The influence of abiotic stress on CIMMYT provitamin A elite maize germplasm

by

Pepukai Manjeru

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Promoters:

Professor Maryke Tine Labuschagne
Doctor John Peter MacRobert
Doctor Angeline van Biljon
Doctor Peter Setimela

SUMMARY

Micronutrient malnutrition, including vitamin A deficiency, affects more than half of the world population, having a major effect on children less than five years old, pregnant and lactating women. The problem is significant in sub-Saharan Africa (SSA) where people subsist mostly on white maize which lacks vitamin A. Vitamin A deficiency is responsible for a number of health disorders that include poor vision and reproduction, and supressed growth and immunity. Biofortification of staple food crops such as maize with β-carotene can be a sustainable approach to address dietary vitamin A deficiency. Orange maize contains high levels of β-carotene, making it an important crop for combating vitamin A deficiency. The SSA region is also prone to various abiotic stresses that impact negatively on maize productivity. To ensure food security in the region, there is a need to breed highly nutritious maize cultivars adapted to the major abiotic stresses experienced in the region. To breed increased provitamin A hybrids, it is important to understand the mode of gene action affecting grain yield and β-carotene expression, and the heritability of β-carotene concentration under the prevailing stresses. There is also a need to determine the stability of provitamin A germplasm for grain yield and nutritional traits such as β-carotene under these stress conditions. In this study, 22 elite provitamin A inbred lines and five yellow drought tolerant inbred testers were crossed following a line × tester crossing design. Thirty hybrids had sufficient seed for replicated trials out of a potential of 110. The 30 hybrids and five checks were evaluated in Zimbabwe under optimum conditions, random drought stress, managed drought stress, combined drought and heat stress, low N stress and low P stress in 2014 and again in 2015. There was significant variation between hybrids for grain yield for all environments, except grain yield under low nitrogen stress. There was a significant interaction between year, environment and genotype for grain yield but no interaction was observed for grain texture. Inbred lines were highly heterotic for grain yield, especially under stress conditions. Narrow sense heritability for grain yield was more than 50% under optimal conditions, managed drought stress, combined and drought and heat stress and low P stress. AMMI and GGE analyses showed that genotype by environment interaction (GEI) was a very important source of maize grain yield variability. The environments were grouped into one mega-environment. The highly significant correlations between the environments suggest that testing can be done in only one environment. Hybrid,

environment, year and GEI effects for β-carotene were highly significant. Beta-carotene concentration was higher under optimum than under stress conditions and was highly significantly correlated with grain yield. Heritability for β-carotene was very high; 97% and 90% under optimum and 70% and 94% under managed drought stress in 2014 and 2015 respectively. General combining ability for β-carotene was significant and specific combining ability was not, emphasising the importance of additive gene action in the expression of the trait. Provitamin A hybrids had β-carotene concentration in the expected range (5-12 µg g^{-1}) for first generation medium to high provitamin A maize genotypes. Lines 6, 7 and 8 can be used for breeding hybrids suitable for all environments except for managed stress conditions. Testers 1 and 2 were ideal for breeding for optimum conditions, managed drought stress, tester 2 for random drought stress and tester 3 for low P stress. Line 8 contributed consistently positively to grain yield, line 3 was favourable under managed drought stress and combined drought and heat stress, lines 6, 7, 8 and 9 were desirable under low N, 6, 7 and 8 under optimum conditions, 4, 6, 7, 8, and 10 under random drought stress, and 3, 8 and 10 under managed drought. The best performing and most stable genotypes for both grain yield and β-carotene can be distributed to SSA farmers for production. These hybrids will go a long way to alleviate vitamin A malnutrition among resource poor households in the region.

Key words: Abiotic stress, orange maize, stability, vitamin A, yield

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.

Pepukai Manjeru   31 January 2017

DEDICATION

To my late father Isaac Dzivakwi Chitereka my hero and source of inspiration.

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ABBREVIATIONS AND SYMBOLS

%	percent
µg g^{-1}	microgram per gram
µL	microlitre
AEA	Average environmental axis
AEC	Average environment coordination
AMMI	Additive Main effects and Multiplicative Interaction
ANOVA	Analysis of variance
ASI	Anthesis Silking Interval
ASV	AMMI Stability Value
BCP	Biofortification Challenge Programme
CGIAR	Consultative Group on International Agricultural Research
CIMMYT	International Centre for Maize and Wheat Improvement
cm	centimetre
crtB	bacterial genes coding for phytoene synthase
crtRB1	β-carotene hydroxylase 1
CV	Coefficient of Variation
DF	Degrees of Freedom
DTMA	Drought Tolerant Maize for Africa
E	Environment
EA	Ear Aspect
EH	Ear Height
EPO	Ear Position
EPP	Number of Ears per Plant
ER	Ear Rot
FAO	Food and Agriculture Organization
FAOSTAT	FAO Statistical Database
FF	Days to 50% silking
g	gram
G x E	Genotype x Environment
G	Genotype
GCA	General Combining Ability
GCA$_f$	Female General Combining Ability
GCA$_m$	Male General Combining Ability
GEI	Genotype x Environment Interaction
GGE	Genotype and Genotype x Environment Interaction
GM	Genetically Modified
GYD	Grain Yield
H^2	Heritability in the broad sense
h^2	heritability in the narrow sense
ha	hectare
HC	Husk Cover
IITA	International Institute of Tropical Agriculture
IPCA	Interactive Principal Component Analysis
IPCC	Intergovernmental Panel on Climate Change
K	Potassium

KCl	Potassium Chloride (murate of potash)
Kg ha^{-1}	Kilogram per hectare
Kg	Kilogram
LSD	Least Significant Difference
m	metre
masl	meter above sea level
Max	Maximum
MF	Days to 50% anthesis
mg	milligram
mg g^{-1}	milligram per gram
Min	Minimum
mM	micro mole
mm	millimetre
MOHCW	Ministry of Health and Child Welfare
MS	Mean Squares
MSE	Mean Square Error
N	Nitrogen
NARS	National Agricultural Research Systems
NE	Number of Ears
NIR	Near Infrared Spectroscopy analyser
nmol g^{-1}	nano moles per gram
NUE	Nitrogen Use Efficiency
ºC	degrees celcius
Optm	Optimum
OPV	Open Pollinated Variety
P	Phosphorus
P$_2$O$_5$	Super Phosphate
PC	Principal Component
PCA	Principal Component analysis
PH	Plant Height
pH	soil acidity or alkalinity
PSY	Phytoene synthase
QTL	Quantitative Trait Loci
rpm	revolutions per minute
R^2	Coefficient of determination
RL	Root Lodging
SCA	Specific Combining Ability
SL	Stem Lodging
SS	Sum of Squares
SSA	Sub-Saharan Africa
SVD	Singular Value Decomposition
t ha^{-1}	ton per hectare
TEX	Grain texture
UNICEF	United Nations Children Fund
USDA ARS	USA Department of Agriculture, Agriculture Research Service
WFP	World Food Programme
WHO	World Health Organisation
Y1	gene coding for phytoene synthase
y1	gene coding for lack of phytoene synthase
YSI	Yield Stability Index

ZIMSTAT	Zimbabwe National Statistics Agency
α	alpha
β	beta
γ	gamma
ζ	epsilon

CHAPTER 1

GENERAL INTRODUCTION

1.1 Maize

Maize (*Zea mays* L.), the American Indian word for corn, meaning literally "that which sustains life" (FAO, 1992), is the third most important cereal crop in the world after wheat and rice (FAOSTAT, 2016). It derives its importance from its various uses which include providing nutrients for humans and animals, serving as an important raw material in industry (Vasal, 2000; Prasanna et al., 2009) for the production of starch, oil and protein, alcoholic beverages, food sweeteners and bio-fuel (FAO, 1992; Watson, 2003). Maize provides carbohydrates, proteins, iron, vitamin A (yellow maize only) and B (except vitamin B-12) and some minerals to human diets (Watson, 2003). According to Edmeades (2008), maize is a main staple food for over 300 million people across the world, mostly in sub-Saharan Africa (SSA) and Latin America. The focus of most maize breeding programmes has been to increase productivity and stability under diverse environments (Edmeades et al., 2011). But since it is a basic staple food for many poor people, particularly those living in developing countries (FAO, 1992), demand for nutritionally rich maize beneficial to human health has gained a lot of interest from both public and private maize breeders (Pollak and Scott, 2005; Berardo et al., 2009).

1.2 Maize grain nutritional quality

Plant breeders have developed specialty maize types with improved grain quality for specific end-uses for human and livestock nutritional needs (such as *opaque-2*, high oil and high β-carotene), and for food and industrial processing (such as waxy and high amylose) (Mason and D'Croz-Mason, 2002). The nutritional composition and quality of maize is influenced by a number of factors including genotype and environment as well as postharvest technology (FAO, 1992; Wilson et al., 2004). Grain composition and the resulting physicochemical properties of grain are determined during seed development and stress during seed development influence grain composition. Substantial research on the influence of production practices on maize grain quality has been conducted, especially fertilizer management and cultural practices. The yellow maize kernel is composed of approximately 61-78% starch, 6-12% protein, 3.1-5.7% oil, 1-3% sugar, 1.1-3.9% ash, 5.8-6.6% pentosans (as xylose), 8.3-11.9% fiber (neutral detergent residue),

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3.3-4.3% cellulose and lignin (acid detergent residue), and 12-36% carotenoids (Watson, 2003). Ordinary maize protein is of poor nutritional quality for humans and monogastric animals, because it is low in lysine and tryptophan (Mertz et al., 1964; Gissa, 2008) and has an undesirable ratio of leucine to isoleucine (Alexander, 1988). The oil in maize increases the caloric value of stock feed, and due to a high degree of unsaturation, is also widely used for human consumption (Perry, 1988). The ash of maize grain contains little calcium (Ca), and although the phosphorous (P) content is relatively high, only 50% is available to monogastric animals (Ertl et al., 1998). Maize protein content and amino acid ratios vary among genotypes and seasons (Earle, 1977; FAO, 1992; Wilson et al., 2004), soil fertility, crop management and climatic conditions (Pierre et al., 1977; Asghari and Hanson, 1984). Tsai et al. (1992) reported that protein yield increases from nitrogen (N) application is accompanied by an increase in the amount of zein present in the endosperm, creating harder, less brittle and more translucent grain. The reduction in biological value of maize protein is, however, compensated for in some cases, since N fertilizer application increases the size of the germ, which has a better amino acid balance than the endosperm (Bhatia and Rabson, 1987).

Production factors that increase grain yield also increase starch concentration of grain, while reducing the grain protein concentration (McDermitt and Loomis, 1981; Rooney et al., 2004). The negative relationship between protein concentration and grain yield is partly associated with the higher energy demand for synthesis of protein than starch (Penning de Vries et al., 1974; Rooney et al., 2004). Moisture stress has a negative effect on maize grain amino acid balance, while low soil N stress has a positive effect (Watson, 2003).

1.3 Maize grain colour

Maize differs significantly in colour from white to yellow, orange, red and brown (Watson, 2003). Colour in maize kernels depends on the level of carotenoid (yellow pigments) or anthocyanin (red and purple pigments) (Ford, 2000). White colour is a result of lack of the two pigments. The yellow colour, attributed to accumulation of carotenoids in the endosperm, has resulted from a gain of a function mutation in the primary biosynthesis reaction at the *y1* or *psy1* locus, which encodes the first rate limiting enzyme in the carotenoid pathway, phytoene synthase (Palaisa et al., 2003). Maize kernels with

white endosperm (*y1y1y1*) lack phytoene synthase, an enzyme required early in the biosynthetic pathway for the synthesis of phytoene (Buckner et al., 1996). The precursors that accumulate in these kernels are colourless, so the endosperm appears white. In Southern Africa white is the predominant grain colour of maize grown and consumed (Muzhingi et al., 2008). Though people prefer white maize over yellow maize, there is little evidence of difference in taste and processing qualities between yellow and white maize (Rubey, 1993; De Groote and Kimenju, 2008). Coloured varieties are mostly flint, which is associated with favourable cooking and processing characteristics (Rubey et al., 1997).

1.4 Carotenoids in maize

Plant carotenoids are 40-carbon isoprenoids with polyene chains that may contain up to 15 conjugated double bonds. The major carotenoids in maize are zeaxanthin and lutein, accounting for 90% of the total carotenoids in yellow maize, with β-carotene and β-cryptoxanthin being present in much smaller amounts (Moros et al., 2002). The molecular structure of vitamin A is identical to one-half of the molecular structure of β-carotene, a provitamin A that is metabolized in the gut and tissues of animal to vitamin A (Sebrell and Harris, 1972; Howe and Tanumihardjo, 2006). In general, any carotenoid pigment that has the vitamin A carbon structure on either end, is a provitamin A. β-cryptoxanthin has about one-half of the provitamin A activity of β-carotene. β-carotene may play an important role in reproduction independent of its role as a provitamin A source (Hemken and Bremel, 1982). The carotenoids are subject to destruction by oxidation, light, minerals, heat, length of storage and other variables (Burt et al., 2010; Boon et al., 2010). The carotenoid content of maize is variable among genotypes and disappears during storage on a logarithmic scale, because it decomposes in the presence of light and oxygen (Watson, 2003).

In plants, carotenoids increase the efficiency of photosynthesis by absorbing blue-green light and transferring this energy to chlorophyll. They protect the photosynthetic apparatus against photo-oxidation. These functions can also be the reason for their properties in humans. Epidemiological studies have shown associations between intake of fruits and vegetables rich in carotenoids and reduced risks of different types of cancer, cardiovascular diseases, and age-related muscular degeneration (Cooper et al., 1999). In

particular, the carotenoids in cereals, lutein and zeaxanthin, play an important role in the prevention of frequently occurring eye diseases like age-related muscular degeneration, cataracts, and retinitis pigmentosa (Fullmer and Shao, 2001). Even though cereal grains contain far fewer carotenoids than most vegetables and fruits, they are consumed frequently in considerable amounts.

1.5 Importance of vitamin A to human health

Vitamin A deficiency affects approximately 140 million children and 20 million pregnant women worldwide. Between 250000 and 500000 children go blind every year, and over 600000 deaths of children annually may be attributed to vitamin A deficiency (West Jr. and Darnton-Hill, 2001; Black et al., 2008). Some 127 million preschool children are vitamin A deficient, which is about one-quarter of all preschool children in high-risk regions of the developing world. Globally, approximately 4.4 million preschool-age children have visible eye damage due to vitamin A deficiency (Black et al., 2008).

Vitamin A deficiency can result in anaemia, weak immunity, stunted growth, damage to mucous membrane tracts, reproductive disorders, xerophthalmia, impaired vision and ultimately blindness and death (Haskell at al., 2004). Children with vitamin A deficiency are often deficient in multiple micronutrients and are likely to be anaemic, have impaired growth, and are at increased risk of severe morbidity from common childhood infections such as diarrhoea and measles (WHO, 2009). Pregnant women with vitamin A deficiency may be at increased risk of mortality. According to a WHO (2009) report on the risk factors responsible for development of illnesses and diseases, vitamin A deficiency ranks 7th among the 10 most important factors in developing countries.

1.6 Biofortification

To capitalize on agricultural research as a tool for public health, in July of 2003 the Consultative Group on International Agricultural Research (CGIAR) established HarvestPlus: the Biofortification Challenge Program (BCP), adding food quality to its agricultural production research paradigm (HarvestPlus, 2007). Biofortification relies on conventional plant breeding and modern biotechnology to increase the micronutrient density of staple crops (Pfeiffer and McClafferty, 2007; Pixley et al., 2010). Biofortification is gaining increasing recognition as an effective means of combating micronutrient malnutrition, particularly amongst the rural poor (Meenakshi et al., 2012).

The technology holds great promise for improving the nutritional status and health of poor populations in both rural and urban areas of the developing world (Graham and Welch, 1996; Graham et al., 1999; 2001; Bouis, 2003). It is a new food-based public health intervention initiative, aimed at controlling micronutrient deficiencies in poor countries (Pfeiffer and McClafferty, 2007). Five maize hybrids and three synthetics were released in 2012 from the International Centre for Maize and Wheat Improvement (CIMMYT) and International Institute of Tropical Agriculture (IITA) biofortification initiative. Three in Zambia, four in Nigeria, and one in Ghana, all with 6-8 ppm provitamin A. The varieties combine competitive grain yield and strong farmer preferences in addition to higher provitamin A content in comparison to commercially available hybrids.

1.7 Effect of abiotic stress on maize grain yield and quality

The major constraints to maize production include both biotic and abiotic factors. The main biotic factors are pests and diseases. The most common abiotic factors are drought, extreme temperatures, low soil fertility (especially low N), high soil aluminium (soil acidity), flooding and salinity (Edmeades et al., 2011). Environmental effects on grain quality are of paramount importance as maize production becomes more focused on end-user traits (Haegele and Westgate, 2007). Heat stress (Wilhelm et al., 1999) and water deficit (Claassen and Shaw, 1970) during grain filling reduces kernel weight. Water stress causes reduction in protein synthesis resulting in reduced grain protein (Wang and Li, 2006; Pierre et al., 2008). Synthesis of starch is another main factor determining grain yield in cereals (Emes et al., 2003). Water stress has varying effects on starch biosynthesis depending upon the crop stage and genotype selection. It is well reported that grain quality attributes depend on a supply of assimilates at anthesis stage (Rotundo et al., 2009; Seebauer et al., 2009) and direct availability of assimilates depends on photosynthetic activity (Kuanar et al., 2010).

1.8 Effect of drought stress on maize production

Water, the main component of a plant body (Ulukan, 2008), is the major abiotic limiting factor for plant growth and development (Zhao et al., 2009; Ji et al., 2010), adversely affecting crop yield and food grain production (Bandurska and Stroinski, 2003). Globally, 160 million ha of maize is under random drought stress conditions and annual yield losses

to drought are estimated at around 25% (Edmeades, 2008) and are greater in subtropical countries that rely on erratic rainfall (Edmeades et al., 2011; Mhike et al., 2011) and can be as high as 70% under extreme conditions compared to well-watered production (Edmeades et al., 1999). In SSA drought affects about 22% of mid-altitude areas and 25% of lowland tropical maize growing regions annually during times of crop production (Heisey and Edmeades, 1999) and this has the direct effect of reducing the attained yield (Edmeades et al., 2006).

Although drought affects all stages of maize growth and production, flowering stage, mostly between tassel emergence and the onset of grain filling is the most susceptible (Grant et al., 1989). This susceptibility is generally attributed to the structure of the maize plant (Magorokosho, 2006). Drought during this period causes a significant grain yield reduction attributed to kernel size reduction (Bolaños and Edmeades, 1993). Drought stress delays silking due to limited assimilates supply, but has no significant effect on timing of anthesis, causing poor male-female flowering synchronization (Cairns et al., 2013a) and also causes kernel and ear abortion (Du Plessis and Dijkhuis, 1967; Nesmith and Ritchie, 1992; Bolaños and Edmeades, 1996) thereby reducing yield. For successful pollination, silks and pollen should not be exposed to a desiccating environment. Pollination may be successful in drought-stressed plants, only to be followed by abortion of the kernels a few days later (Westgate and Bassetti, 1990).

The reduction in mean seasonal precipitation under climate change conditions implies that the water available for irrigation purposes would also be reduced (Edmeades, 2008). Given the lack of water and its cardinal role in crop production, it follows that tolerance to drought and efficient water usage should be assigned the highest priority in developing future crops (Edmeades, 2008). Irrigation cannot be the answer because demand for water is increasing, precipitation is reducing due to climate change and energy needed to pump the water is increasing (Makado et al., 2006). As a rough rule of thumb, it has been estimated that 25% of losses due to drought can be eliminated by genetic improvement in drought tolerance, and a further 25% by application of water-conserving agronomic practices, leaving the remaining 50% that can only be met by irrigation (Edmeades, 2008). A successful maize cultivar must be able to withstand some variation in rainfall from year to year (Bänziger et al., 2000).

1.9 Effect of heat stress on maize production

The problem of drought is worsened by occurrence of high temperatures (Edmeades et al., 2011; Cairns et al., 2013b). Climate change models indicate that levels of greenhouse gases are likely to increase global average surface temperatures by 1.5 to 4.5°C over the next 100 years (IPCC, 2007). With a temperature increase of 2°C, the wet zones of Zimbabwe (with a water surplus) will decrease by two-third from 9% to about 2.5% and the drier zones will double in area (Downing, 1992). Downing (1992) further predicted that an increase in temperature of 4°C will reduce the summer water-surplus zones of Zimbabwe to less than 2%. Maize yield reduction of 50% by the year 2020 and 90% revenue fall by 2100 is projected because of elevated temperatures (Boko et al., 2007).

