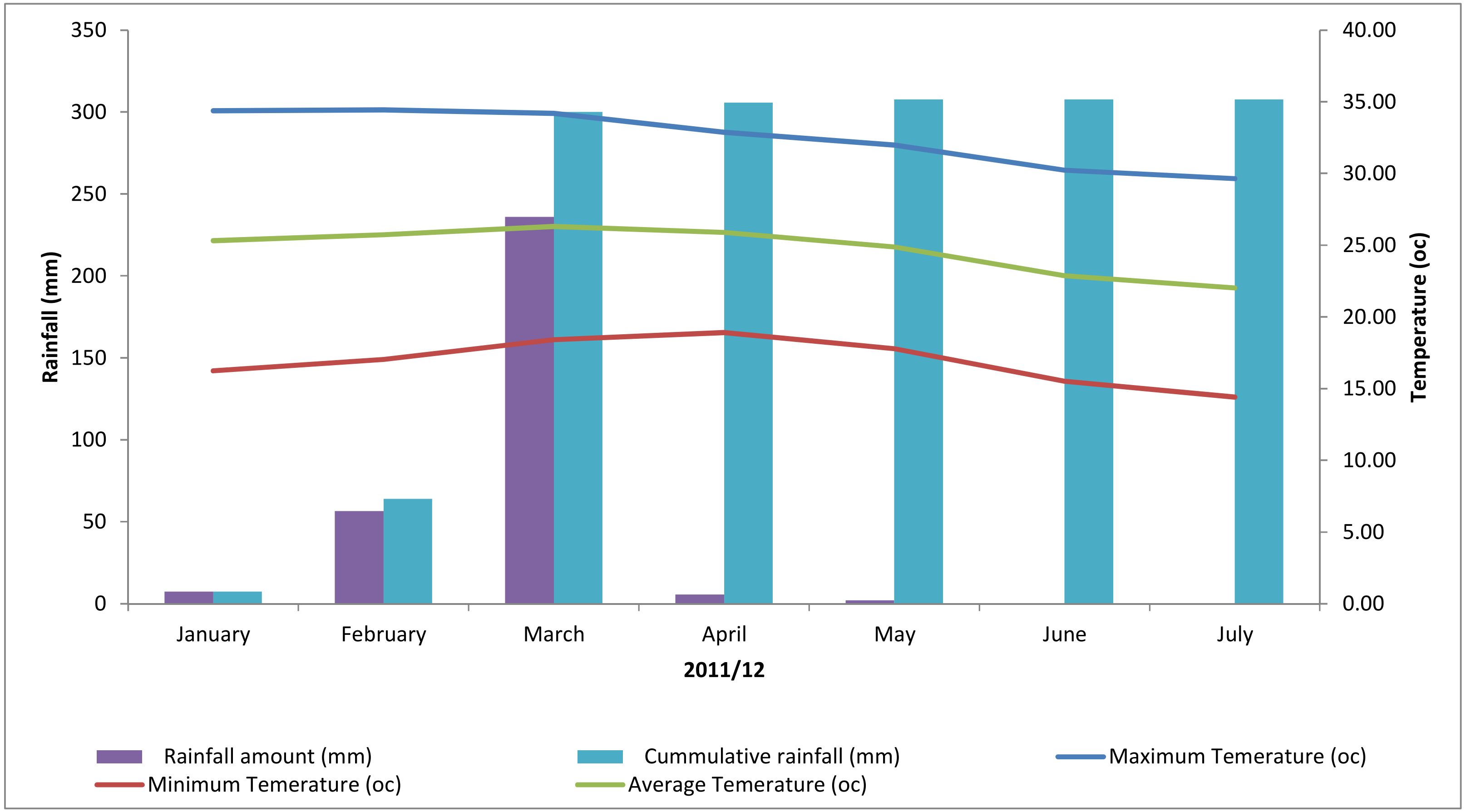


Figure A3 Chitedze, Malawi 2011/12 weather data

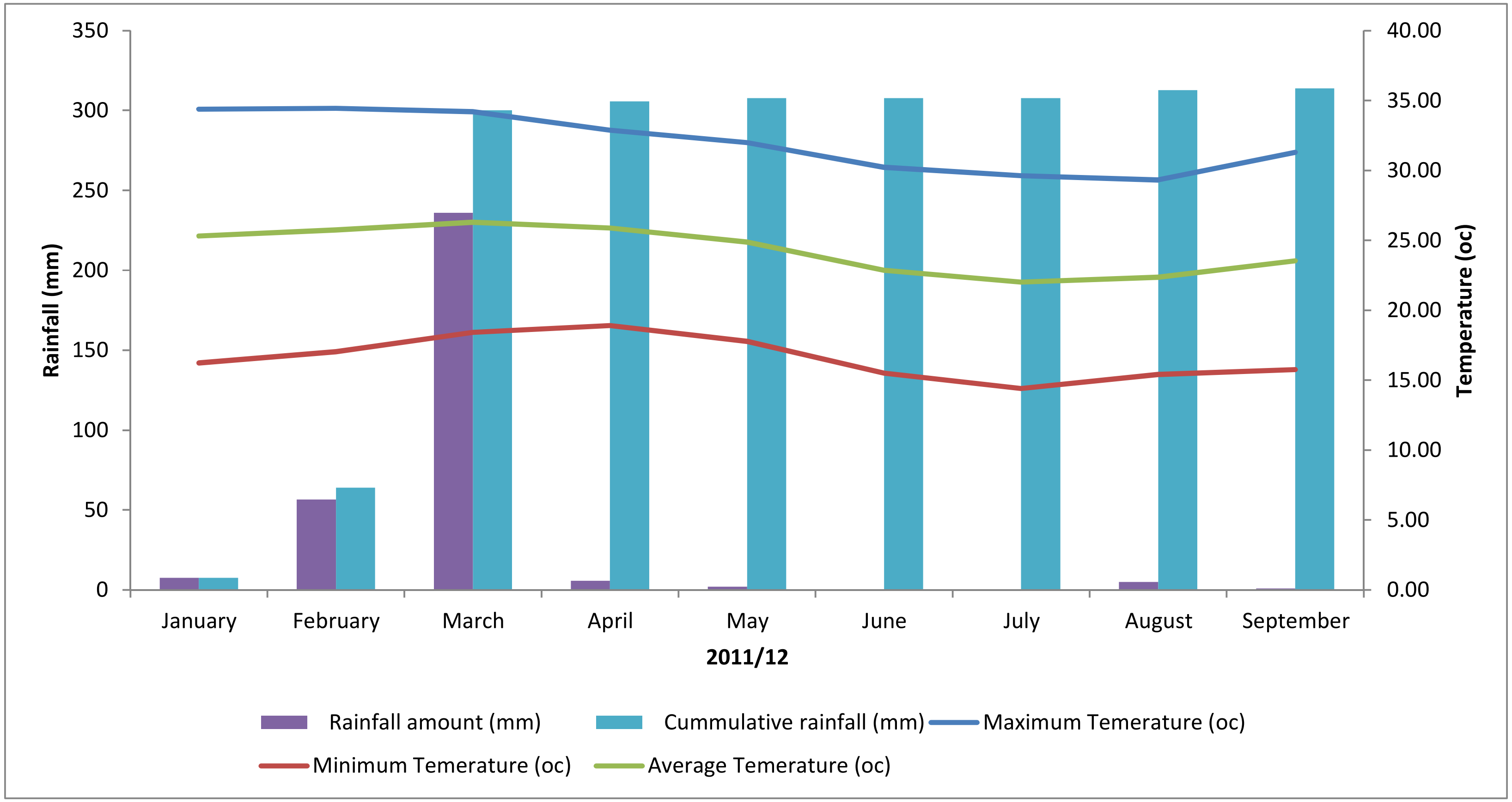
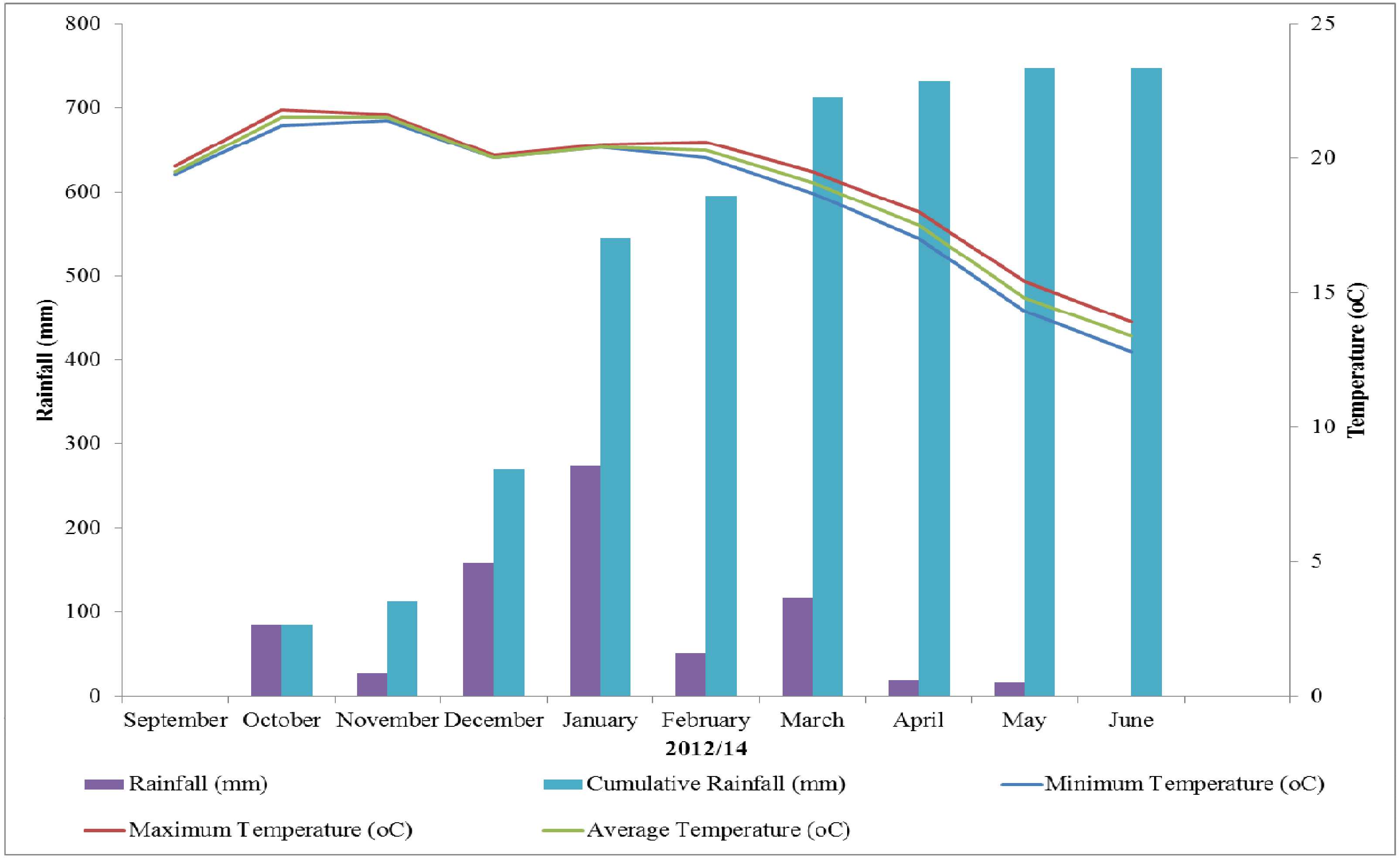


Figure A4 GART, Zambia weather data 2011/12


800
700
600
500
400
300
200
100
0
Rainfall (mm)
25
20
15
10
5
0