Heat stress affects all the growth stages of maize. Optimal temperatures for maize growth vary between day and night, day temperature ranging from 25-30ºC, while night temperatures range between 17-23ºC (Zaidi and Singh, 2005). High temperatures for a number of days during the growth of maize cause a lot of morphological, anatomical, physiological and biochemical changes in the crop (Cairns et al., 2013a). Cairns et al. (2013a) defined heat stress as temperatures above a threshold level that results in irreversible damage to crop growth and development and is a function of intensity, duration and rate of increase in temperature. Thomson et al. (1966) demonstrated that a temperature increase of 6ºC during grain filling stage caused 10% yield reduction. Dale (1983) observed a negative inverse relationship between maize yield and temperature rise from 32ºC during this sensitive period. Lobell et al. (2011) showed that for every degree day in excess of 30ºC maize loses yield by 1% and 1.7% under optimum growing conditions and drought stress respectively.

High temperatures during the flowering stage causes loss of yield through reduction of grain number and weight (Cairns et al., 2013a). Under heat stress conditions, the number of successfully ovules fertilised is heavily reduced (Schoper et al., 1987) because pollen production and viability is compromised. The position of the tassel gives maximum exposure to heat stress, which damages the pollen, leading to lack of pollen viability. Pollen produced under high temperatures has reduced viability and *in vitro* germination (Schoper et al., 1987).

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Heat stress during the grain filling stage affects grain development and the mass of the grain is reduced because the number of the endosperm cells formed is less (Jones et al., 1984). During this stage, heat stress affects cell division, sugar metabolism and starch biosynthesis, thereby reducing dry matter accumulation in the grain (Monjardino et al., 2005). Maize grain mass is a function of rate and duration of grain filling, both of which are affected by temperature. High temperatures hasten grain filling and also reduce endosperm starch content, resulting in poorly filled grains with reduced mass. Heat stress affects the conversion of sugars to storage products.

Walker (1969) reported that heat stress also affects germination, early seedling development and vegetative stages of maize ontogeny. High temperature reduces the percent germination, which has an effect on overall plant population. It also affects the early growth stages by reducing root and shoot amount by about 10% for each degree increase from 26ºC to 35ºC when growth is severely retarded. The poor growth is attributed to poor reserve mobilisation and reduced protein synthesis (Riley, 1981).

High temperatures also delay canopy closure, reducing its capacity to intercept photosynthetic active radiation and competeness with weeds. Watt (1972) showed that temperatures above 35ºC affect maize leaf elongation rate, leaf area, shoot biomass and photosynthetic carbon dioxide assimilation rate. Elongation of the first internode and overall shoot growth of maize is the most sensitive processes of the vegetative stage to high temperatures (Weaich et al., 1996)

1.10 Effect of low N stress on maize production

Beside moisture stress, most maize in developing countries is produced under low N conditions (McCown et al., 1992; Oikeh and Horst, 2001) because of low N status of tropical soils, low N use efficiency in drought-prone environments, high price ratios between fertilizer and grain, limited availability of fertilizer, and low purchasing power of farmers (Bänziger et al., 1997). Declining soil fertility, particularly N deficit, is the most severe and widespread constraint to smallholder maize productivity and to long-term food security in SSA (Waddington and Heisey, 1997). Efficient N management is the most challenging aspect of tropical smallholder agriculture in SSA including Zimbabwe (Giller et al., 1997; Chikowo et al., 2004). Mineral fertilizer use in smallholder cropping systems

remains insufficient to meet crop N demand on a sustainable basis, partly because of prohibitive costs and/or lack of availability. N use efficiency is affected by N supply, genotype and other growth factors (other nutrients, radiation, water, soil pH). N stress reduces crop photosynthesis by reducing leaf area development and leaf photosynthesis rate and by accelerating leaf senescence. Maize plants responded to N deficiency by increasing total root length and altering root architecture by increasing the elongation of individual axial roots and enhancing lateral root growth, but with a reduction in the number of axial roots (Jones et al., 1986; Chun et al., 2005). Sub-optimal N affects the N-rich carbon dioxide assimilation enzymes which can limit maize production (Jones et al., 1986).

1.11 Effect of low P stress on maize production

Phosphorus is one of the major macronutrients required for optimal growth of maize; however it is the least available in most soils (Raghothama, 1999) with 30 to 40% of soils in the world being deficient in P (Batjes, 1997). Low soil P negatively affects maize productivity by diminishing photosynthetic carbon dioxide fixation rate (Batjes, 1997; Wang et al., 2007) and the expansion of the photosynthetic leaf surface (Zhu and Lynch, 2004). Phosphorus affects root development and root volume affecting the plant's capacity to draw other nutrients and water. The ability of a genotype to take up more P in deficient soils (high P uptake efficiency) and produce more dry matter for a given quantity of P (high P use efficiency) make it adapted to low P stress (Raghothama, 1999). High P uptake efficiency is related to the development of a robust rooting system allowing a plant to explore a larger volume of soil for nutrients and the changes in root physiology giving the plant enhanced capacity to draw P at lower concentrations in the soil solution or from insoluble inorganic or organic forms (Marschner, 1995). Many researchers observed that low P stress leads to a higher root/shoot ratio (Anghinoni and Barber, 1980; Fredeen et al., 1989; Rosolem et al., 1994).

1.12 Problem statement

Maize is an important food security crop serving millions of households in SSA. However, the ordinary white maize is considerably decient in vitamin A content causing malnultrition to young children and pregnant and lactating women. Further, productivity of maize is low in SSA due to several abiotic, biotic and socioeconomic constraints. Heat,

drought, low nitrogen and low phosperous stresses are among the major abiotic constraints affecting production and productivity of maize in the region. Ensuring stable maize yields in an era where climate change threatens traditional production practices is a challenge to most smallholder farmers and has become a vital concern in global food security (van Oosten et al., 2016). The SSA region had experienced serious droughts of unusually long duration and crop yield were drastically reduced leading to femaine. The world population is also growing increasing the pressure on agricultural land to yield more nutritious food and pushing farmers into producing crops in marginal areas. Therefore, there is need for systematic breeding of maize to develop improved cultivars with increased β-carotene content to circumvent vitamin A deficiency and to enhance abiotic stress tolerance to boost productivity. This will go a long way towards addressing vitamin A malnutrition especially in the rural poor communities that subsits on maize.

1.13 Objectives

The major objective of this study was to study abiotic stress tolerance and nutritional value of CIMMYT provitamin A elite maize germplasm. The specific objectives of this study were:

1. To evaluate agronomic performance of provitamin A maize hybrids under abiotic stress and optimal conditions and select promising genotypes with enhanced grain yield and provitamin A concetration

2. To estimate heterosis and combining ability of provitamin A and drought tolerant inbred lines for grain yield, and agronomic traits under abiotic stress and optimal conditions and identify lines and testers to use for breeding provitamin A rich maize cultivars

3. To study genotype-environment interaction (GEI) and stability analysis for grain yield in the single cross hybrids produced from CIMMYT provitamin A and drought tolerant inbred lines under abiotic stress and optimal conditions and identify stable high yielding hybrids with high provitamin A concentration for specific environents or diverse environments

4. To determine genotype by environment interaction and stability analysis for provitamin A carotenoids under different abiotic stress and optimal conditions in provitamin A rich maize genotypes and identify the best environment for production of provitamin A rich maize

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5. To determine the relationship between provitamin A carotenoid concentration and grain yield and texture and find out if it is possible to improve the traits simultaneously.

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CHAPTER 2

LITERATURE REVIEW

2.1 Abstract

Micronutrient deficiency, including vitamin A, has been identified as a major health problem affecting about 50% of the world population and with a greater impact on developing countries whose diets are mainly cereal-based. Most strategies to improve mineral nutrition have been less successful because of political, socio-economic, infrastructure-related and technical constraints which are a common feature in most developing counties. Biofortification of staple crops like maize has been proposed as one of the most cost effective and feasible approaches to combat this problem. Both animal and human studies have shown that provitamin A from biofortified crops is highly bioavailable and has the capacity to improve vitamin A status of vulnerable groups. Since most people subsist on maize in the sub-Saharan Africa region, which is heavily affected by vitamin A deficiency especially among children and pregnant women, it should be the ideal source of vitamin A. With the exception of golden rice which is transgenic, the rest of the biofortified crops have received considerable acceptance by most communities. Negative perceptions associated with yellow maize does not affect orange maize, which is, for example, well-liked in rural Zambia. With proper policy frameworks and full commercialization of provitamin A maize, such as encouraging farmers to start large scale production and consumption, provitamin A maize can address the problem of vitamin A deficiency among poor nations with maize-based diets.

2.2 Introduction

Several global demographic health surveys estimate that one third of the world's population does not meet their physical and intellectual potential because of vitamin and mineral deficiencies. Vitamin A deficiency affects about 50% of the world population, with a much greater impact in developing countries whose diets are mainly cereal-based (Graham et al., 2001; WHO, 2010). It has been identified as a major health problem among low and middle income countries (West and Darnton-Hill, 2001; Zimmermann and Qaim, 2004; Naqvi et al., 2009; Meenakshi et al., 2012). Vitamin A is an essential

nutrient, which is generally provided by retinyl esters in meat and dairy products and provitamin A carotenoids in plants (Chao et al., 2011). Globally, vitamin A deficiency places 140 to 250 million people at risk for a number of health problems (Harjes et al., 2008). Vitamin A malnutrition leads to night blindness and increases the risk of child and maternal mortality (WHO, 2010) and also weakens the immune system of children, thus exposing them to other opportunistic diseases. The World Health Organisation (WHO) estimates that deficiencies in vitamin A rank among the top 10 leading causes of death in developing countries through several diseases (WHO, 2002; WHO, 2009).

Vitamin A malnutrition is estimated to affect approximately one third of children under the age of five around the world (WHO, 2002; WHO, 2009) and is estimated to claim the lives of 670,000 children under five annually (Black et al., 2008). Approximately 44 - 50% preschool children in the South Asian region suffer from severe vitamin A malnutrition (WHO, 2009). More than half of preschool children in Zambia are at risk of vitamin A deficiency (Micronutrient Initiative, 2009) and it accounts for 6% of all deaths and 5% of the total disease burden among preschool children (Black et al., 2008). Sixty four percent of 1 to 9 year old children in South Africa suffer from vitamin A deficiency (Labadarios et al., 2007). Thirty-four percent of women of child-bearing age, 35% of children under 5 years of age and 18% of school going children (between 6 and 14 years) in Zimbabwe are vitamin A deficient with serum retinal levels below 0.70 µmol dl^{-1} (Muzhingi, et al., 2008). Unfortunately, most people affected by vitamin A malnutrition do not show clinical symptoms, nor are they themselves aware of the deficiency, a phenomenon called "hidden hunger" (World Food Programme, 2006). Vitamin A malnutrition is more serious in populations subsisting on cereals and tubers as staple food crops because these food sources are deficient of provitamin A carotenoids.

Micronutrient deficiencies contribute to the degenerative cycle of poverty by limiting disposable income in households where people are too weak to work effectively because of hunger (WHO, 1999). Lack of adequate disposable income limits the capacity of parents to provide nutritious food to their families, leading to malnutrition, which negatively affects the health and normal development of children. Micronutrient deficiency prevalence is increasing due to a reduction in food diversity as a result of the effect of population increase on land pressure. As pressure for land increases, people tend to concentrate on energy giving crops, mostly cereals, because they are the most

productive, reliable and profitable (Welch and Graham, 1999). Unfortunately, cereals are poor in micronutrients. Various non-governmental organisations (NGOs) proposed the use of nutrition gardens to address the problem of micronutrient malnutrition. However, the initiative is limited by lack of land, space and water for irrigation due to frequent droughts. In urban areas, low income earners have no access to land for vegetable gardens and they normally buy vegetables. This results in insufficient vegetables being consumed to meaningfully address the problem of vitamin A deficiency. This leaves biofortification of staple food crops as one of the few sustainable ways of alleviating vitamin A deficiency, particularly on a large scale (Hotz and McClafferty, 2007).

Industrial fortification of maize flour only benefits the urban populations who buy processed maize meal and is of little benefit to rural people who generally take their maize to the mill, without any additions. Furthermore, during times of economic challenges, companies do not normally fortify maize meal, since it increases the production costs. Currently, maize meal fortification is not a policy in many countries in Africa. Companies that fortify maize meal in countries like Zimbabwe do it as a marketing strategy, and they normally receive very little support, if any, from government. There is very little nutritional education from the Ministry of Health of several developing countries and other public health practitioners to educate the people to buy the fortified maize meal, which at times is slightly more expensive than unfortified meal. African governments put more emphasis on supplementation, which unfortunately is not very sustainable because of poor funding, governance and infrastructure (Graham et al., 2001). In most cases the supplementation is donor driven, which is again unsustainable and unreliable. The most cost effective and feasible approach to combat the detrimental effects of dietary deficiencies in sub-Saharan Africa is therefore to biofortify the staple food crops. Biofortification is a process by which crops are purposefully bred for higher nutritional density (Graham et al., 2001; Fraser and Bramley, 2004). The aim of this review is to uncover the value of maize as candidate for biofortification.

2.3 Importance of maize

Maize is a very important crop in the world because of its various uses as a food crop, animal feed and as an important raw material in industry (Vasal, 2000; Prasanna et al., 2009). It is a staple food for more than 1.2 billion people in sub-Saharan Africa and Latin

America and is regarded as a vital crop in the perspective of global nutrition (IITA, 2010; Nuss and Tanumihardjo, 2010). Maize is very important for food security in Southern Africa accounting for an average of 36% of all caloric intake in the region (Grant et al., 2012). It is important both as a human staple and as animal feed and is often used as an infant weaning food by resource poor households, with no additional animal products. In many countries, the crop can be an ideal source of dietary supplement, as it provides carbohydrates, proteins, iron, vitamin A (yellow and orange maize only) and B (except vitamin B12) and some minerals to the human diet. Most food stuffs that contain vitamin A, such as fruits, vegetables and animal products, are expensive and most poor households cannot afford them, but all households take at least three meals of maize meal per day (Mashingaidze, 2004).

2.4 How can vitamin A deficiency be addressed?

Because of the significant importance of maize as a basic staple food for large population groups, particularly in developing countries, and its low nutritional value, including micronutrient deficiency, many efforts have been made to improve its nutritional value (FAO, 1992). In the developed world, micronutrient deficiency is addressed by diet diversification, food fortification and supplementation. All people must have access to a varied diet, rich in fruits and vegetables but this is, however, limited by seasonality of crops, affordability and low bioavailability of green leafy plant carotenoids (van Lieshout et al., 2001; West et al., 2002). Diversification, fortification and supplementation are, unfortunately, less effective in developing countries because of insufficient funding, poor governance, poor distribution networks (FAO/WHO, 2001), political, socio-economic and technical constraints that are prevalent in these counties (Darnton-Hill and Nalubola, 2002).

Micronutrient fortification is the deliberate addition of micronutrients that are essential into food products during processing. Processing is not widely recognized as a means of improving nutritional value. There have been many efforts to fortify maize, with outstanding results, but unfortunately fortification has not been implemented widely (FAO, 1992). This approach, however, may become important in the future as more people consume industrially processed foods, which can be more easily and efficiently fortified. About 25% to 50% of additional Vitamin A in the diet of the average European

comes from fortified food products (UNICEF, 2009).

Dietary diversification leads to consumption of foods rich in vitamin A (World Food Program, 2006). Non-animal sources of vitamin A which contain pre-formed vitamin A, account for more than 80% of intake for most individuals in the developing world (UNICEF, 2009). Increase in consumption of vitamin A rich animal products, vegetables and fruits can prevent vitamin A malnutrition. However, many people in the drier parts of Zimbabwe do not have access to fresh vegetables and fruits throughout the year (Gadaga et al., 2009). Educating people on the value of vitamin A rich foods also helps the population to appreciate the value of some foods they think are not important (UNICEF, 2009).

As an oral form, the supplementation of vitamin A is effective for lowering the risk of morbidity, especially from severe diarrhoea, and reducing mortality. Studies have shown that vitamin A supplementation of children under five who are at risk of vitamin A malnutrition can reduce mortality by 23% (Beaton et al, 1993). High dose of vitamin A supplementation given to lactating mothers in the early months can provide the breast fed infant with an appropriate amount of vitamin A through breast milk. However the coverage rate of supplementation is disappointing. Vitamin A supplementation coverage rate (% of children ages 6-59 months) in Zimbabwe was 34% in 2013, its highest value over the past 11 years was 83% in 2007, while its lowest value was 20% in 2004 (World Bank, 2013).

Although environment and cultural practices may be partly responsible, the variability of various chemical compounds is of genetic origin; thus composition can be changed through appropriate manipulation. Hence crops can be bred to increase the nutritional value, a process called biofortification. However efforts in this direction have concentrated on carbohydrate composition and on quantity and quality of oil and protein. Staple crop biofortification is thus a promising and potentially feasible intervention to alleviate micronutrient deficiency in developing countries (Combs et al., 1996; Welch et al., 1997; Graham et al., 2001).

2.5 Maize as a source of provitamin A carotenoids

Clinical studies by Tang et al. (2009) and Li et al. (2010) have shown that β-carotene biofortified rice (Golden rice) as well as β-carotene biofortified maize are effective sources of provitamin A. Taking from these observations improvement in provitamin A content of yellow maize has received increased interest in recent years in an effort to overcome vitamin A malnutrition resulting from the consumption of white maize in poor communities who cannot afford animal products, and sufficient fruits and vegetables. Maize grain carotenoid concentrations are among the highest produced in cereals (Howitt and Pogson, 2006) and display considerable natural variation for carotenoid composition, including vitamin A precursors α-carotene, β-carotene, and β-cryptoxanthin (Harjes et al., 2008). Yellow or orange maize contains a significant level of provitamin A carotenoids in the endosperm (Buckner et al., 1990). In yellow and orange maize, provitamin A carotenoids include α-carotene, β-carotene and β-cryptoxanthin, but concentrations are low, ranging from 0 to 1.3, 0.13 to 2.7 and 0.13 to 1.9 nmolg^{-1} respectively (Kurilich and Juvik, 1999). Recently developed orange maize varieties have β-carotene levels of about 15 µg g^{-1} (HarvestPlus, 2007) and even as high as 25 µg g^{-1} (USDA, 2007). This can support about 57% of daily needs of vitamin A required by human beings (Pixley et al., 2010).

Plant-based carotenoids are widely recognized for their antioxidant and nutritional value, which include provitamin A activity (Johnson, 2002). Upon symmetrical breakdown, provitamin A carotenoids produce one or two retinyl groups which are the structural base for vitamin A molecules. There is no limit to the amount of plant based carotenoids that can be safely taken in contrast to the toxic levels caused by excessive intake of vitamin A (Tanumihardjo, 2008).

Chao et al. (2011) proposed that the potential impact of carotenoid enhancement should be judged against benchmarks, which include the importance of particular crops in terms of global food security and the amount of food that must be consumed to achieve the reference daily intake of vitamin A. Seventy-seven percent of maize produced in sub-Saharan Africa (except South Africa) is used for human food and only 12% serves as animal feed (Grant et al., 2012). Maize is consumed in large quantities, about three times a day in many settings in Africa, Latin American and Asia (Rooney and Serna-Saldivar, 1987; Rooney and Serna-Saldivar, 2003; Mashingaidze, 2004) and is often used as an

infant weaning food in many countries in sub-Saharan Africa (Menkir et al., 2008).The dietary habits of many Africans where maize is consumed for all three meals a day, makes maize a good candidate for biofortification (Li et al., 2007). Shifting consumption from white maize to provitamin A orange maize would reduce vitamin A deficiency among vulnerable children and expecting mothers, without problems of overdose because the body would regulate how much provitamin A to convert into vitamin A (Lindqvist and Verba, 2009). This will take advantage of the consistent daily consumption of large amounts of staple food crops by the poor, especially women and children, who are most vulnerable. This provides a low cost option for preventing or controlling vitamin A deficiency among the groups (Chowdhury et al., 2009).

2.6 Status and acceptance of provitamin A maize in Southern and Eastern Africa

The sub-Saharan region of Africa is a leader in the cultivation and consumption of white maize (IITA, 2010) which lacks provitamin A carotenoids (FAO, 1992; Johnson, 2000). It is unfortunate that yellow maize is unpopular among consumers in southern Africa (Muzhingi et al., 2008) and is presumed to have little or no human consumption demand (Rubey, 1993). Rich in oils, carotenoids and fructose, yellow maize easily goes rancid and produces undesirable odours and flavour. It is also commonly perceived as a "poor man's" grain because it is associated with food aid (Tschirley and Santos, 1994; Muzhingi et al., 2008). Although people prefer white maize over yellow maize, there is little evidence of differences in taste and processing qualities between yellow and white maize, except that coloured varieties are often flint, which is actually often associated with favourable cooking and processing characteristics (De Groote and Kimenju, 2008).

Consumer acceptability plays a crucial role for provitamin A maize to be meaningful in alleviating vitamin A deficiency in maize-based diets. Although biofortified staple foods are inexpensive, locally adapted and offer long term solution to diet deficiencies, cultural preferences may limit their acceptance (Harjes et al., 2008). Muzhingi et al. (2008) found that nutritional education can potentially counter the negative perception on yellow maize consumption in countries like Zimbabwe, especially if targeted at low income household level. This will make people benefit from the nutritional value of orange maize and reduce vitamin A malnutrition.

Literature comparing acceptance of white maize with orange maize is limited. Most studies are comparing white maize with yellow maize except one study by Stevens and Winter-Nelson (2008), which includes white, yellow and orange varieties of maize. The study by Muzhingi et al. (2008) on consumer acceptability of yellow maize in urban and rural Zimbabwe found that more than 94% of households were willing to consume yellow maize if they knew it was more nutritious than white maize. Meenakshi et al. (2010) found that the negative perception associated with yellow maize does not affect orange maize which is well liked in rural Zambia. Nuss et al. (2012) also observed quick adoption of orange maize in Zambia in the form of thin and thick porridge. A successful intervention to introduce β-carotene rich, orange sweet potato in Mozambique, where only white sweet potato was previously cultivated, suggests that orange-coloured staple foods can be acceptable, and their regular consumption results in improved vitamin A status (Howe and Tanumihardjo, 2006).Orange-fleshed sweet potato production and use also spilled into neighbouring countries such as Zimbabwe, Malawi and South Africa. Results on acceptability of orange maize research in Mozambique suggest that orange maize meal may be as preferred as white and that no price discounts are likely to be necessary to promote its consumption. In addition, families with young children and those that do not consume diets rich in animal products are more likely to accept orange maize. Because of the perceived acceptability and the potential to address vitamin A deficiency among poor households, Tumuhimbise et al. (2013) suggested that it is time to fully commercialise provitamin A crops by encouraging farmers to start large scale production and consumption.

For provitamin A to be widely accepted there is need to develop strategies for taking the information of its benefits to the target people through nutritional education and advocacy. There is also a need to make it more available in shops and price it strategically lower than white maize, to make it affordable for the poor communities (Pillary et al., 2011), like what South Africa did with its nutritionally fortified brown bread. Unfortunately pricing it lower than white maize may result in stigma, as people might view it as food for people who cannot afford to pay a premium price for white maize. So this approach needs to be seriously scrutinized. It might be more effective to price it the same but intensify nutritional education on the benefits of eating orange maize.

Several authors suggested that nutritional information can influence consumer acceptance of orange maize. Muzhingi et al. (2008) found that nutritional information is the single most important factor in determining a household's decision to purchase nutritionally enhanced maize; a nutrition campaign can significantly alter consumers' perceptions and lead to a much higher probability that non-white maize would be consumed. A study done in Nairobi gave evidence of a substantial reduction in the discount for commercially fortified yellow maize flour as a consequence of nutrition education (De Groote and Kimenju, 2008). They found that while there is an interest in commercially fortified maize, the average premium for fortification is less than half the discount on yellow maize. However, what is worrying is the observation that poorer people tend to place lower premiums on nutritionally enhanced foods (Morawetz et al., 2006; De Groote and Kimenju, 2008) as they seem to be more worried about addressing the caloric needs. This may mean pricing orange maize cheaper than white one might make it adopted by the poor households wanting to address energy need and subsequently address vitamin A deficiency at the same time.

Effective nutrition campaigns can be conducted using various methods which include food labelling, mass media, theatre and community leaders. In developed countries, health information is typically conveyed through the use of written labels and the literature suggests that premiums for health labelling can be significant (Kinnucan et al. 1997). The use of labelling is not practical in poor communities, given low levels of literacy, costs of labelling, and the fact that maize is sold from farmer to farmer. Using community leaders, extension workers, non-governmental organisations (NGOs) and radio messages for conveying health information is more realistic in this case (Zimicki, 1997).

There is also need to address the problem of unacceptable organoleptic properties of yellow maize (Muzhingi et al., 2008) to enhance its acceptability. This can be achieved through breeding by strategically selecting against high oil content, which causes the crop to quickly go bad if not properly stored. There is also a need to carry out research on the best on-farm storage conditions for the crop; so that it will not lose its provitamin A carotenoids due to degradation and also that it will not produce undesirable characteristics in storage.

To enhance acceptability by farmers there is need also to research the agronomic performance and stability of provitamin A maize across varying environments, so that the provitamin A maize varieties will not yield less than varieties currently grown by farmers. Provitamin A maize hybrids developed in Zambia are agronomically sound. Li et al. (2007) reported that the positive nutritional and acceptance results observed will need to be coordinated with comprehensive breeding and seed distribution efforts to realize the potential of provitamin A biofortified maize. One possibility is to counter negative perceptions of increased β-carotene content with other new traits that farmers find useful. Governments may also subsidise the production of provitamin A maize to encourage cultivation and consumption by resources-poor consumers. Economists typically assume that adoption by farmers is *prima facie* evidence that it provides them with benefits (Dawe and Unnevehr, 2007).

In order to have maximum impact on biofortification, high yielding varieties are needed to convince the poor farmers to grow it, even though the target consumer is in no position to pay a higher price for quality. Researchers found that it is possible to combine the high-density trait with high yield, unlike protein content and yield that are negatively correlated. The micronutrient traits are stable across environments and the genetic control is relatively simple (Suwarno et al., 2014). For example, high iron density in rice is linked closely with aromaticity, a single gene trait, making selection easy in early generations (Graham et al., 1997). Given equal or superior agronomic performance of the orange maize varieties, they may attract a premium in the market. Consumer education, extension, properly designed policies encouraging adoption, mitigating the higher seed cost, and lessons learned from sweet potato are all part of the desired policy mix to enhance adoption (Tumuhimbise et al., 2013).

2.7 Effect of maize storage and processing on retention of carotenoids

Maize can be stored from one harvest to the next, which is equivalent to 6-12 months, depending on the number of growing seasons per year in any given agro-ecological zone. The highly unsaturated structure of carotenoids makes them susceptible to post harvest degradation by heat, oxygen and light (De Moura et al., 2013). The mechanisms of carotenoid degradation may involve: the reaction of carotenoids with heat (thermal degradation), atmospheric oxygen (auto-oxidation) and light (photo degradation), as well

as degradation by the interactions of carotenoids with singlet oxygen, acid, metals and free radicals. In food systems, the degradation mechanisms are more complex (Boon et al., 2010). After 6 months of storage, Weber (1987) reported the average total carotenoid retention among four inbred lines of maize as 58%. The genotype with the lowest initial total carotenoids content (27.4 µg g^{-1}dry weight) showed the highest retention (67%). Burt et al. (2010), while studying two genotypes over a period of 18 months, observed that total carotenoids remained constant for the first three months and declined significantly by six months and then remained stable, giving a total loss of 35-40%. There is a need to carry out more research on the effect of drying on carotenoid retention in maize. There is also a need to evaluate different genotypes under different storage conditions including storing at room temperature, on-farm storage, storage in darkness and under light conditions. Burt et al. (2010) stated that the understanding of genotype effect has the potential to guide the development of high carotenoid maize inbred lines with good stability during drying and storage.

The carotenoids are stored in the endosperm; therefore they are less subjected to milling losses. Milling provitamin A biofortified maize into mealie meal resulted in a higher retention of carotenoids compared to milling into samp (Pillary et al., 2011). This is very encouraging because most poor households consume maize in the form of porridge made from mealie meal. However, the study demonstrated that provitamin A retention in maize is affected by the cooking method and therefore cooking methods that result in a good retention of provitamin A need to be identified and recommended. Their research further observed that fermentation does not adversely affect the retention of provitamin A carotenoids in porridges prepared with high β-carotene maize.

2.8 Provitamin A maize breeding

To draw maximum benefit from agricultural research as a vehicle for addressing public health issues, in July of 2003 the Consultative Group on International Agricultural Research (CGIAR) established HarvestPlus: the Biofortification Challenge Program (BCP), adding food quality to its agricultural production research programme (Pixley et al., 2010). Biofortification research is a comprehensive programme that spans from genetic crop improvement to research on the impact of biofortified crops on human health (Haas et al., 2005; van Jaarsveld et al., 2005) and is conducted mainly under the auspices

of HarvestPlus. The focus of HarvestPlus is three micronutrients, iron, zinc and vitamin A that have been identified by WHO as limiting for most poor households. Scientists generally agree that exploiting the genetic variation in crop plants for micronutrient density is one of the most powerful tools available to change the nutrient balance of a given diet on a large scale. With this realisation the BCP was set up to carry out research on possible ways to address iron, zinc and vitamin A deficiency among poor people.

Maize displays considerable natural genetic variation for carotenoid composition, with some lines accumulating as much as 66 mg g^{-1} (Harjes et al., 2008), which means selection for provitamin A in maize is possible. Researchers at the Agricultural Research Service of the USA identified genetic sequences in maize associated with higher levels of β-carotene (vitamin A precursor). It was discovered that breeders can crossbreed certain variations of maize with the aim of producing a crop with an 18-fold increase in β-carotene (USDA ARS, 2010).

Historically, work on maize carotenoids has been limited to available material rather than germplasm developed for high carotenoid levels (Kurilich and Juvik, 1999). Of interest now is research on improving carotenoid content, which has been conducted by several groups. For example, Egesel et al. (2003) were able to determine combining ability for several Corn Belt dent inbred lines and discovered that selection for improved carotenoid content can yield improved varieties. Suwarno et al. (2014) found that provitamin A concentration in maize is controlled primarily by additive gene action. However, the significant environmental effects for total provitamin A concentration they observed represents a challenge to developing cultivars with widespread impact on vitamin A malnutrition. Harjes et al. (2008) demonstrated the power of targeting specific steps of the metabolic pathway in order to achieve the desired carotenoid profile and high β-carotene maize and they showed the potential for marker assisted selection to improve carotenoid content within the breeding pools. Early research had indicated that maize carotenoid content in the grain varies considerably and breeding maize for high provitamin A is possible. Genetic variation for specific carotenoid content has been reported in maize lines adapted to the tropics (Harjes et al., 2008; Menkir et al., 2008).

Experimental evidence from association and linkage populations in maize demonstrates that the gene encoding β-carotene hydroxylase 1 (*crtRB1*) underlies a principal

quantitative trait locus (QTL) associated with β-carotene concentration and conversion in maize kernels (Babu et al., 2012). The *crtRB1* alleles associated with reduced transcript expression correlate with higher β-carotene concentrations (Yan et al., 2010). These alleles are rare in frequency and unique to temperate germplasm, but CIMMYT has successfully introduced the alleles into some tropical germplasm. The implementation of this programme requires backcross selection to convert African adapted white germplasm to orange (Pixley et al., 2011; Chandler et al., 2013). Under the best scenario, the *crtRB1* gene variations can increase concentration of β-carotene from a little above zero, to about 57% of the micronutrient target (15 mg g^{-1} β-carotene) (Babu et al., 2012). HarvestPlus has determined that this would improve poor people's nutrition and health (Pixley et al., 2010).

Suwarno et al. (2014) also conducted a study to assess the heritability of visual scores for relative intensity of orange kernel colour and they identified genetic markers associated with orange colour. They identified visually scored kernel colour to have a moderately high heritability and identified five common QTLs and six rare QTLs for intensity of orange colour. Notably, half of them coincided with carotenoid biosynthetic genes. Their results indicate that breeders can have flexibility to select for orange kernel colour visually and/or with gene-specific markers. The moderately high heritability of visual scores for relative intensity of orange kernel colour indicates that this trait should respond favourably to phenotypic selection. The identification of strong positive effect QTLs in the vicinity of carotenoid biosynthetic pathway genes *y1*, *zds1*, and *lcyE* implies that phenotypic selection for dark orange colour will likely result in higher amounts of total carotenoids in the maize kernel. However, the discovery of only weak positive effect QTLs in close proximity to *zep1* and *ccd1* underscores the need to further explore and characterize genetic diversity at these two loci and search for more favourable alleles (Suwarno et al., 2014).

The absence of vitamins in cereals means the corresponding metabolic pathway is absent, truncated or inhibited in the endosperm, hence genes encoding enzymes free from feedback need to be introduced (Christou and Twyman, 2004; Zhu et al., 2007). Maneesha et al. (2008) found that over-expression of the bacterial genes *crtB* (for phytoene synthase) and *crtl* (for the four desaturation steps of the carotenoid pathway catalysed by phytoene desaturase and ζ-carotene desaturase in plants), under the control

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of 'super γ-zein promotor' for endosperm expression, resulted in an increase of total carotenoids of up to 34-fold with preferential accumulation of β-carotene in the maize endosperm. The β-carotene trait was found to be reproducible over at least four generations.

Phenotypic selection for orange colour should be effective, simple and low cost for converting white or yellow grain maize germplasm to orange. To further enhance β-carotene levels, this phenotypic selection could be combined with marker assisted selection for favourable *crtRB1* alleles. Genotyping single kernels and selecting for favourable alleles at the six loci would further expedite breeding efforts by assuring that the most desirable genotypes are selected before planting in winter or summer nurseries (Suwarno et al., 2014). They proposed that this approach could be combined with a strategy that uses a set of genome-wide markers to rapidly select against the undesirable genetic background of less adapted or lower yielding orange donor lines.

Although current genetic results and strategy are encouraging, they need to be placed in context as part of an overall biofortification effort encompassing breeding infrastructure, seed distribution, societal acceptance, dietary habits and nutritional impact. Available information on some of these issues is encouraging (De Groote and Kimenju, 2008). A plant breeding strategy, if successful, will not eliminate the need for supplementation, fortification, dietary diversification and disease reduction programmes in the future, to combat micronutrient malnutrition. Nevertheless, this strategy does hold great promise for significantly reducing recurrent expenditures required for these higher costs short-run programmes by significantly reducing the numbers of people requiring supplementation.

The importance of provitamin A maize varieties as sources of vitamin A in a breeding programme also depends on the stability of expression of these compounds across different growing conditions. Limited information is available on the effect of different growing conditions and its interaction with the genotype on provitamin A carotenoids content in sub-Saharan Africa.

2.9 Conventional versus transgenic breeding on maize biofortification: challenges and opportunities

One of the significant disadvantages of conventional breeding compared to transgenic strategies, is its reliance on alleles already in the species gene pool (Zhu et al., 2007). But the major advantage of conventional breeding is that it uses intrinsic properties of the crop. As a result there are few regulatory requirements. The dependence on the existing gene pool will mean that a long time is needed to develop a new variety. Traits might need to be introgressed from wild relatives, which might take a longer period of time. Because of these setbacks, biofortification programmes based on conventional breeding have met marginal success (Naqvi et al., 2009). For example, polymorphism at the *lcye* locus in maize was shown to alter the flux between the α-carotene and β-carotene branches of the carotenoids pathway, potentially allowing breeding for enhanced β-carotene levels (Harjes et al., 2008). However, such a QTL based approach would require years of conventional breeding to achieve a significant enhancement in locally adapted lines grown by subsistence farmers in the developing world.

Gene expression analysis suggests that increased accumulation of β-carotene is due to an up-regulation of the endogenous lycopene β-cylase. This set the stage for designing transgenic methods to generate provitamin A-rich maize. Modification of *crtl* as well as phytoene synthase (*PSY)* boosts kernel provitamin A content in maize (Maneesha et al., 2008). Transgenic approach advantages are rapid; unconstrained by a genepool; target expression in inedible organs and are applicable directly to elite lines. However, for transgenic biofortification strategies to be successful, there are also regulatory and public perception issues to overcome, such as the current negative perception of genetically modified (GM) foods in most developing countries. These should be addressed purely through science-based analysis and divorced from socio-political and regional economic interests, for example, through the oversight of independent, NGO-sponsored panels (Zhu et al., 2007).

NGOs have a lot of influence on adoption of technologies by farmers and consumers. These NGOs are unlikely to support GM maize because they mostly advocate organic farming. The NGO's objections are due to ethical or ideological considerations, not scientific scepticism (Dawe and Unnevehr, 2007). So there is need for educational campaigns targeting farmers and the general public. Most importantly, nutritionally

enhanced crops should be available to those most in need without intellectual property constraints and licensing restrictions, which are often in place for commercial use in the western communities.

Transgenic approaches offer the most rapid way to develop high-nutrient commercial lines. Transgenic strategies differ from other approaches in that novel genetic information is introduced directly into the plant's genome (Zhu et al., 2007). The best biofortification strategies should include genetic engineering in conjunction with conventional breeding, particularly when the direct enhancement of local lines is required (Naqvi et al., 2009). However, adoption will depend on legalisation of GM organisms. From a political standpoint, in Africa and Asia, there are still very few advocates for GM technology. Government agents mostly promote organic farming. Any biofortification effort needs the support of National Research Centres and NGOs in order to be designed and targeted appropriately. If NGOs who work with the poor households embrace it, it will be easily accepted. Any biofortification of a staple crop using GM technology will encounter greater political resistance, as well as more challenges in safety assessments and delivery, than non-GM approaches.

While conventional breeding will most likely never allow us to reach the high levels possible with transgenics, it can achieve the substantial and important increments necessary to improve human nutrition (Pixley, 2010). In the case of maize, CIMMYT and other HarvestPlus partners are developing high provitamin A maize lines and have identified lines with 15 ug g^{-1} of provitamin A. This is far lower than the 60 ug g^{-1} achieved transgenically, but it is much higher than the 0 ug g^{-1} found in white maize, or 1.5 ug g^{-1} common in yellow maize (Pixley, 2010). Naqvi et al. (2009) developed elite inbreds in which three vitamins were increased, specifically in the endosperm, through simultaneous modification of three separate pathways. The kernels produced contained 169-fold the normal amount of β-carotene, six-fold the normal amount of ascorbate and double the amount of folate. The vitamins produced remained stable up to the T$_3$ homozygous generation. This means the development of nutritionally complete cereals to address deficiency of important nutrients in resource-poor households is possible.

In Zambia, where conventionally-bred provitamin A maize was first released, HarvestPlus has estimated that up to 50% of the recommended dietary allowance for

vitamin A could be met by eating 15 ug g^{-1} HarvestPlus orange-maize instead of non-biofortified lines. Transgenic maize, which can have up to 169 fold of provitamin A carotenoids, can address 100% of daily needs of vitamin A. This suggests that increasing the amount by using transgenic approaches could meet 100% of the recommended dietary allowance. Additionally, other micronutrients not present in maize could also be incorporated using transgenic approaches. This may be true, but once a transgenic line is obtained, at least three expensive and time-consuming actions are required; several years of conventional breeding are needed to assure that the transgenes are stably inherited and that it does not result in inadvertent, undesirable associated effects. Concurrent conventional breeding is needed to incorporate the transgenic line into varieties which also perform well agronomically, so that famers will want to grow it. There is also an expensive and lengthy process of research to document and defend the human and environmental safety of transgenic crops, and to obtain the legal approvals to release and commercialize these lines (Pixley, 2010). Furthermore, in quite a number of countries there currently is no legal framework that allows commercial release of transgenic maize varieties. Thus, while transgenic approaches are clearly the fastest way to "prove the concept" that biofortified crops, and in this case multi-micronutrient-biofortified crops, are possible, they may not be the quickest to obtain usable products and desired impact.

2.10 Analysis of provitamin A carotenoids to support breeding

Accurate assessment of provitamin A carotenoids in maize must be performed to direct breeding efforts. Carotenoid analysis of foods is inherently difficult due to a large number of naturally occurring carotenoids, highly variable composition of foods, wide ranges of carotenoid concentrations, and isomerization and degradation of carotenoids prior to and during analysis. Breeding for carotenoids requires a high throughput screening method, which is cheap to run. If the colour intensity has a link with the amount of provitamin A carotenoids, rapid screening will be possible, since genetic gain in carotene content will be visually estimated with accuracy (Simon, 1992). There is need to do profiling research to see the relationship of colour intensity and carotenoids concentration of grains, so that breeders can exploit this for selection for high carotenoid genotypes. Selection indices are very often used in selection for high-yielding genotypes under drought stressed and irrigated conditions (Cattivelli et al., 2008). However, colour intensity might give an

indication of total carotenoid content only, without indicating the amount of provitamin A carotenoids and other carotenoids, including non-provitamin A.