Temperature (oC)
September
October
November
December
January
February
March
April
May
June
2012/14
Rainfall (mm)
Cumulative Rainfall (mm)
Minimum Temperature (oC)
Maximum Temperature (oC)
Average Temperature (oC)

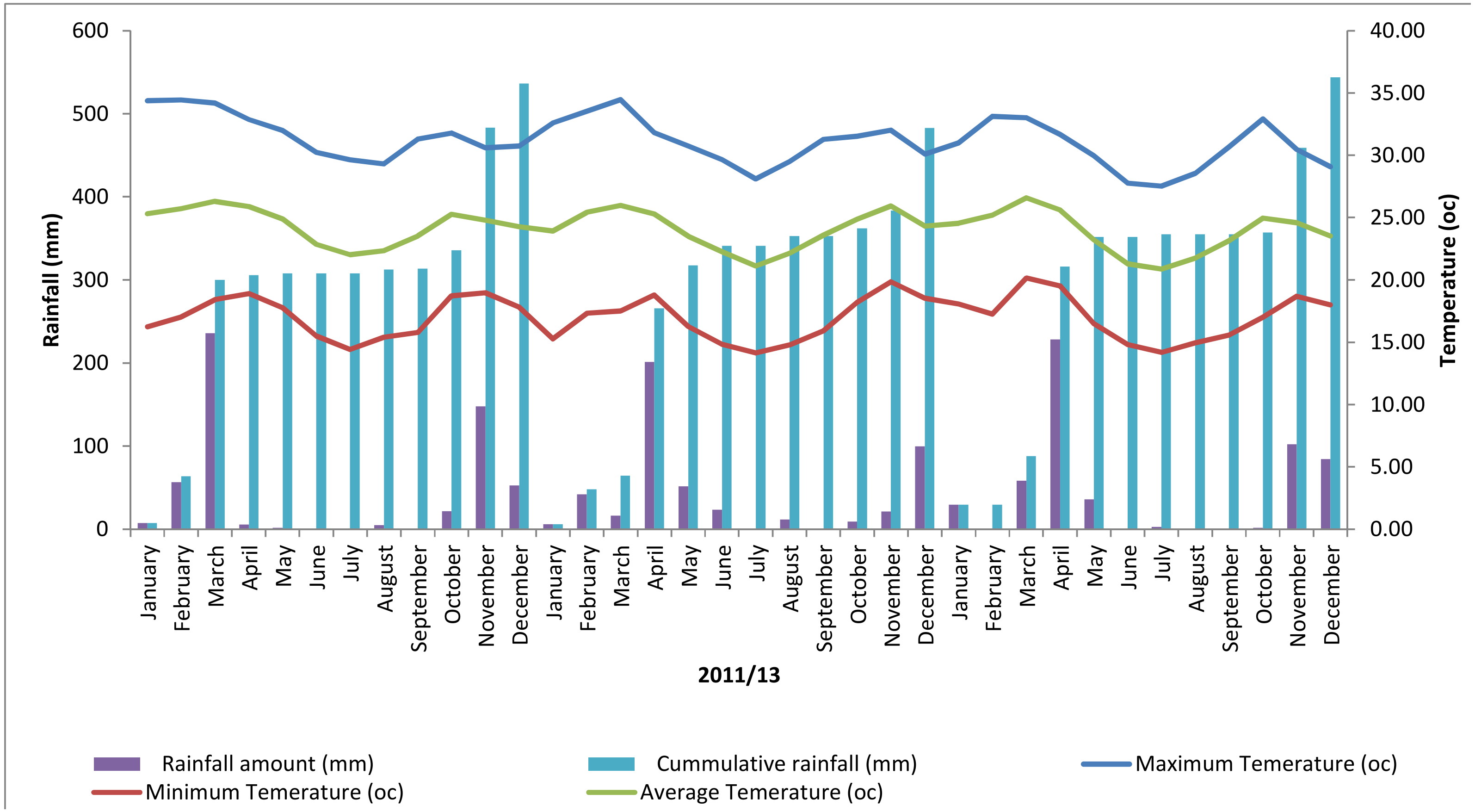


Figure A6 Kiboko, Kenya weather data 2011/12