2.11 Bioavailability of provitamin A carotenoids from maize

Both animal and human nutrient studies have shown that provitamin A from biofortified crops is highly bioavailable and has the capacity to improve vitamin A status. The total amount of a micronutrient in plant food does not represent the actual micronutrient content of the food which is utilizable by the consumer. In human nutrition terms, bioavailability is commonly defined as the amount of a nutrient in a meal that is absorbable and can be utilized by the person eating the meal (van Campen and Glahn, 1999). Total nutrient concentration is not the objective, but utilizable nutrient in the human gut. Micronutrients can occur in various chemical forms of differing proportions in plant foods and their amounts vary depending on numerous factors including the growth environment, plant species, genotype, and cultural methods and management practices used to grow the plant. These forms have characteristically different solubilities and reactivities with other plant constituents and other meal components. There are multiple interactions occurring between micronutrients in plant foods and other plant substances once the food is consumed, such as with other interacting nutrients and chemical substances which can either inhibit (such as anti-nutrients) or enhance (such as promoters that can increase absorption and/or utilization) micronutrient bioavailability.

Additionally, many other interacting factors, both genetic and environmental, affect micronutrient bioavailability to the consumer, such as food processing methods, meal preparation techniques, and an individual's personal characteristics (such as gender, age, genetic predisposition, ethnic background, economic status, physiological state, nutritional and disease status). Thus, determining micronutrient bioavailability in plant foods is beset with difficulties and uncertainties (House, 1999). Reports (Garcia-Casal et al., 1998; Garcia-Casal and Layrisse, 1999; Layrisse et al., 1997; 1998) indicated that fortifying cereal-based diets with vitamin A or β-carotene and Fe(II)-fumarate, enhanced the bioavailability of the Fe to humans dramatically (e.g. β-carotene increased Fe bioavailability more than three fold in rice-based meals and more than 1.8 fold in wheat and corn-based meals). Li et al. (2010) found that β-carotene in biofortified maize has good bioavailability as a plant source of vitamin A. They observed a bioconversion ratio

of β-carotene to retinol of 7:1 and Muzhingi et al. (2011) observed a ratio of 3:1. Based on these results it can safely be concluded that biofortification of maize as an intervention to combat vitamin A deficiency among vulnerable groups is a feasible option.

Heying et al. (2013) observed that consumption of daily provitamin A carotenoids by sows during gestation and lactation increased liver retinol status in weanling piglets, illustrating the potential for provitamin A carotenoids consumption from biofortified staple foods to improve vitamin A reserves. They concluded that frequent intake of provitamin A carotenoids from biofortified maize may sustain adequate vitamin A status in deficient populations if widely adopted as their staple food. A study in India found a 54% reduction in childhood mortality in children who were given small weekly doses of preformed vitamin A, which represented achievable daily consumption amounts from food (Rahmatthullah et al., 1990). Biofortified maize adequately maintained vitamin A status in Mongolian gerbils and was as effective as β-carotene supplementation (Howe and Tanumihardo, 2006). They found that provitamin A carotenoids in maize are as bioavailable as β-carotene supplements in a vitamin A depleted gerbil model.

2.12 Genotype x environment iteraction

A good cultivar needs to posses high and stable yield potential over a wide range of environmental conditions (Eberhart and Russel, 1969; Wricke and Weber, 1986; Becker and Leon, 1988, Fasoula and Fasoula, 2002). The basic cause for differences between genotypes in their yield stability is a wide occurrence of GEI. The change in rank and the relative differences over a range of locations is defined statistically as GEI, which is a differential genotypic expression across environments (Becker and Leon, 1988; Sharma, 1998; Kang, 1998; Janick, 1999). The knowledge of GEI can help to reduce the cost of extensive genotype evaluation by eliminating unnecessary testing sites and by fine-tuning breeding programs. Various biotic and abiotic stresses have been implicated as causes of GEI. Improving genotype resistance/tolerance to different stresses to which they would likely be exposed might minimize GEI (Kang, 1998). Performance in a range of environments, both in favourable and stress environments is what is needed in a cultivar (Falconer 1989).

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2.13 Line x tester mating design

To breed high yielding varieties, breeders often face the problem of selecting parents and crosses. The line × tester analysis method introduced by Kempthorne (1957) cited by Sharma (2006) has been applied to provide a systematic approach for the detection of suitable parents and crosses for investigated characters. The mating design is one of the powerful tools available to estimate the combining ability effects and aids in selecting desirable parents and crosses for exploitation in pedigree breeding (Rashid et al., 2007; Basbag et al., 2007; Jain and Sastry, 2012). This design involves hybridization between lines (f) and wide based testers in one to one fashion generating f x m = fm hybrids (Sharma, 2006). It provides both full-sibs and half-sibs simultaneously (Nduwumuremyi et al., 2013). It is used in estimating various types of gene actions important in the expression of quantitative traits (Sharma, 2006; Rashid et al., 2007). Line x tester analysis provides information about general combining ability (GCA) and specific combining ability (SCA) effects (Sharma, 2006). GCA ability is attributed to additive type of gene effects, while SCA is attributed to nonadditive type of gene actions. Nonadditive action is not reliably fixable whereas additive gene action is reliably fixable (Xiang and li, 2001; Yan and Hunt, 2002).

2.14 Combining ability

Combining ability has been defined as the performance of a line in hybrid combinations (Kambal and Webster, 1965). The final evaluation of inbred lines can be best determined by hybrid performance, it plays an important role in selecting superior parents for hybrid combinations and in studying the nature of genetic variation (Hallauer and Miranda, 1988; Duvick, 1999. Sprague and Tatum (1942) introduced the concepts of GCA and SCA. They defined GCA as the average performance of a line in hybrid combinations, while SCA as those instances in which certain hybrid combinations are either better or poorer than would be expected of the average performance of the parent inbred lines included. GCA is associated with additive effects of the genes, while SCA is related to dominance and epistatic effects (non-additive effects) of the genes. Sprague and Tatum (1942) found that GCA was relatively more important than SCA for unselected inbred lines, whereas SCA was more important than GCA for previously selected lines for influencing yield. GCA effects quantitatively measure the comparative performance of parents and cross combinations in relation to one another.

2.15 Heterosis

Heterosis is the genetic expression of the superiority of a hybrid in relation to its parents (Miranda Filho, 1999). Several studies on maize have shown that inbred lines from diverse stocks tend to be more heterotic than crosses of inbred lines from same variety (Vasal, 1998). Saxena et al. (1998) also reported that manifestation of heterosis usually depends on the genetic divergence of the two parental lines. Based on parents used, two major types of estimation of heterosis are reported in literature: 1) Mid-parent or average heterosis (MPH), which is the increased vigor of the F1 over the mean of two parents; 2) High-parent or better parent heterosis (HPH), which is the increased vigor of the F1 over the better parent (Sinha and Khana, 1975; Jinks, 1983). For HPH, the term heterobeltiosis has been suggested to describe the increased performance of the hybrid over the better parent (Fonseca and Patterson, 1968). Maize hybrids typically yield two to three times as much as their parental lines.

Heterosis is dependent not only on the parent combinations but also on the effect of environmental conditions and species as well as the trait under consideration (Knight, 1973; Jinks, 1983; Chapman et al., 2000). Young and Virmani (1990), reported that the higher heterosis in rice under stress environment than in a favorable environment. Betran et al. (2003) reported extremely high expression of heterosis in maize under stress, especially under severe drought stress because of the poor performance of inbred lines under these conditions. There is a lack of information about the magnitude of heterosis in provitamin A hybrids developed from provitamin A rich lines and drought tolerant yellow lines when tested in different environments.

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CHAPTER 3

AGRONOMIC PERFORMANCE OF PROVITAMIN A MAIZE HYBRIDS UNDER ABIOTIC STRESS AND OPTIMAL ENVIRONMENTAL CONDITIONS

3.1 Abstract

Drought, heat and low soil fertility stress are among the most important abiotic stressors that reduce maize yields in sub-Saharan Africa. Thirty single cross provitamin A hybrids and five checks were evaluated for grain yield performance under different abiotic stress and optimum conditions in 2014 and 2015. The experiments were conducted in Zimbabwe under six different environmental conditions; drought, a combination of drought and heat, low N, low P, random drought stress and optimum conditions. Hybrids performed significantly different for grain yield, except under low N stress. Grain texture of the hybrids was also significantly affected by environment. There was a significant interaction between year, environment and genotype for grain yield but no interaction was observed for grain texture. The inconsistency in genotype ranking observed in this study points to the existence of genotype x environment interaction, hence there is need to carry out grain yield stability analysis to find stable hybrids. Since hybrids performed variably across environmental conditions, it is possible to select hybrids with good yield potential for specific target environments. Entry 8, which ranked in the top ten in all the environments with grain yield of 9.38 t ha^{-1}, 4.11 t ha^{-1}, 1.75 t ha^{-1}, 3.35 t ha^{-1}, 4.32 t ha^{-1} under optimum, random drought stress, managed drought stress, combined drought and heat stress and low N respectively, should be considered for release as a cultivar for all the conditions.

3.2 Introduction

Abiotic stresses such as drought, salinity, heat, N and P deficiencies, and aluminium (Al) toxicity, provide a major limitation to crop yield throughout the world (Cavatte et al., 2012). Bray et al. (2000) reported that these abiotic stresses account for yield reduction in annual crops of about 51-82%, depending on the crop and timing of the stress. Biologically, stress is considered as a significant deviation from the ideal conditions in which plants are grown, preventing them from expressing their full genetic potential for growth, development, and reproduction. Because of the present scenario of global climate

changes and considering that major advances in agriculture were designed for environments favourable to the ''Green Revolution'', crop performance under adverse conditions in marginal environments, which has often been overlooked, is currently the subject of constant debate (Cavatte et al., 2012).

Globally, 160 million ha of maize is produced under random drought stress conditions and annual yield losses to drought are estimated at around 25% (Edmeades, 2008) and are greater in subtropical countries that rely on erratic and unpredictable rainfall (Heisey and Edmeades, 1999; Mhike et al., 2011). Edmeades et al. (1999) estimated yield loss due to drought to be around 17% annually, with regional losses reaching 70% under extreme conditions compared to well-watered production. Drought reduced maize production in Zimbabwe by about 70% between 1981 and 1982 (Rukuni et al., 2006). In 2001-2003, drought left about seven million people malnourished in Zimbabwe, and the nation imported more than two million ton of maize (Rukuni et al., 2006). In the 2014/2015 season, maize yields in Zimbabwe were reduced by 49.04% from 1 456 000 t in the previous year to about 742 000 t because of a prolonged mid-season drought and during the 2015/2016 season, El nino induced drought reduced the yields by a further 52.83% to give an annual yield of 350 000 t (USDA, 2016). Between 2003 and 2005 the World Food Program spent US$ 1.5 billion to meet food deficiencies due to drought and crop failure in Africa (World Food Program, 2006). With most maize in the developing world grown under random drought stress conditions and the proportion of maize grown in marginal areas increasing, breeding for tolerance to drought and other abiotic stresses is now a major breeding objective of most breeding programmes in the world (Ndhlela, 2012).

Zimbabwe has in the last decade shown a remarkable shift in seasons, and annual rainfall reduction of about 5-20% from the 1961-1990 average (Chasi, 2008). This is set to further decrease due to global warming, which causes climate change. The Intergovernmental Panel on Climate Change (IPCC) Fourth Assessment Report (2007) and Zimbabwe's Initial National Communication on Climate Change (INC, 2010) suggest that by 2050 temperatures and rainfall over the country will be 2-4°C higher and 10-20% less than the 1961-1990 baseline, respectively. Battisti and Naylar (2009) also reported that by the end of the 21st century, temperatures during the normal growing season will be higher than the most extreme seasonal temperatures recorded in the previous 100 years. This calls for the need to develop cultivars that are tolerant to raised temperatures, to combat hunger.

Soil fertility degradation was also reported to be one of the most important constraints to food production in SSA, including Zimbabwe (Sanchez et al., 1997; Mapfumo and Giller, 2001). Seventy percent of Zimbabwe's soils are sandy and of granitic origin (Thompson and Purves, 1981; Nyamangara et al., 2000). They are inherently low in N, P and sulphur (Grant, 1981) as well as low in organic matter content (Giller et al., 1997) and prone to leaching. A map of the soils of Zimbabwe is shown in Figure 3.1. However, resource poor smallholder farmers still till these soils for cultivation of maize and other crops, with very little or no external nutrient additions (Mtambanengwe and Mapfumo, 2005). The effectiveness of applied fertilisers is also affected by deficiencies of micronutrients (Zingore et al., 2008) and low pH [$CaCl_2$] (< 5.0). Soil nutrient deficiencies can be addressed through application of inorganic fertilisers which are beyond reach of most resource poor smallholder farmers (Nyamangara et al., 2000).

Seventy-seven percent of soils in the smallholder sector of Zimbabwe are acidic and have a potential problem of Al toxicity (Nyamangara et al., 2000) which may override benefits from N and other nutrients. Fertiliser use in SSA is negligible, with smallholder farmers in Zimbabwe applying only 18 kg ha^{-1} of mineral fertiliser, which is far below the recommended 300 kg ha^{-1} (Murwira and Mukamuri, 1998). The farmers rely mostly on low input agricultural systems, which produce very little output, resulting in food insecurity (Mtambanenge and Mapfumo, 2005). Since SSA, including Zimbabwe is dominated by low-input agricultural systems; it is of paramount importance to develop maize hybrids that produce reasonable yields under these systems.

There is need to develop hybrids that are tolerant to all the prevailing stresses, to maximise farmer returns and for food security. The farmer wants cultivars that produce a satisfactory yield when subjected to stress conditions, but that have a high productivity under ideal growing conditions. The objective of this chapter was to evaluate the agronomic performance of provitamin A single cross hybrids across experimental sites under drought stress, heat stress, combined drought and heat stress, low N stress, low P stress, random drought and optimum growing conditions.

3.3 Materials and methods

3.3.1 Study sites

The trials were conducted in Zimbabwe, a developing country located between latitudes 15° and 22° south and longitudes 26° and 34° east (FAO sub-Regional Office for East and Southern Africa, 2000). The country was divided into five natural regions or agro-ecological regions (Figure 3.2) based on the amount of rainfall and agricultural productivity (Vincent and Thomas, 1961; Mugandani et al., 2012). The description of the natural regions is presented in Table 3.1. The specific study sites where trials were conducted are summarised in Table 3.2 and shown in Figure 3.2.
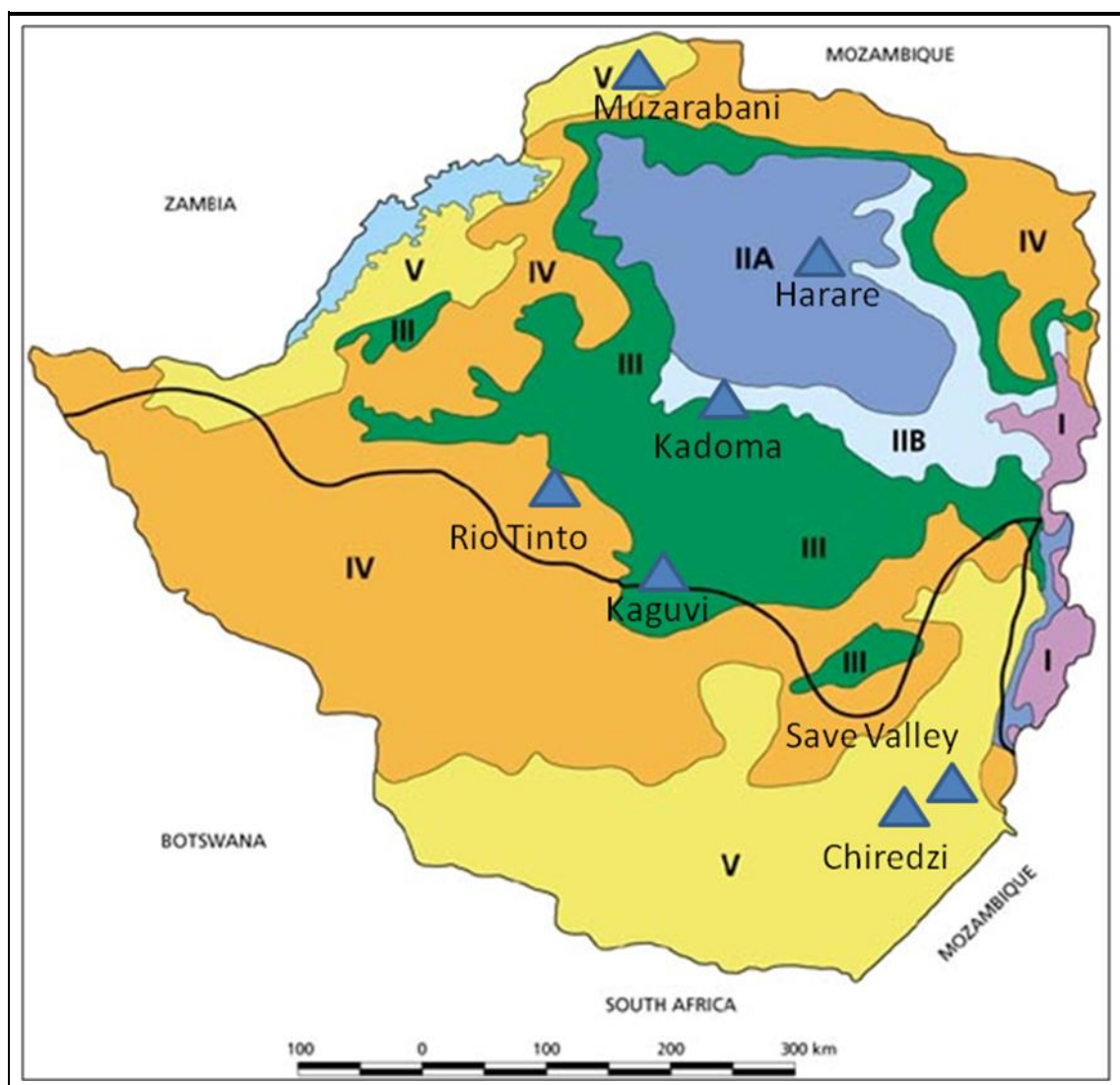
Figure 3.1 Natural regions of Zimbabwe map

Source:http://www.fao.org/docrep/009/a0395e/a0395e06.htm accessed 26/05/2016
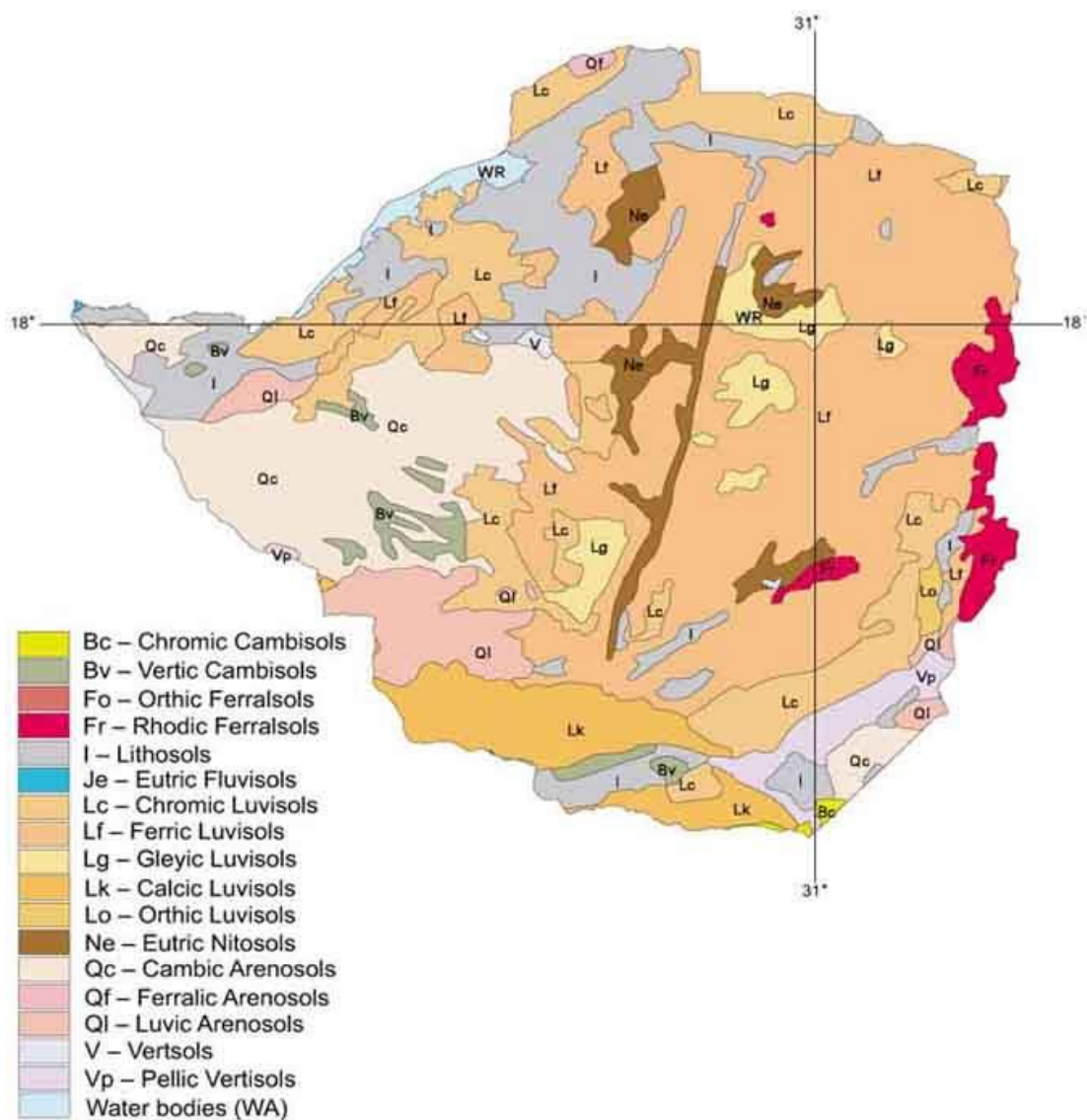
Figure 3.2 Dominant soil map of Zimbabwe

Source: http://www.fao.org/docrep/009/a0395e/a0395e06.htm. Accessed 26/05/2016.

Table 3.1 Description of the natural regions of Zimbabwe

Natural Region	Area (000 ha)	% total land area	Annual rainfall (mm)	Mean temperature ranges	Farming systems
I	613	1.56	More than 1000. Rains throughout the year and temperatures are relatively low	Annual 15-18ºC, maximum 19-23ºC and minimum 10-12ºC	Suitable for dairy farming forestry, tea, coffee, fruit, beef and maize production
II	7 343	18.68	700-1000. Rainfall confined to summer	Annual 16-19ºC. maximum 19-23ºC and minimum 10-13ºC	Suitable for intensive farming, based on maize, tobacco, cotton and livestock
III	6 855	17.43	500-800. Relatively high temperatures and infrequent, heavy falls of rain, and subject to seasonal droughts and severe mid-season dry spells	Annual 18-22ºC. maximum 23-26ºC and minimum 11-15ºC	Semi-intensive farming region. Suitable for livestock production, together with production of fodder crops and cash crops under good farm management
IV	13 010 036	33.03	450-650. Rainfall subject to frequent seasonal droughts and severe dry spells during the rainy season	Annual 18-24ºC, maximum 19-26ºC and minimum 11-20ºC	Semi-extensive region. Suitable for farm systems based on livestock and resistant fodder crops. Forestry, wildlife/tourism
V	10 288	26.2	<450. Very erratic rainfall. Northern low veldt may have more rain but the topography and soils are poor	Annual 21-25ºC, maximum 26-32ºC and minimum 14-18ºC.	Extensive farming region. Suitable for extensive cattle ranching. Zambezi Valley is infested with tsetse fly. Forestry, wildlife/tourism

Vincent and Thomas (1961), FAO (2006), Mugandani et al. (2012)

Table 3.2 Description of study sites

Site	[+]Agro ecological region	Planting season	[+]Average annual rainfall (mm)	Latitude	Longitude	Altitude (masl)	[*]Soil type	Environment
CIMMYT Harare (HA)	IIa	Summer	820	17°43'S	31°05'E	1 480m	Harare 5E series	Optimum
CIMMYT Harare (HA)	IIa	Summer	820	17°43'S	31°05'E	1 480m	Harare 5E series	Low N stress
CIMMYT Harare (HA)	IIa	Summer	820	17°43'S	31°05'E	1 480m	Harare 5E series	Low P stress
Chiredzi Research Station	V	Winter	500	21°02'S	31°58' E	433m	Triangle E series	Managed drought stress
Chiredzi Research Station	V	Winter	500	21°02'S	31°58' E	433m	Triangle E series	Combined drought and heat stress
Kaguvi Training Centre	III	Summer	650	19°11'S	29°48'E	1440	Clay	Random drought stress
Save Valley Experimental Station	V	Winter	500	20°2'S	32°21'E	444m	Sabi 4U.2	Managed drought stress
Kadoma Research Station	III	Summer	800	18°32'S	30°90' E	1155m	Clay	Random drought stress
Rio Tinto Agricultural college	IV	Summer	650	18° 26'S	18° 44'E	1020m	Clay	Optimum
Rattray Arnold Research Station	IIa	Summer	865	17°40' S	31°05' E	1369m	Harare 5G2 series	Optimum

[+] Vincent and Thomas (1961), [+]Mugandani et al. (2012), [*]Nyamapfene (1991)

3.3.2 Germplasm

Twenty-two elite orange maize inbred lines were obtained from CIMMYT-Mexico and five elite drought tolerant yellow maize inbred lines were obtained from CIMMYT-Zimbabwe's Drought Tolerant Maize for Africa (DTMA) programme. They were crossed following the line x tester scheme at Muzarabani, in the winter of 2012 and 2013. The five elite drought tolerant yellow maize inbreds were used as testers. Pedigrees of these parental materials are listed in Appendix 1. Thirty single crosses were successfully produced out of a potential of 110 hybrids. The 30 successful experimental hybrids with four local check hybrids (two white and two yellow) and an orange hybrid from Zambia (Appendix 2) were evaluated under different environments as indicated in Table 3.1.

The low N stress, low P stress as well as optimum growing conditions and random drought stress trials were planted in the summer of the 2013/2014 and 2014/2015 seasons. A separate parental trial was also established at each site close to the hybrid trial in both seasons.

Managed drought trials were planted at Chiredzi Research Station and Save Valley Experimental Station in the winter of 2014 and 2015. There was no significant rainfall at the two sites in winter and all the water was supplied through irrigation, making it easy to manage the amount of water applied. The combined heat and drought stress trial experiment was planted at Chiredzi Research Station on 15 August 2014 and the same day in the 2015 season, so that susceptible flowering stage coincides with the hottest months of October and November.

3.3.3 Agronomic practices

Managed drought and combined heat and drought stress trials were done under irrigation in the winter season. Irrigation for these trials was withheld 14 days before flowering to target stress during flowering. Thereafter only survival irrigation was applied to prevent the crop from total wilting. This stress level delays silking and causes ear abortion in non-stress tolerant genotypes. Such stress levels achieve an anthesis silking interval (ASI) of between 4 to 8 days and 0.3 to 0.7 ears per plant (Bänziger et al., 2000).

The low N stress trial was planted at CIMMYT-Harare in a field depleted of N through continuous cultivation of maize in summer and irrigated wheat in winter with no addition

of N fertilisers and removal of all the residues after harvest. The low P site was a field known to be inherently low in soil P at CIMMYT-Harare. Optimum trials were planted in the summer of each season and received supplementary irrigation to ensure that no water stress occurred.

Weed management was done at all sites by using herbicides and handhoeing to remove late emerging weeds. Maize stalk borer (*Busseola fusca)* was controlled by applying endosulfan 1% granules at a rate of 2 kg ha^{-1} in a mixture of 2:1 (sand:pesticides) when needed.

Recommended fertilizer levels for maize for each location were applied. At Harare (except in the low P and low N stress trials), Kadoma Research Station, Kaguvi Training Centre, Rio Tinto Agricultural College and Rattray Arnold Research Station sites compound D [N (8%): P (14%): K (7%)] was applied as basal fertiliser at a rate of 400 kg ha^{-1}. Ammonium nitrate top dressing was applied at the rate of 400 kg ha^{-1} split applied 200 kg per application at the optimum trials planted at Harare as well as the trials at Rattray Arnold Research Station, Rio Tinto Agricultural College, Kadoma Research Station and Kaguvi Training Centre. The first application was done four weeks after crop emergence and the second application at six weeks after crop emergence.

In the low N stress trial, muriate of potash (KCl) and super phosphate (P$_2$O$_5$) were applied as basal fertiliser at the same rate of P and K as applied in the optimum trials and there was no top dressing fertiliser applied.

In the low P stress trial, KCl and ammonium nitrate were applied as basal fertiliser and the same rate of N and K was applied as in the optimum trials. Ammonium nitrate top dressing was applied at a rate of 400 kg ha^{-1} split applied 200 kg ha^{-1} per application. The first application was done four weeks after crop emergence and the second application at six weeks after crop emergence.

At Save Valley Experimental Station and Chiredzi Research Station sites 300 kg ha^{-1} compound D was applied as basal dressing followed by 200 kg ha^{-1} ammonium nitrate split application in equal splits of 100 kg ha^{-1} at four and six weeks after crop emergence as top dressing.

3.3.4 Experimental design

The 30 hybrids and five hybrids checks were laid out as a 5 x 14 incomplete lattice design at all the sites. A separate trial of 13 parents with two more inbreds were laid out as a 3 x 5 incomplete lattice at all the sites and environments and planted adjacent to the hybrid trials. The design used was more efficient than a randomised complete block design, as it maintains homogeneity of experimental units and as a result, gives improved precision. The plot size for both the hybrids and their parents was 1 x 4 m row. Inter-row spacing of 0.75 m and in-row spacing of 0.25 m was used. Two kernels were planted per station and thinned to one plant per station three weeks after emergence to achieve a plant population of approximately 53 000 plants ha^{-1}.

3.3.5 Measurements

Grain texture, which is an important aspect considered in grain processing and grain yield, was measured according to the CIMMYT guidelines for collecting data from trials. Texture (TEX) was measured on a scale of 1-5 with 1 being flint and 5 being dent. Grain yield (GYD) in t ha^{-1} was calculated from shelled grain weight per plot adjusted to 12.5% grain moisture.

3.3.6 Data analysis

Analysis of variance (ANOVA) was done on measured and derived agronomic traits using Agrobase software (Agronomix Software, 2013) and GenStat$^{®}$ 17th Edition statistical software (VSN International, 2015). ASI was first normalised using ln$\sqrt{}$(ASI + 10) as suggested by Bolaños and Edmeades (1996). Single environment analysis was done followed by combined environment analysis.

3.4 Results

3.4.1 Grain yield and texture performance of 30 provitamin A maize single cross hybrids tested across six environments in 2014

In 2014, genotype differences for yield were highly significant ($P < 0.01$) at all environments, except low N stress, which was not significant (Table 3.3). The genotypes contributed the largest percentage to total sum of squares when compared with other sources of variation, except for the random drought trial (Table 3.3). Entry 31, a local check genotype, ranked first with a grain yield of 10.72 t ha^{-1} under optimum conditions, followed by another check, hybrid 34 which yielded 10.39 t ha^{-1} (Table 3.4). Entry 8, an

experimental provitamin A hybrid, yielded 9.01 t ha^{-1} t ha^{-1} which was significantly higher than entry 32, a local check hybrid which yielded 6.51 t ha^{-1}. Entries 8, 7, and 16 which yielded 9.01 t ha^{-1}, 8.46 t ha^{-1}, and 8.39 t ha^{-1} respectively, also ranked significantly higher than entry 35, a provitamin A cultivar released in Zambia, which ranked 16th under optimum conditions with a grain yield of 7.02 t ha^{-1}. There was a significant ($P < 0.05$) interaction between location and genotype. Entry 29, an experimental provitamin A hybrid with a average grain yield of 5.05 t ha^{-1}, ranked higher than entries 35 and 32, local check hybrids, under random drought stress, although they were not significantly different from the other three local check hybrids. Entries 8, 3 and 12 yielded significantly higher than three checks, entries 34, 33 and 35, except under managed drought stress.

Under optimum and random drought stress conditions there was also significant interaction between location and genotype (Table 3.3). Under a combination of managed drought and heat stress, entries 8, 3 and 23 were the top ranking and they ranked significantly higher than entries 31, 32 and 33, the local checks (Table 3.4). However they did not differ significantly from entries 34 and 35, a local check and a provitamin A check hybrid, respectively. Entry 6, an experimental provitamin A hybrid, ranked in the top five under low N stress, but was not significantly different from the four local checks, but yielded significantly higher than entry 35, a provitamin A cultivar. Under low P stress, entries 24 and 6 ranked significantly higher than checks 32 and 33. Entry 26 also ranked higher than entry 33, a local check hybrid. Entries 6 and 24 ranked higher than entries 32 and 33 but did not differ significantly from entries 31 and 34, which are local checks. Average grain yield under low P stress was 11.23% less than under optimum conditions at Harare. Grain yield was reduced by 55.82% under low N stress when compared to the optimum environment at Harare. When compared to low P stress, grain yield for low N stress at Harare in 2014 was 50.23% lower. Grain yield under managed drought stress was 4.21% higher when compared to yields under a combination of drought and heat stress at Chiredzi in 2014.

Genotypes were also significantly different ($P < 0.05$) for grain texture (Table 3.5) when evaluated under random drought, managed drought stress, combined drought and heat stress, low N stress and low P stress. However the genotypes were not significantly different from each other in texture under optimum growing conditions. Location and

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genotype effects were significant under optimum, random drought and drought stress conditions. Under managed drought and combined drought and heat stress, the grain texture was mostly dent. Under low P stress and low N the texture of the genotypes was mostly flint. There was also a significant interaction ($p < 0.05$) between location and genotypes for grain texture (Table 3.5) under optimum condition, random drought stress and managed drought stress.

3.4.2 Grain yield and texture performance of 30 provitamin A maize single cross hybrids tested across six environments in 2015

In 2015, genotypes performed significantly different ($P < 0.05$) under all environments except under low N stress (Table 3.6). Like in 2014, the percentage contribution of entry to total variation was higher than any other source of variation except under random drought.

Under optimum conditions (Table 3.7), entries 8 and 6 of the 30 provitamin A maize single cross genotypes ranked significantly higher than three checks, entries 32, 33 and 35 and not significantly different from the highest yielding checks, entries 31 and 34. The yield of hybrids under optimum conditions ranged from 1.19 to 11.46 t ha^{-1} (Table 3.7). Location and entry effects were not significant under optimum conditions (Table 3.6). Under random drought conditions, location and entry effects were highly significant. Entries 11 and 14, which are experimental hybrids, had yields not significantly different from the highest yielding local check (entry 31). The two hybrids were also not significantly different from entry 35, the provitamin A cultivar which ranked the highest. Under managed drought stress conditions entry 23, an experimental provitamin A hybrid, yielded significantly higher than all five checks (Table 3.7). Entry 13 also yielded significantly higher than four checks. Entries 15, 20 and 11 also yielded significantly higher than check hybrids 34, 35 and 32. There was also a significant interaction between location and genotypes for grain yield in 2015 (Table 3.6). Under a combination of drought and heat stress only one check hybrid (entry 35) yielded significantly lower than the highest yielding experimental provitamin A hybrids (entries 10, 14, 23 and 7, Table 3.7). Under low N stress there were no significant yield differences between the genotypes. Grain yield under optimum conditions was 27.27% higher than yields under low N stress at Harare in 2015.

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Table 3.3 Analysis of variance for grain yield of 30 provitamin A maize single cross hybrids and five checks grown in Zimbabwe under different stress and optimum conditions in 2014

Source	Environment																	
	Optimum			Random drought			Managed drought stress			Combined drought and heat stress			Low N stress			Low P stress		
	DF	SS	MS	DF	SS	MS	DF	SS	MS	DF	SS	MS	DF	SS	MS	DF	SS	MS
Block	2	7.847	3.923	3	6.29	2.10	2	10.72	5.36**	1	1.37	1.37	1	0.72	0.72	1	0.11	0.11
Location	1	34.641	34.64**	2	452.25	226.13**	1	0.97	0.97									
Entry	34	517.52	15.22**	34	264.58	7.78**	34	47.76	1.41**	34	84.85	2.50**	34	36.88	1.09	34	182.75	5.38**
Location * Entry	34	1.67.68	4.93**	68	267.76	3.94*	34	19.93	0.59									
Residual	68	121.41		102	277.99		68	37.52	0.55	34	29.72		34	24.10		34	39.45	
Total	139	849.09		209	1269.12		139	116.90		69	115.95		69	61.69		69	222.31	
CV%		20.84			43.04			53.39			30.00			29.51			18.92	

* $p < 0.05$, **$p < 0.01$, SS = sum of squares, MS = mean squares, CV = coefficient of variation, DF = degrees of freedom

Table 3.4 Mean grain yield and texture performance of 30 provitamin A maize single cross hybrids tested across six environments in 2014 in Zimbabwe

	Optimum			Random drought			Managed drought stress			Combined drought and heat stress			Low N			Low P		
Rank	GYD	Entry	TEX	GYD	Entry	TEX	GYD	Entry	TEX	GYD	Entry	TEX	GYD	Entry	TEX	GYD	Entry	TEX
1	10.72	31	3.0	7.34	31	2.3	2.88	3	3.5	6.20	8	4.0	4.68	32	2.7	9.60	31	1.8
2	10.39	34	3.0	5.60	34	2.6	2.48	12	3.2	5.27	3	4.0	4.12	6	1.4	9.45	34	2.3
3	9.01	8	2.9	5.52	33	2.8	2.29	31	3.4	4.52	1	4.0	4.09	33	1.9	8.33	24	3.5
4	8.46	7	2.5	5.05	29	2.7	2.07	8	2.8	4.38	34	4.0	4.01	31	2.2	7.87	6	3.0
5	8.39	16	2.6	5.00	35	3.0	1.96	5	3.0	4.28	7	3.0	3.98	34	2.9	7.38	26	2.0
6	8.36	33	2.4	4.96	32	2.2	1.94	32	3.0	4.16	35	3.0	3.33	8	3.6	7.27	16	1.8
7	7.90	29	2.8	4.93	16	1.7	1.90	16	2.9	4.13	23	2.0	3.33	30	2.7	6.80	1	1.5
8	7.87	27	2.9	4.83	8	2.6	1.81	11	3.4	4.00	20	4.0	3.30	26	3.2	6.60	23	3.3
9	7.81	28	3.5	4.77	19	2.2	1.75	23	3.9	3.86	11	4.0	3.25	28	2.2	6.60	35	3.5
10	7.80	26	2.9	4.39	14	2.9	1.72	18	3.2	3.70	10	3.0	3.21	7	2.1	6.54	28	3.3
11	7.71	1	2.5	4.31	7	2.5	1.61	10	2.7	3.66	4	4.0	3.16	1	2.2	6.33	7	2.3
12	7.37	14	2.5	3.99	30	2.3	1.61	7	3.2	3.56	12	4.0	3.07	23	2.7	6.31	27	2.5
13	7.35	30	3.0	3.92	4	1.5	1.60	28	2.8	3.42	25	4.0	3.05	12	2.3	6.09	8	3.0
14	7.26	6	3.3	3.85	10	3.0	1.56	14	3.0	3.41	17	4.0	3.05	17	2.4	6.06	3	3.5
15	7.15	19	2.8	3.78	6	2.4	1.49	2	3.2	3.31	6	3.0	3.05	14	2.3	6.01	29	1.5
16	7.02	35	2.9	3.76	9	2.3	1.48	30	3.2	3.28	14	3.5	3.01	19	2.6	5.95	21	1.5
17	6.87	24	2.6	3.76	28	2.2	1.45	19	2.6	3.27	2	2.5	2.94	25	2.8	5.73	25	1.8
18	6.51	32	2.9	3.74	18	2.8	1.44	1	2.9	3.20	13	4.0	2.94	13	1.8	5.73	32	3.0
19	6.51	23	2.8	3.71	23	2.7	1.42	20	3.7	3.20	16	4.0	2.86	27	2.7	5.54	33	2.5
20	6.44	5	3.1	3.66	24	2.7	1.34	26	3.2	2.77	31	4.0	2.76	24	2.2	5.25	5	3.0
21	5.90	25	2.5	3.61	11	2.2	1.25	24	3.5	2.69	22	3.5	2.65	5	2.3	5.24	9	2.0
22	5.56	4	2.8	3.51	3	2.1	1.20	13	3.8	2.66	18	4.0	2.62	21	2.3	5.00	17	2.0
23	5.27	3	3.0	3.45	20	2.1	1.19	17	3.0	2.61	26	4.0	2.53	16	2.9	4.91	4	3.8
24	5.08	22	3.0	3.39	27	3.0	1.18	35	3.9	2.59	28	4.0	2.49	15	2.8	4.87	20	4.0
25	5.06	17	3.3	3.29	5	2.7	1.09	34	4.3	2.58	5	4.0	2.30	3	2.7	4.83	14	2.3
26	4.82	20	2.6	3.16	12	1.9	1.09	9	3.3	2.36	27	3.5	2.29	20	2.9	4.78	30	1.5
27	4.64	10	2.1	3.12	17	1.8	1.09	15	3.5	2.17	24	3.5	2.12	10	2.2	4.52	19	2.0
28	4.62	9	3.6	3.01	21	3.3	0.96	27	3.5	2.16	15	3.5	2.11	35	3.0	4.38	12	3.0
29	4.10	21	2.8	2.90	25	3.3	0.84	33	3.8	2.03	32	3.5	2.09	11	2.6	4.30	15	3.0
30	3.99	12	2.8	2.89	1	2.8	0.74	4	3.5	1.92	33	3.0	2.09	18	3.2	4.22	13	3.8
31	3.89	2	3.5	2.58	15	3.4	0.61	6	3.7	1.86	9	3.5	1.99	22	3.3	3.91	18	4.3
32	3.75	15	3.4	2.57	26	3.8	0.60	21	4.0	1.61	29	5.0	1.98	4	3.7	3.75	11	4.3
33	3.74	18	2.6	2.07	22	2.6	0.38	22	3.2	1.53	19	3.5	1.90	2	4.1	3.59	22	3.5
34	3.71	11	3.3	1.91	2	2.9	0.34	25	3.9	1.45	30	5.0	1.80	29	3.8	3.13	10	4.8
35	3.43	13	2.5	1.88	13	1.7	0.34	29	2.8	1.28	21	2.5	1.67	9	2.4	2.34	2	1.5
Grand mean	6.41		2.9	3.84		2.5	1.39		3.3	3.12		3.6	2.85		2.4	5.69		2.7
P-Value	<0.001		0.0453	<0.001		<0.001	<0.001		0.03	0.0015		<0.001	0.11		<0.001	<0.001		<0.001
LSD	1.58		0.63	1.58		0.69	0.88		0.84	1.58		0.66	1.42		0.67	1.82		0.76
CV%	20.84		18.77	43.04		28.41	53.39		21.90	30.0		8.69	29.51		16.79	18.92		16.53

GYD = Grain yield, LSD = Least significant difference, CV = Coefficient of variation, TEX = Texture

Table 3.5 Analysis of variance for grain texture of 30 provitamin A maize single cross hybrids and five checks grown in Zimbabwe under different stress and optimum conditions in 2014

Source	Environment																	
	Optimum			Random drought			Managed drought stress			Combined drought and heat stress			Low N stress			Low P stress		
	DF	SS	MS	DF	SS	MS	DF	SS	MS	DF	SS	MS	DF	SS	MS	DF	SS	MS
Block	2	4.42	2.21*	3	0.15	0.05	2	3.35	1.67	1	1.37	1.37	1	0.36	0.36	1	0.18	0.18
Location	1	22.00	22.00**	2	3.69	1.844*	1	0.40	0.40									
Entry	34	15.99	0.47*	34	54.60	1.61**	34	29.69	0.87*	34	84.85	2.85*	34	21.59	0.64**	34	58.24	1.71**
Location * Entry	34	16.19	0.48*	68	75.48	1.11**	34	31.29	0.92*									
Residual	68	19.71	0.29	102	52.85	0.52	68	34.53	0.51	34	29.73		34	5.39		34	6.95	0.20
Total	139	78.31		209	186.77		139	99.25		69	115.95		69	27.34		69	65.36	
CV%	18.77			28.41			21.90			29.56			16.79			16.53		

** $p < 0.01$, * $p < 0.05$, SS = sum of squares, MS = mean squares, CV = coefficient of variation, DF = degrees of freedom

Table 3.6 Analysis of variance for grain yield of 30 provitamin A maize single cross hybrids and five checks grown in Zimbabwe under different stress and optimum conditions in 2015

	Environment														
Source	Optimum			Random drought			Managed drought stress			Combined drought and heat stress			Low N stress		
	DF	SS	MS	DF	SS	MS	DF	SS	MS	DF	SS	MS	DF	SS	MS
Block	2	3.85	1.94	3	5.05	1.68	2	5.05	2.53*	1	5.26	5.26*	1	0.30	0.30
Location	1	50.52	50.52**	2	648.70	324.35**	1	10.53	10.53**						
Entry	34	488.19	14.36**	34	119.11	3.50**	34	41.51	1.22**	34	75.63	2.22*	34	106.53	3.13
Location * Entry	34	63.64	1.87	68	223.45	3.29**	34	27.02	0.80*						
Residual	68	162.68	2.39	102	155.85	1.53	68	34.03	0.50	34	40.84	1.20	34	68.75	2.02
Total	139	768.89		209	1152.16		139	118.14		69	121.73		69	175.58	
CV%	23.99			35.75			57.17			50.56			30.28		

** $p < 0.01$, * $p < 0.05$, SS = sum of squares, MS = mean squares, CV = coefficient of variation, DF = degrees of freedom

Table 3.7 Mean grain yield (t ha^{-1}) and grain texture performance of 30 provitamin A maize single cross hybrids tested across six environments in 2015 in Zimbabwe

Rank	Optimum			Random drought			Managed drought stress			Combined drought and heat stress			Low N		
	GYD	Entry	TEX	GYD	Entry	TEX	GYD	Entry	TEX	GYD	Entry	TEX	GYD	Entry	TEX
1	11.46	31	2.1	4.56	35	2.4	2.61	23	2.5	3.98	32	3.5	7.77	34	1.8
2	9.75	8	2.2	4.51	11	1.0	2.29	13	3.5	3.89	10	3.5	6.96	10	2.3
3	9.17	34	2.3	4.47	14	2.6	2.10	15	3.8	3.58	31	3.3	5.98	16	2.5
4	9.00	6	2.1	4.36	31	2.9	2.09	20	3.3	3.39	14	3.0	5.97	27	1.8
5	8.67	7	1.8	4.32	18	2.5	2.07	18	3.0	3.33	23	1.0	5.93	31	2.5
6	8.46	10	1.5	4.19	16	2.8	1.89	11	3.0	3.32	7	1.5	5.81	32	1.8
7	7.95	12	1.5	4.17	32	1.5	1.56	31	1.8	3.25	33	1.0	5.71	17	1.8
8	7.24	16	2.0	4.13	20	2.9	1.48	7	3.6	3.21	8	3.5	5.71	9	2.0
9	7.20	32	2.1	4.11	4	2.5	1.43	8	2.9	2.96	16	3.5	5.69	6	2.0
10	7.10	3	2.1	3.99	19	2.5	1.43	28	3.3	2.90	20	3.0	5.66	8	2.0
11	7.04	26	1.8	3.96	33	2.4	1.37	33	2.3	2.87	18	3.5	5.52	33	1.8
12	7.00	1	2.1	3.89	12	2.8	1.34	12	3.3	2.73	15	3.0	5.45	7	2.5
13	6.89	23	2.1	3.87	10	3.0	1.34	14	3.9	2.72	12	3.5	5.09	25	3.0
14	6.57	13	2.0	3.77	15	2.0	1.32	17	4.0	2.64	27	3.5	4.95	2	3.0
15	6.54	17	2.1	3.76	27	2.8	1.32	1	2.8	2.58	24	2.8	4.84	3	2.8
16	6.45	18	1.9	3.73	13	2.4	1.30	26	3.1	2.47	11	3.5	4.79	26	2.3
17	6.44	33	1.6	3.72	17	2.3	1.29	10	2.1	2.43	3	2.0	4.75	35	2.0
18	6.36	9	2.6	3.67	7	2.9	1.25	5	3.8	2.30	28	3.5	4.66	4	2.3
19	6.34	27	2.1	3.53	23	2.9	1.18	24	3.5	2.24	13	3.5	4.58	24	3.0
20	6.12	20	2.4	3.39	8	2.9	1.12	19	3.8	2.14	34	3.8	4.54	18	2.8
21	6.12	35	2.0	3.33	25	2.5	1.04	34	3.0	1.82	17	3.3	4.30	23	1.8
22	6.12	11	1.9	3.29	2	2.3	1.03	25	3.9	1.68	19	3.0	4.29	13	1.5
23	6.06	15	2.4	3.17	6	2.8	0.99	3	4.0	1.60	25	3.5	4.13	11	2.3
24	5.97	25	2.1	3.16	21	2.9	0.98	27	3.6	1.50	1	3.5	4.08	1	2.8
25	5.84	5	2.1	3.09	34	2.3	0.93	16	3.6	1.42	35	3.0	4.08	20	1.8
26	5.72	24	1.9	3.04	1	2.6	0.91	2	2.5	1.42	22	3.0	4.00	15	1.8
27	5.12	29	1.9	3.00	26	1.9	0.89	35	3.5	1.41	4	2.0	4.00	21	2.0
28	4.91	4	2.4	2.87	9	2.5	0.86	4	3.9	1.22	29	3.5	3.87	14	2.5
29	4.83	28	2.0	2.72	28	2.8	0.77	32	3.6	1.17	2	3.5	3.76	5	1.8
30	4.74	21	2.4	2.70	22	2.1	0.69	6	2.0	1.15	30	3.3	3.47	12	2.5
31	4.70	2	3.5	2.65	24	3.1	0.68	22	4.1	1.02	21	4.0	3.42	19	1.8
32	4.66	19	3.9	2.44	30	3.5	0.61	9	4.3	0.76	9	4.5	3.34	29	3.0
33	4.56	22	2.6	1.90	29	3.0	0.60	21	4.3	0.68	26	3.8	3.30	28	2.5
34	3.34	30	3.4	1.83	3	3.0	0.45	29	4.0	0.10	6	4.5	2.90	22	1.5
35	1.19	14	1.5	1.78	5	1.4	0.14	30	2.4	0.00	5	1.0	1.08	30	2.0
Grand mean	6.27		2.2	3.33		2.5	1.21		3.3	2.13		3.1	4.56		2.2
P-Value	<0.001		<0.001	<0.001		<0.001	<0.001		<0.001	0.038		0.001	0.103		0.001
LSD	0.52		0.43	1.41		0.55	1.06		0.86	2.23		0.98	2.89		0.68
CV%	23.82		18.6	35.68		15.39	60.90		18.42	50.56		15.49	30.26		15.32

GYD = Grain yield, LSD = Least significant difference, CV = Coefficient of variation, TEX = Texture

Table 3.8 Analysis of variance for grain texture on 30 provitamin A maize single cross hybrids and five checks grown in Zimbabwe under different stresses and optimum conditions in 2015

Source	Environment																	
	Optimum			Random drought			Managed drought stress			Combined drought and heat stress			Low N stress			Low P stress		
	DF	SS	MS	DF	SS	MS	DF	SS	MS	DF	SS	MS	DF	SS	MS	DF	SS	MS
Block	2	0.35	0.17	3	0.061	0.020	2	0.59	0.29	1	0.06	0.06	1	0.29	0.29	1	0.01	0.004
Location	1	0.05	0.05	2	379.94	189.97**	1	9.26	9.26**									
Entry	34	38.49	1.13**	34	23.60	0.69**	34	62.40	1.84**	34	62.89	1.85**	34	14.52	0.43**	34	13.95	0.41**
Location * Entry	34	14.89	0.44**	68	43.15	0.64**	34	32.24	0.95**									
Residual	68	6.03	0.09**	102	10.31	0.10	68	24.91		34	7.69	0.23	34	3.84	0.11	34	1.62	0.05
Total	139	59.81		209	457.95		139	129.40		69	70.64		69	18.65		69	15.58	
CV%	13.73			18.94			18.34			15.49			15.32			11.20		

* p < 0.01, *p < 0.05, SS = sum of squares, MS = mean squares, CV = coefficient of variation, DF = degrees of freedom

Contrary to the 2014 season, grain yield under a combination of drought and heat stress was higher than yield under managed drought stress (Table 3.7). There was a highly significant difference ($p < 0.05$) between the textures of the genotypes in all environments (Table 3.8). Under optimum conditions grain texture did not differ significantly. However location effect on texture was significant for the random drought environment and under drought stress.

3.4.3 Grain yield performance of 30 provitamin A maize single cross hybrids tested across six environments in 2014 and 2015

Combined ANOVA across the two seasons showed genotypes performing significantly different ($P < 0.05$) under all the environments (Table 3.9). Year effect was significant ($P < 0.05$) under random drought stress and managed drought stress as well as a combination of drought and heat stress. Year by location interaction was also significant ($P < 0.05$) under managed drought stress and highly significant ($P < 0.01$) under random drought stress conditions and low N stress. The genotypes yielded significantly different in the different environments across years under optimum, random drought stress and combined drought and heat stress and performed similar under managed drought stress and low N stress. Grain yield under low N stress at Harare was reduced by 44.27% for the two seasons. Grain yield under a combination of drought and heat stress was higher by 34.27% when compared to yields under drought stress only.

Entry 31 ranked first when genotypes were cultivated under optimum and under random drought stress (Table 3.10). Under optimum conditions, entries 8, 7, 6, 16 and 26 performed better than three check hybrids 33, 32 and 35, however, they were outyielded by entry 31 and 34 that ranked first and second, respectively. When genotypes were cultivated under random drought conditions, provitamin A hybrids performed poorly compared to four checks (entries 31, 35, 33 and 32). The genotypes that ranked higher were only better when compared to local check entry 34. Provitamin A hybrid entry 23 ranked first when genotypes were evaluated under managed drought stress conditions, followed by entry 3, a provitamin A hybrid. It was very encouraging that 16 provitamin A hybrids performed better than four checks (entries 32, 33, 34 and 35) under managed drought stress. Entry 8 ranked in the top 10 hybrids in all the environments. Under combined drought and heat stress environments it ranked second to entry 32, a local check. Under low N stress two local checks, entries34 and 33 ranked the highest.

The average grain yield of genotypes in 2014 and 2015 under optimum conditions was 6.43 t ha^{-1} ranging from 4.28 to 11.09 t ha^{-1} (Table 3.10). The yield difference under random drought stress was 43.27% lower than under optimum condition. Yield was reduced by 79.55%, 68.42% and 44.27% under managed drought stress, combination of drought and heat stress and low N stress respectively, when compared with genotype performance under optimum conditions.

Table 3.9 Analysis of variance for grain yield of 30 provitamin A maize single cross hybrids and five checks grown in Zimbabwe under different stress and optimum conditions in 2014 and 2015

Source	Environment									
	Optimum		Random drought		Managed drought stress		Combined drought and heat stress		Low N stress	
	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
Replication	1	2.69	1	0.26	1	0.07	1	0.74	1	2.14
Year	1	0.076	1	14.95**	1	1.659	1	165.30**	1	172.66**
Location	1	84.40**	2	860.22**	1	8.95**				
Entry	34	676.05**	34	239.48**	34	58.03**	34	82.18**	34	70.99*
Year*Location	1	0.75	1	240.84**	1	2.55*				
Year*Entry	34	329.66**	34	144.34**	34	29.95	34	100.44**	34	64.00
Location * Entry	34	132.36**	68	223.26*	34	23.40				
Year*Location*Entry	34	99.02	34	267.89**	34	24.69				
Residual	135	293.13	176	444.91	135	87.43	69	80.37	69	84.69
Total	275	1618.14	351	2436.15	275	236.75	139	429.03	139	394.45

** $p < 0.01$, *$p < 0.05$, SS = sums of square, MS = mean squares, DF = degrees of freedom

Table 3.10 Mean grain yield performance and texture of 30 provitamin A maize single cross hybrids and five checks hybrids tested across six environments in 2014 and 2015 in Zimbabwe

	Optimum			Random drought			Managed drought stress			Combined drought and heat stress			Low N		
Rank	GYD	Entry	TEX	GYD	Entry	TEX	GYD	Entry	TEX	GYD	Entry	TEX	GYD	Entry	TEX
1	11.09	31	2.6	5.85	31	2.4	2.18	23	2.7	4.27	32	3.8	5.74	34	1.9
2	9.78	34	2.6	4.78	35	1.0	1.94	3	1.5	3.35	8	3.8	4.86	33	2.3
3	9.38	8	2.6	4.74	33	2.6	1.93	31	2.9	3.19	34	3.6	4.50	10	2.6
4	8.57	7	2.3	4.57	32	2.9	1.91	12	2.9	3.06	23	3.5	4.39	6	2.1
5	8.13	6	2.2	4.56	16	2.5	1.89	18	3.2	3.01	1	1.5	4.33	16	2.1
6	7.82	16	1.9	4.43	14	2.8	1.85	11	2.7	2.68	11	2.3	4.32	8	1.8
7	7.42	26	2.2	4.38	19	1.5	1.76	20	1.6	2.63	3	1.5	4.26	31	2.0
8	7.40	33	2.4	4.35	34	2.9	1.75	8	2.8	2.60	16	3.8	4.24	9	2.4
9	7.35	1	2.8	4.11	8	2.5	1.75	13	2.2	2.50	20	3.8	4.24	32	2.1
10	7.11	27	2.5	4.06	11	2.5	1.60	5	3.0	2.35	10	3.0	4.09	17	2.6
11	6.85	32	2.1	4.03	18	2.4	1.59	15	2.5	2.28	12	3.8	3.80	25	2.0
12	6.70	23	2.3	4.02	4	2.8	1.54	7	2.5	2.21	33	3.5	3.79	7	2.5
13	6.57	35	2.7	3.99	7	3.0	1.51	28	2.7	2.14	7	3.8	3.75	27	3.3
14	6.55	10	2.7	3.86	10	2	1.45	10	2.8	2.14	14	3.8	3.72	35	2.8
15	6.51	29	2.4	3.79	20	2.8	1.45	14	2.8	2.13	2	2.9	3.67	24	3.0
16	6.32	28	2.4	3.62	23	2.4	1.42	16	2.4	2.10	13	3.5	3.66	26	2.3
17	6.29	24	2.1	3.58	27	2.3	1.38	1	2.5	2.08	18	2.3	3.58	4	1.9
18	6.19	3	2.8	3.52	12	2.9	1.36	32	3.0	2.08	35	3.8	3.48	23	2.4
19	6.14	5	2.4	3.48	29	2.8	1.32	26	3.1	1.93	27	3.8	3.37	29	2.6
20	5.97	12	2.8	3.48	6	2.9	1.28	19	2.6	1.91	6	3.9	3.36	5	2.6
21	5.93	25	2.3	3.42	17	2.5	1.25	17	2.4	1.88	31	3.4	3.20	11	1.8
22	5.91	19	2.3	3.31	9	2.3	1.21	24	2.3	1.83	4	3.5	3.17	3	1.6
23	5.80	17	2.7	3.24	28	2.8	1.20	2	2.4	1.79	5	3.8	3.14	1	2.3
24	5.49	9	2.6	3.22	30	2.9	1.11	33	3.2	1.71	25	3.8	3.10	20	2.5
25	5.47	20	2.7	3.18	15	2.3	1.06	34	2.6	1.70	17	3.5	3.08	15	2.1
26	5.34	30	2.3	3.16	24	2.6	1.04	35	2.3	1.59	22	3.3	3.07	28	2.1
27	5.23	4	2.0	3.11	25	1.9	0.97	27	2.0	1.59	24	2.8	3.06	18	1.8
28	5.09	18	3.0	3.09	21	2.5	0.85	9	3.2	1.31	26	3.5	3.04	21	2.4
29	5.00	13	2.4	2.97	1	2.8	0.81	30	3.4	1.30	28	3.5	2.96	13	1.9
30	4.91	11	2.6	2.80	13	2.1	0.80	4	2.3	1.08	15	3.1	2.95	2	2.5
31	4.91	15	3.5	2.79	26	3.1	0.69	25	2.9	1.02	19	3.8	2.92	12	2.0
32	4.82	22	3.6	2.67	3	3.5	0.65	6	3.6	0.98	30	4.8	2.89	19	2.5
33	4.42	21	2.6	2.60	2	3.0	0.60	21	2.9	0.93	9	3.6	2.85	14	2.8
34	4.30	2	3.3	2.54	5	3.0	0.53	22	2.9	0.89	21	4.8	2.75	22	2.1
35	4.28	14	2.0	2.38	22	1.4	0.39	29	1.6	0.81	29	1.8	2.07	30	2.1
Grand mean	6.43		2.5	3.65		2.5	1.31		2.6	2.03		3.4	3.58		2.27
P-Value	<0.001		<0.001	<0.001		<0.001	<0.001		<0.001	0.005		<0.001	0.031		0.002
LSD	0.34		0.92	1.17		0.63	0.78		1.18	1.52		0.87	1.56		0.69
CV%	22.29		18.36	40.01		23.88	60.34		17.77	53.18		12.76	30.90		21.62

GYD = Grain yield, LSD = Least significant difference, CV = Coefficient of variation, TEX = Texture

Table 3.11 Across season analysis of variance for grain texture of 30 provitamin A maize single cross hybrids and five checks grown in Zimbabwe under different stresses and optimum conditions in 2014 and 2015

	Optimum			Random drought stress			Managed drought stress			Combined drought and heat stress			Low N stress		
Source	DF	SS	MS	DF	SS	MS	DF	SS	MS	DF	SS	MS		SS	MS
Rep	1	1.10	1.10*	1	0.02	0.02	1	0.57	0.57	1	0.01	0.01	1	0.11	0.11
Year	1	32.63	32.63**	1	0.02	0.02	1	0.04	0.04	1	10.17	10.17**	1	0.86	0.86
Loc	1	11.14	11.14**	2	34.18	17.10**	1	10.27	10.27**						
Entry	34	40.66	1.20**	34	65.97	1.94**	34	58.38	1.72**	34	68.43	2.01**	34	19.06	0.56*
Year x loc	1	10.91	10.91**	1	53.59	53.59**	1	1.26	1.26						
Year x entry	34	12.02	0.35*	34	24.03	0.71*	34	27.10	0.80**	34	11.68	0.34*	34	9.51	0.28
Loc x entry	34	11.33	0.33*	68	81.95	1.21**	34	23.07	0.68*						
Year x loc x entry	34	16.57	0.49**	34	24.88	0.73*	34	28.40	0.84**						
Residual	135	29.69	0.22	176	63.36	0.36	135	47.13	0.35	69	12.72	0.19	69	16.64	0.24
Total	275	166.05	0.60	351	347.99	1.00	275	196.24	0.71	139	103.02	0.75	139	46.19	0.33

* $p \leq 0.001$, **$p \leq 0.05$ SS = sums of square, MS = mean squares, loc = location, DF = degrees of freedom

3.4.4 Grain texture performance of 30 provitamin A maize single cross hybrids tested across six environments across years

Analysis of variance for grain texture (Table 3.11) showed that year, location, entry, year x location and year x location x entry were highly significant ($p < 0.01$) under optimum conditions. Year x entry and location x entry were significant ($p < 0.05$). Under random drought stress condition location, entry, year x location and location x entry were highly significant and year x entry and year x location x entry were significant ($p < 0.05$). Location, entry, year x entry and year x location x entry were highly significant ($p < 0.001$) and location x entry were significant ($p < 0.05$) under managed drought stress conditions. Under combined drought and heat stress year and entry were highly significant ($p < 0.001$) and year x entry was significant ($p < 0.05$). Only entry was significant ($p < 0.05$) under low N stress.

3.5 Discussion

Maize breeders in both private and public institutions prioritise total grain yield to make farming more profitable (Haegele and Westgate, 2007). In Zimbabwe, the maize cultivars on the market have a potential to yield more than 10 t ha^{-1}. In this study genotypes performed significantly different ($p < 0.05$) for grain yield when evaluated under optimum conditions, random drought stress, managed drought stress, a combination of managed drought and heat stress and low P stress in both 2014 and 2015. However, yield did not differ significantly for genotypes when cultivated under low N stress. This means that there is genetic variability for grain yield among all the tested environments, except under low N stress.

Yield reduction under low N stress was 44.27% when compared to optimum conditions at CIMMYT Harare. This was comparable with yield reduction of 37-78% observed by Bänziger et al. (1997). Contribution of genotypes to total variation was high under all conditions except under random drought stress, meaning the differences in yield between genotypes was an effect of genotype rather than environment. Under this scenario, selection will be effective, since variation is genetic.

The yields obtained were higher than the national grain average of about 1 t ha^{-1} obtained by smallholder farmers in Zimbabwe. However, they were lower than the potential of the

current cultivated varieties in Zimbabwe, which is above 10 t ha^{-1}. Average grain yield under managed drought was 1.31 t ha^{-1} which was 79.55% lower than yields obtained under optimum conditions. These results are in agreement with findings reported by Edmeades (2008) who estimated yield reduction of more than 25% under random drought stress in subtropical countries that rely on erratic rainfall or up to 70% under extreme conditions. Drought stress reduces yield of maize and other grain crops by reducing canopy absorption of incident photosynthetic active radiation, reducing radiation use efficiency and reducing harvest index (Earl and Davis, 2003). Silk growth and kernel number appear to depend directly on the flow of photosynthetic products during the three weeks of extreme sensitivity bracketing flowering (Schussler and Westgate, 1995). In this study the crop was water stressed from two weeks before flowering to maturity. Drought stress during that period causes grain yield losses due to kernel size reduction and ear abortion (Bolaños and Edmeades, 1996). It also interferes with pollination, which reduces the number of grains formed. Experimental provitamin A hybrids 23, 8, 11, 3, 20 and 12, which ranked under the top 10 genotypes under managed drought stress and under combined drought and heat stress, need to be further evaluated and released as hybrids under those conditions. The gave average yield above 1.40 t ha^{-1} and 2.90 t ha^{-1}, under managed drought stress and combined drought and heat stress respectively.

Yield under a combination of drought and heat stress was also very low, about 51.75% of yield under optimum growing conditions. High temperatures, like drought stress, affect grain yield by reducing the number and weight of kernels (Cairns et al., 2013). Under heat stress conditions the number of successful ovules fertilised is reduced (Schoper et al., 1987) because pollen production and viability is compromised. The position of the tassel gives maximum exposure to heat stress, which damages the pollen, leading to lack of pollen viability (Magorokosho, 2006). Thomson et al. (1966) demonstrated that a temperature increase of 6ºC during grain filling stage causes about 10% yield loss. Dale (1983) observed a negative inverse relationship between maize yield and temperature rise from 32ºC during this sensitive period. Lobell et al. (2011) showed that for every degree day in excess of 30ºC, maize loses yield by 1% and 1.7% under optimum growing conditions and drought stress respectively. During grain filling, heat stress affects cell division, sugar metabolism and starch biosynthesis, thereby reducing dry matter accumulation in the grain (Monjardino et al., 2005). Maize grain mass is a function of rate and duration of grain filling, both of which are affected by temperature. High

temperatures hasten grain filling and also reduce endosperm starch content, resulting in poorly filled grains with reduced mass.

Though it is generally known that high temperatures during the grain filling stage in maize reduces maize yields, in this study grain yield under a combination of drought and heat stress in both 2014 and 2015 and under combined analysis of data from 2014 and 2015, was higher than under managed drought stress. This may be explained by thermo-tolerances reported by Sung et al. (2003). Managed drought stress trials were planted in July when the temperatures were still cool so it did not acquire thermo-tolerance to protect it from the vagrancy of moisture stress and elevated temperatures at the critical reproductive and grain filling stages. However the trial for combined drought and heat stress was planted mid-August when temperatures were already elevated so they were exposed to high temperatures from the early stages during the vegetative stage and maybe high temperatures activated genes that made them tolerant to higher temperatures during the reproductive and grain filling stages. In 2015 there were also higher temperatures than in 2014 as a result of a heat wave which was experienced in the country in the months of September and October. This observation is of importance to plant breeding, considering the perceived effects of climate change on temperature regimes.

The inconsistency in genotype ranking observed in this study points to the existence of genotype x environmental interaction (GEI), hence there is need to evaluate the genotypes for their interaction with the different environments. Several studies reported significant GEI for maize grain yield (Bänziger et al., 2000; Bänzinger and Diallo, 2004; Kamutando et al., 2013). It is also prudent to evaluate the stability of the genotypes across the different environments and seasons. There were highly significant yield differences across locations and years, indicating that the prevailing conditions in the two years were different at all the locations. De Souza et al. (2009) also observed a significant effect of environment on maize grain yield. The differences in prevailing growing conditions across years and locations give rise to significant GEI, resulting in inconsistency in genotype ranking in the different years and environments. GEI also indicates genotypes that are stable and unstable for production in fluctuating environments, which normally exists in the smallholder farmer's fields, as they rely on erratic rainfall with little or no external inputs. However, it is disappointing that across all locations not one experimental provitamin A hybrid showed a consistent yield advantage, which is a big challenge to

plant breeders who want to select stable hybrids. Voltas et al. (2002) showed that GEI significantly reduced the relationship between the phenotype and genotype, because the phenotype cannot depict the value of the genotype. Because of this, it is important to quantify the influence of the environment and GEI interaction on the phenotype to improve the effectiveness of selection and the usefulness of hybrids. It is also imperative to study the yield stability of genotypes across diverse environments. Experimental hybrids performed differently by performing well in one environment and poorly in another. This means it might be possible to select hybrids with good yield potential for specific target environments.

Genotypes in this study were significantly different for grain texture at all six environments that were evaluated in 2014 and 2015 and combined for 2014 and 2015 except under random drought stress for 2014. Grain texture, refers to hardness (flintiness) or softness (dent) of the kernel. Flint kernels have relatively higher percentages of amylopectin starch formed by branched chain high molecular weight glucose molecules. Soft kernels have a relatively higher percentage of amylose starch formed by straight chain glucose molecules. Regular maize contains 70-76% amylopectin and 24-28% amylose (Watson, 2003). Gazza et al. (2008) reported that grain texture was affected by kernel weight. The different environmental factors in this study, such as drought stress, low N stress and a combination of drought and heat affect kernel weight, which might be the cause of texture variability. Most maize cultivars in Zimbabwe are dent or semi-dent, reflecting the preferences of the market. This may be because it is cheaper to process dent maize into maize meal. Dent maize types are also high yielding and yield is the main trait looked for by farmers. Most of the experimental hybrids were semi-dent, moderate or semi-flint, which means they would be acceptable for Zimbabwean farmers, who are currently cultivating mostly dent cultivars.

3.6 Conclusions

Agronomic evaluation of the provitamin A hybrids under different environmental conditions showed that experimental hybrids performed significantly different in all the environments, except under low N stress. Selection of improved experimental hybrids can be done under all environments except under low N stress. There was significant GEI, resulting in inconsistence in ranking of experimental hybrids in different in environments. There is need to study the stability of these experimental hybrids in these environments. It

is possible to select experimental hybrids targeted for specific environments. Most experimental hybrids have moderate texture which should make them acceptable to farmers because of ease of processing and storage.

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CHAPTER 4

HETEROSIS AND COMBINING ABILITY OF PROVITAMIN A AND DROUGHT TOLERANT INBRED LINES FOR GRAIN YIELD UNDER ABIOTIC STRESS AND OPTIMAL CONDITIONS

4.1 Abstract

Maize is an important staple crop in SSA. However, its production is seriously constrained by various abiotic stresses occurring during its ontogeny, drastically reducing its yield. What is also disturbing is that current cultivated and consumed maize in the SSA region is of poor nutritional value and devoid of vitamin A. To have maximum gain in maize breeding programmes for abiotic stress and high nutritional quality and develop effective breeding strategies for stress tolerance and nutritional quality, it is important to understand gene action for grain yield and quality under the prevailing stresses. Information on general combining ability (GCA) and specific combining ability (SCA) of maize grain yield has been well documented both in temperate and tropical germplasm. However, little information is available on combining ability of provitamin A inbred lines under various abiotic stresses prevalent in SSA agricultural systems. Combining ability and heterosis of provitamin A elite parental inbred lines and drought tolerant elite inbred testers for grain yield was estimated to identify the best parents to use for hybrid and synthetic cultivar development under abiotic stress. Twenty-two elite provitamin A lines and five elite drought tolerant testers were crossed following a line × tester scheme and 30 single cross hybrids were generated out of a potential of 110. The 30 hybrids and five checks were evaluated in Zimbabwe under optimum conditions, random drought stress, managed drought stress, combined drought and heat stress, low N stress and low P stress in 2014 and 2015. Results for combining ability analysis showed highly significant (P < 0.01) effects for both GCA and SCA, meaning that both additive and non-additive gene actions were important in expression of grain yield under all the abiotic stresses and optimum conditions. Variances due to SCA were larger than that due to both line and tester GCA combined, which means non-additive gene action was most important in yield. There was a positive relationship between grain yield and SCA. This was also reflected in high heterosis and consequently higher yields. Higher heterosis was expressed under stress conditions than under optimum conditions. Narrow sense heritability was high (> 50%) under optimal conditions, managed drought stress, combined and drought

and heat stress and low P stress, indicating the importance of additive genetic variance in the expression of grain yield under those conditions. Lines 6, 7 and 8 and tester 1 and 2 should be used when breeding for higher potential areas. The parents to consider when breeding for random drought stress, were lines 4, 8, 9, 7, 10, 6 and tester 2. The ideal parents for managed drought stress were lines 3, 8 and 10 and tester 1 and 2, for a combination of drought and heat stress lines 3, 8, 7, 10 and 4 were desirable. Hybrids that perform well under low N stress should contain lines 6, 7, 9, 8, and 5 as parents. Lines 6, 4, 7, 3, 8 and 1 and tester 3 had positive GCA values under low P stress conditions, suggesting they can be desirable parents when breeding for varieties that do well under these conditions.

4.2 Introduction

Population growth and climate change are forcing resource poor subsistence farmers to grow maize under adverse conditions that are arid with poor soil fertility (Prasanna, 2011). Edmeades et al. (2011) reported that about 85% of the 160 million ha of maize grown throughout the globe is rainfed and is exposed to random stress. The rainfed production system is exposed to adverse climatic condition which may be exacerbated by the ongoing climate change (Zaidi and Cairns, 2011; Cairns et al., 2013). Drought is recognized as the most important constraint across the rainfed production systems. Resource poor households in SSA, however, cannot afford irrigation infrastructure and artificial fertilisers to boost their production systems and negate aridity and poor soil fertility (Hulme, 1996; IPCC, 1998; Jiri et al., 2015) leaving development of varieties tolerant to these stresses as the only viable option (Edmeades et al., 2011).

The major abiotic stresses common in SSA are low N and drought stress (Bänziger et al., 1999), and acid soils which render P unavailable (Pandey et al., 2007), resulting in low P stress. Temperatures during the growing season are also increasing and are also expected to reduce maize yields (Battisti and Naylor, 2009; Lobell et al., 2011). This highlights the need to develop provitamin A maize which is tolerant to drought, high temperature and low soil fertility stress. Development of provitamin A maize varieties tolerant to the prevailing abiotic and biotic stress is the only way to guarantee their adoption by resource poor farmers. Breeding elite maize germplasm with tolerance to multiple abiotic stresses is, however, a challenge (Edmeades et al., 2011) and very little is known about stress

combinations and comparing the effects of different stresses is an important step forward in understanding plant behaviour in the field (Laurentius et al., 2008). This calls to the need to test provitamin A maize under different stress conditions to see how they perform, and come up with appropriate recommendations for breeders and farmers. Concurrent screening for drought and low N can lead to the development of superior germplasm with tolerance to both stresses (Bänziger et al., 2006). Significant spill-over of tolerance of one abiotic stress to another, has also been observed, for example, from drought to low N tolerance (Zaidi et al., 2004; Bänziger et al., 2006) and from drought to water logging stress tolerance (Zaidi et al., 2008). The use of managed stress environments and/or wide area testing has contributed significantly to gain in maize yields (Bänziger et al., 2000; Campos et al., 2006). Selection in hot environments under drought identifies recombinants with superior heat tolerance during the sensitive flowering period (Edmeades et al., 2011). All these findings suggest that it is possible to come up with provitamin A varieties tolerant to more than one stress or multiple stresses found in the field.

Maize provides food, feed and nutritional security in some of the world's poorest regions in Africa, Asia, and Latin America (Prasanna, 2011). More than 310 million people subsisting on maize (Shiferaw et al. 2011) live on income of less than US$ 2 per day (Prasanna, 2011) and because of low incomes, these people cannot afford to diversify their diets and to purchase animal products. Micronutrient malnutrition, caused by inadequate consumption or utilization of iron, zinc or vitamin A, compromises the health of these people (Pixley et al., 2011). These people also consume white maize, which is devoid of vitamin A. If these people cultivate and consume provitamin A maize, vitamin A malnutrition will be greatly alleviated. Since more than 85% of the maize produced worldwide is used directly for food and feed, mostly by poor people, enhancement of nutritional quality of the crop is an important breeding objective.

Maize exhibits wide genetic diversity (Liu et al., 2003) and also considerable natural variation for kernel carotenoids (Wurtzel, 2004; Harjes et al., 2008), with some lines accumulating as much as 66.0 µg g^{-1} (Harjes et al., 2008). However, most yellow maize grown and consumed throughout the world has only 0.5 to 1.5 µg g^{-1} β-carotene. Recently developed provitamin A maize varieties have β-carotene levels of about 15 µg g^{-1} (Pixley et al., 2007; Pixley et al., 2011) and even as high as 25 µg g^{-1} (USDA, 2007). This can

support about 57% of daily needs of vitamin A required by human beings (Pixley et al., 2010).

Breeding maize cultivars with high nutritional quality has been one of CIMMYT's goals for many years (Krivanek et al., 2007; Gunaratna et al., 2010). HarvestPlus and its partners, including CIMMYT, IITA and ZARI (Zambian Agriculture Research Institute) have been working for more than a decade on provitamin A biofortification of maize to help alleviate vitamin A malnutrition, which affects millions of people living in low and medium income countries (IITA, 2010). CIMMYT's maize biofortification programme is primarily devoted to inbred line and hybrid development. The general breeding strategy is to cross elite lines from breeding programmes in Africa, Mexico and elsewhere with source lines that have 10-20 $\mu g\ g^{-1}$ of provitamin A carotenoids (Pixley et al., 2011). Since lines with high provitamin A carotenoids are found in temperate germplasm, to benefit SSA, which is most affected by vitamin A malnutrition, there is need to test them for combining ability and heterosis under various abiotic stresses affecting farmers in the region. This will assist in designing a plant breeding programme and elucidating the breeding value of these parental lines for development of adapted provitamin A maize enriched hybrids and synthetic varieties (Panhawar et al., 2008).

Both GCA and SCA are powerful tools used by breeders in selecting best parents for further crosses to develop hybrids and synthetic cultivars (Mahgoub, 2004; Shukla and Pandey, 2008) and there is need to study them in provitamin A germplasm. The objective of this study was to estimate combining ability and heterosis of provitamin A lines under abiotic stress conditions prevalent in most smallholder farmer agricultural systems, so as to identify useful parents to breed provitamin A hybrids or synthetic varieties adapted for smallholder growing conditions. The abiotic stress tolerant provitamin A varieties developed will go a long way in mitigating vitamin A deficiency among poor populations in low and middle income countries whose diets are based on maize with little, if any animal products.

4.3 Materials and methods

The study was conducted at the sites and environments describe in Chapter 3 Section 3.3. The same genotypes presented in Appendix 1 and 2 were analysed.

4.3.1 Statistical analysis

ANOVA for every environment and combined environments were computed using Fieldbook based R-Statistic package (R Core Team, 2015). Genotypes were taken as fixed effects whilst replicates and incomplete blocks were taken as random effects. R programme for line x tester analysis was used to calculate the GCA and SCA effects according to the method of Singh and Chaudhary (1977). The line x tester model used was as follows.

$$Y_{ijkl} = \mu + a_i + b_{kj} + g_i + g_j + s_{ij} + \varepsilon_{ijkl}$$

where,

Y_{ijkl} = observed value from each experimental unit

μ = population mean

a_l = location effect

b_{kl} = block or replication effect within each location

g_i = GCA for the ith parental line

g_j = GCA effect of the jth tester

s_{ij} = SCA for the ijth F1 hybrid

ε_{ijkl} = residual effect

Heterosis was estimated only for Harare optimum and low N stress and Chiredzi managed drought stress. For drought stress, Chiredzi Research Station data was considered. Mid-parent heterosis was calculated as the difference between the F1 hybrid mean and the average of its parents (Falconer and Mackay, 1996) as follows:

$$MPH = \frac{F1 - MP}{MP} \; x \; 100$$

where F1 is the mean of the F1 hybrid performance,

$$MP = \frac{P1 + P2}{2}$$

in which P1 and P2 are the means of the inbred parents, respectively.

Broad sense heritability of all the traits was calculated using the broad sense formula according to Hallauer and Miranda (1995).

$$H^2 = \frac{\delta_g^2}{\delta_g^2 + \delta_{gxe}^2 + \delta_e^2}$$

where:

H^2 = broad sense heritability,

δ^2 = variance component for genotype effects

δ^2_{g+e} = variance component for interaction between genotype and environment,

δ^2_e = variance component for residual effects.

Narrow sense heritability of all the traits was calculated using the narrow sense heritability formula according to Hallauer and Miranda (1995).

$$h^2 = \frac{gca}{gca + sca + error}$$

where:

h^2= narrow sense heritability

gca = General combining ability

sca= Specific combining ability

4. 4 Results

4.4.1 Combining ability of provitamin A maize elite inbred lines and elite drought tolerant testers across environments and seasons

ANOVA for combining ability analysis across locations (Table 4.1) showed highly significant variances ($P < 0.01$) for line GCA, tester GCA, SCA, location x entry, location x GCA, location x SCA in both 2014 and 2015. Only location effect was highly significant ($P < 0.01$) across seasons, entry and GCA_f effects were significant ($p < 0.05$) and GCA_m, SCA, location x entry, location x GCA_f, location x GCA_m and location x SCA were not significant. The highly significant GCA_f and GCA_m mean squares in both 2014 and 2015 suggest that the line and tester combinations were not consistent across sites. Both line GCA and testers GCA were highly significant in both 2014 and 2015, suggesting that both lines and testers contributed significantly to grain yield across sites in both seasons. Across seasons only line GCA was significant. Across season analysis

suggests that GCA_m was consistent across sites, but GCA_f was not consistent. The highly significant ($P < 0.01$) location x entry interaction in both 2014 and 2015 suggests that the performance of the genotypes was variable across the sites, but across season analysis suggested otherwise. The significant GCA and SCA variances in both 2014 and 2015 means they are both important for grain yield. However, across season analysis suggests that only GCA_f was important and GCA_m and SCA were not important. SCA mean squares were larger than both GCA_f and GCA_m mean squares in both years, suggesting that non-additive effects played a more important role than additive effects in the expression of grain yield in maize grown under optimum conditions.

The highly significant ($P < 0.01$) in 2014 and 2015, and significant ($p < 0.05$) across season mean squares for entry suggests that the different combinations of lines and testers exhibited significant difference for grain yield. Significant location by entry GCA interaction points to the existence of GEI calling for the need to carry out grain yield stability analysis across sites in both seasons. However, across season there was no GEI, since location x entry GCA were not significant (Table 4.1). Line x tester (SCA) variance was significant, meaning non-additive gene action was also important in grain yield expression across sites in both seasons. Line x tester variance was higher than both line and tester contribution, meaning both additive and non-additive gene action was very important in grain yield expression. Location x line and location x tester interaction were also significant, implying that there was also a GEI of lines and testers across environments.

Table 4.1 Mean squares for combining ability across sites in 2014, 2015 and across years

	2014	2015	Across years
Source of variation	MS	MS	MS
Loc replication	2.50*	2.42	0.37
Loc	222.39**	281.35**	715.13**
Entry	8.43**	8.67**	9.81*
GCA_f	9.60**	8.22**	13.64*
GCA_m	6.75**	26.56**	14.99
SCA	7.43**	7.50**	8.42
Loc x Entry	3.18**	2.69**	7.35
Loc x GCA_f	3.71**	3.25**	6.41
Loc x GCA_m	6.99**	7.35**	17.22
Loc x SCA	2.57**	1.84*	6.90
Residuals	1.30	1.36	6.02

MS = mean squares, GCA_f = female general combining ability, GCA_m = male general combining ability, * $p < 0.05$, ** $p < 0.01$

GCA values for lines 6, 7 and 8 were positive but not significant ($p > 0.05$) under optimum conditions, which mean they contributed to grain yield in the environment. GCA values for grain yield across sites showed that lines 6, 7 and 8 contributed positively to grain yield in both 2014 and 2015 (Table 4.2). Lines 9 and 10, which had positive GCA values in 2014, had negative values in 2015 and line 3, which had a negative GCA value in 2014, had a positive GCA value in 2015. Lines 3, 6, 7 and 8 had positive nonsignificant GCA across seasons. Lines 1, 2 and 5 had negative GCA values in both 2014 and 2015, therefore these three lines contributed negatively to grain yield in both years. However, the GCA values were not significant. Line 10 had a GCA value of zero across seasons, suggesting that it had no net contribution to grain yield. Lines 6, 7, and 8 with positive though nonsignificant GCA in 2014, 2015 and across seasons should be considered for breeding for high yields for high potential areas.

All the testers showed positive GCA values in 2014 season (Table 4.3), however, in 2015 season and across seasons, tester 3 showed negative GCA values suggesting it contributed negatively to grain yield, however, it was not significant in 2015 and on average across seasons.

Table 4.2 Across site GCA effects of lines for grain yield (t ha^{-1}) planted at 10 sites in Zimbabwe in 2014, 2015 and across years

	2014			2015			Across years		
Line	Line mean	Line GCA	GCA rank	Line mean	Line GCA	GCA rank	Line mean	Line GCA	GCA rank
1	3.09	-0.35	8	3.43	-0.07	6	3.25	-0.21	8
2	2.68	-0.75	10	3.13	-0.36	8	2.91	-0.56	10
3	3.22	-0.21	7	3.88	0.39	3	3.55	0.08	4
4	3.66	0.23	5	3.12	-0.37	9	3.39	-0.08	6
5	3.01	-0.43	9	3.22	-0.27	7	3.12	-0.35	9
6	3.94	0.51	2	3.84	0.35	4	3.89	0.42	2
7	3.67	0.23	4	4.10	0.60	1	3.88	0.41	3
8	3.99	0.56	1	3.96	0.47	2	3.98	0.51	1
9	3.68	0.25	3	2.80	-0.69	10	3.28	-0.18	7
10	3.44	0.01	6	3.49	-0.00	5	3.47	0.00	5

GCA = general combining ability

Table 4.3 Across site GCA effects of testers for grain yield (t ha^{-1}) planted at 10 sites in Zimbabwe in 2014 and 2015

	2014			2015			Across years		
Tester	Tester mean	Tester GCA	Tester GCA rank	Tester mean	Tester GCA	Tester GCA rank	Tester mean	Tester GCA	Tester GCA rank
1	3.63	0.16	1	3.63	0.14	2	3.64	0.17	1
2	3.26	0.16	3	3.79	0.29	1	3.52	0.05	2
3	3.42	0.16	2	3.06	-0.43	3	3.24	-0.23	3

GCA = general combining ability

4.4.2 Combining ability of provitamin A maize elite inbred lines and elite drought tolerant testers under optimum conditions in 2014 and 2015

Location effect was significant ($p < 0.05$) and highly significant ($P < 0.01$) in 2014 and 2015 respectively (Table 4.4). Entry was highly significant ($P < 0.01$), suggesting that genotypes performed differently and it is possible to select the genotypes under optimal environments. Both line and tester GCA was highly significant both in 2014 and 2015,

suggesting that the lines and testers contributed differently to yield. SCA was also highly significant ($P < 0.01$), suggesting the importance of non-additive gene action on grain yield expression. Location x entry was highly significant ($P < 0.01$), suggesting the genotypes performed differently in the two optimal locations. The larger magnitude of GCA_f and GCA_m mean squares compared to location x GCA_f and location x GCA_m mean squares suggests that interaction effects may be of relatively little importance. Location x GCA_f interaction was highly significant in 2014 ($P < 0.01$) and not significant in 2015. Location x GCA_m interaction was significant ($p < 0.05$) in 2014 and not significant in 2015.

4.4.3 Combining ability of provitamin A maize elite inbred lines and elite drought tolerant testers under random drought stress conditions in 2014 and 2015

Location effect was highly significant ($P < 0.01$) in both years and entry was highly significant ($P < 0.01$) in 2014 and significant ($p < 0.05$) in 2015 (Table 4.4). Significant differences ($P<0.05$) were observed among the entries. GCA_f was not significant in 2014 and highly significant ($P < 0.01$) in 2015 and contrary to GCA_f, GCA_m was highly significant ($P < 0.01$) in 2014 and not significant in 2015. SCA was not significant. Location x entry was significant ($p < 0.05$). Loc x GCA_f was highly significant ($P < 0.01$) in 2014 and not significant in 2015 and on the other hand location x GCA_m was significant in both years ($p < 0.05$). Location x SCA was significant ($p < 0.05$) in 2014 and highly significant ($P < 0.01$) in 2015. Six lines had positive GCA values, ranking from the highest 4, 8, 9, 7, 10 and 6 (Table 4.6). These lines contributed positively to grain yield under random drought stress and should be considered as parents when breeding for higher yields under random drought stress. Tester 1 and 3 had negative GCA values, suggesting that they contributed to reduced yield under random drought stress conditions (Table 4.7). When breeding for higher yields under random drought stress conditions, tester 2 should be used as one of the parents since it contributed positively to grain yield.

4.4.4 Combining ability of provitamin A maize elite inbred lines and elite drought tolerant testers under managed drought stress conditions in 2014 and 2015

Combining ability analysis revealed highly significant differences ($P < 0.01$) between entries in both years (Table 4.4). Location was, however, not significant in 2014 and highly significant in 2015. GCA_f was highly significant ($P < 0.01$) in both years but GCA_m was significant ($p < 0.05$) in 2014 and highly significant ($P < 0.01$) in 2015.

Significance GCA suggests the importance of additive gene action in maize grain yield expression under drought stress. SCA effects were significant ($p < 0.05$) in both years, suggesting importance of non-additive gene action in grain yield expression under managed drought stress. Location x entry interaction was not significant in 2014, however, significant differences ($p < 0.05$) were observed in 2015. Significant location x GCA_f interaction was observed in 2015 and the interaction was not significant in 2014. GCA_m interaction was similar in both years. Location x SCA interaction was also not significant in 2014 but was significant ($p < 0.05$) in 2015. For individual trials under stress conditions, entry, GCA_f and GCA_m variances were significant under combined heat and drought stress in 2014 under low N stress in 2015 and low P stress in 2014 (Table 4.5). Lines 3, 8 and 10 also had positive GCA for yield under managed drought stress (Table 4.6), suggesting they may be considered as parents when breeding cultivars tolerant to drought stress. Tester 1 and 2 contributed positively to grain yield under managed drought stress condition (Table 4.7). Tester 3 with negative GCA value might not be considered as a parent when breeding for drought tolerant cultivars.

Table 4.4 Mean squares for combining ability for optimum, random drought stress and managed drought stress for 2014 and 2015

	Optimum		Random drought stress		Managed drought stress	
Source of variation	2014	2015	2014	2015	2014	2015
Replication	3.19	1.16	2.14	0.94	4.56**	1.75*
Loc	19.29*	44.81**	282.30**	177.53**	0.95	6.63**
Entry	11.99**	11.95**	3.45*	4.21*	1.43**	1.37**
GCA_f	14.53**	14.20**	1.66	7.10**	4.19**	3.85**
GCA_m	32.74**	32.15**	21.23**	0.60	1.10*	2.19**
SCA	8.63**	8.59**	2.40	2.81	1.29*	0.93*
Loc x Entry	5.17**	1.52**	3.14*	3.63*	0.62	0.84*
Loc x GCA_f	7.97**	1.82	4.27**	2.49	0.26	1.76*
Loc x GCA_m	5.09*	3.81	5.95*	4.52*	0.10	0.71
Loc x SCA	3.40*	1.11	2.24*	4.36**	0.92	0.78*
Residuals	1.93	2.22	1.54	1.76	0.54	0.38

GCA_f = line general combining ability, GCA_m = tester general combining ability, SCA = specific Combining ability * $p < 0.05$, ** $p < 0.01$, loc = location

4.4.5 Combining ability of provitamin A maize elite inbred lines and elite drought tolerant testers under combined drought and heat stress conditions in 2014 and 2015

Significant differences ($p<0.05$) were observed among the entries in 2014, but not in 2015 (Table 4.5). GCA_f was significant in 2014 and not significant in 2015. Highly significant differences ($P < 0.01$) were observed for GCA_m in 2014 and in 2015 it was significant ($p < 0.05$). SCA was not significant in both years, suggesting predominantly additive gene action in expression of grain yield under combined drought and heat stress. Under combined drought and heat stress lines 3, 8, 7, 10 and 4 were desirable for yield increase, since they had positive GCA values (Table 4.6). However the GCA values were not significant. All the testers did not contribute to yield (Table 4.7).

4.4.6 Combining ability of provitamin A maize elite inbred lines and elite drought tolerant testers under low N stress conditions in 2014 and 2015

There were significant differences ($p < 0.05$) between entries, GCA_f , GCA_m, and SCA in 2015 (Table 4.5), however in 2014 they were all similar. Under low N stress lines 6, 7, 9, 8 and 5 had positive nonsignificant GCA values, suggesting that they contributed positively to higher yields (Table 4.6). All the testers had a GCA value of zero,

suggesting that they made no significant contribution to grain yield under low N stress (Table 4.7).

4.4.7 Combining ability of provitamin A maize elite inbred lines and elite drought tolerant testers under low P stress conditions in 2014

Highly significant differences ($P < 0.01$) were found for entries, GCA_f, and GCA_m in 2014 (Table 4.5). SCA was not significant. There was no data to analyse for 2015. Under low P stress lines 6, 4, 7, 3, 8 and 1 had positive GCA values (Table 4.6) hence they contributed positively to grain yield. Only tester 3 had positive GCA (Table 4.7), suggesting that it may be the only tester worth considering as a parent when breeding for low P stress.

Table 4.5 Mean squares for combining ability for Harare low N stress, low P stress and Chiredzi combined drought and heat stress for 2014 and 2015

	Combined drought heat stress		Low N stress		Low P stress
Source of variation	2014	2015	2014	2015	2014
Replication	1.20	4.16	2.35*	4.99*	0.00
Entry	2.56*	2.09	0.64	2.69*	4.05**
GCA_f	2.67*	2.44	0.63	2.36*	6.92**
GCA_m	10.31**	4.33*	1.00	7.99*	10.03**
SCA	1.64	1.67	0.60	2.27*	1.95
Residuals	0.97	1.28	0.51	1.09	1.21

GCA_f = line general combining ability, GCA_m = tester general combining ability, SCA = specific combining ability, * $p < 0.05$, ** $P < 0.01$

Table 4.6 General combining ability effects of lines for grain yield (t ha^{-1}) at six environments planted in Zimbabwe in 2014 and 2015

	Optimum		Random drought stress		Managed drought stress		Combined drought and heat stress		Low N stress		Low P stress	
Line	GCA	rank	GCA	rank	GCA	rank	GCA	rank	GCA	rank	GCA	rank
1	-0.20	8	-0.03	7	-0.05	6	-0.04	6	-0.30	9	0.05	6
2	-0.42	10	-0.21	10	-0.11	7	-0.09	7	-0.55	10	-1.43	10
3	-0.06	5	-0.20	8	0.77	1	0.40	1	-0.22	8	0.17	4
4	-0.33	9	0.17	1	-0.18	8	0.07	5	-0.05	6	0.48	2
5	-0.18	7	-0.20	9	-0.04	4	-0.23	9	0.03	5	-0.25	8
6	0.65	1	0.06	6	-0.19	9	-0.20	8	0.70	1	1.53	1
7	0.40	2	0.08	4	-0.04	5	0.18	3	0.45	2	0.34	3
8	0.32	3	0.14	2	0.48	2	0.33	2	0.06	4	0.07	5
9	-0.06	4	0.11	3	-0.57	10	-0.53	10	0.08	3	-0.10	7
10	-0.13	6	0.08	5	0.03	3	0.12	4	-0.20	7	-0.85	9

GCA = general combining ability, N = nitrogen, P = phosphorous, t ha^{-1} = ton per hectare

Table 4.7 General combining ability effects of testers for grain yield (t ha^{-1}) at six environments planted in Zimbabwe in 2014 and 2015

Tester	Optimum		Random drought stress		Managed drought stress		Combined drought and heat stress		Low N stress		Low P stress	
	GCA	rank	GCA	rank	GCA	rank	GCA	rank	GCA	rank	GCA	rank
1	0.40	1	-0.04	2	0.16	2	0.00	1	0.00	1	-0.01	2
2	-0.41	3	0.20	1	0.19	1	0.00	1	0.00	1	-0.56	3
3	0.01	2	-0.16	3	-0.35	3	0.00	1	0.00	1	0.56	1

GCA = general combining ability, N = nitrogen, P = phosphorous, t ha^{-1} = ton per hectare

4.4.8 Combining ability of provitamin A maize elite inbred lines and elite drought tolerant testers analysed across seasons

Location was not significant across all environments. Entry and GCA$_f$ were highly significant (P < 0.01) under optimum, managed drought stress and combined drought and heat stress and were not significant under random stress and low N stress (Table 4.8). The highly significant entry suggests that the genotypes performed differently and one can select desirable genotypes in all the environments. GCA$_m$ was highly significant (P < 0.01) under optimum, managed drought stress and significant (p < 0.05) under combined drought and heat stress, but was not significant under random stress and low N stress. The significant GCA suggests the importance of additive gene action in expression of grain yield. SCA was highly significant (P < 0.01) under optimum and significant (p < 0.05) under managed drought stress and combined drought and heat stress; this suggests the importance of non-additive gene action in expression of grain yield in maize. However, SCA was not significant under random drought stress and low N stress, suggesting that non additive gene action was not important in expression of grain yield under random drought stress and low N stress. Location x entry and location x GCA$_f$ were highly significant (P < 0.01) under optimum and not significant in the other four environments. Since both GCA and SCA were significant it means both additive and non-additive gene action were important. Location x GCA$_m$ was highly significant (P < 0.01) under optimum and significant (p < 0.05) under combined drought and heat stress but not significant in the other three environments. Location x SCA was highly significant (P < 0.01) under optimum condition and significant (p < 0.05) under managed drought stress, but was not significant in the other environments. Line 8 contributed positively to grain

yield across all environments (Table 4.9). Lines 1 and 2 contributed negatively to grain yield across all the environments. Line 3 was favourable under managed drought stress and combined drought and heat stress and unfavourable under optimum, random drought stress and low N stress. Line 4 had negative GCA under optimum, managed drought stress and low N stress, suggesting it contributed negatively to grain yield in those environments. Lines 6, 7, 8 and 9 contributed positively to grain yield under low N stress and the rest of lines contributed to reduced yields. The ideal lines under optimum conditions were 6, 7 and 8, which had positive GCA; the rest of the lines had negative GCA, suggesting they contributed negatively to grain yield under optimum conditions. Lines 4, 6, 7, 8, 8, and 10 contributed positively to grain yield under random drought stress and lines 1, 2, 3 and 5 had negative GCA values, suggesting that they were unfavourable. Under managed drought stress lines 3, 8 and 10 should be considered as parents since they had positive GCA values. Lines 3, 4, 7, 8 and 10 had positive GCA values, suggesting they contributed positively to grain yield under the environment.

Under optimum conditions, tester 1 and 2 had positive GCA (Table 4.10), suggesting they were good parents to use when breeding for higher yield under optimum condition. Only tester 2 was favourable under random drought stress. Under managed drought stress tester 2 was desirable and tester 3 was undesirable. Tester 1 and 2 had positive GCA values under combined drought and heat stress, suggesting they are favourable parents to use when breeding for tolerance to combined drought and heat stress environment. Line had positive GCA values and the other two had negative GCA values suggesting that only line 1 was a good parent for breeding hybrids with tolerance to low N stress.

Table 4.8 Mean squares for combining ability across seasons of provitamin A and drought tolerant lines grown in optimal and different abiotic stress conditions

	Optimum	Random drought stress	Managed drought stress	Combined drought and heat stress	Low N stress
	MS	MS	MS	MS	MS
Source of variation	MS	MS	MS	MS	MS
Replication	1.07	1.50	0.41	2.69	3.67
Loc	0.94	2.43	1.05	34.45	135.70
Entry	13.08**	4.17	1.94**	3.35**	1.35
GCA_f	18.98**	5.14	6.59**	4.34**	1.59
GCA_m	31.73**	12.72	2.99**	6.94*	3.30
SCA	7.95**	2.72	1.09*	2.46*	1.01
Loc x Entry	10.86**	3.50	0.85	1.30	1.98
Loc x GCA_f	9.70**	3.84	1.41	0.76	1.40
Loc x GCA_m	32.70**	8.98	0.41	7.69*	5.70
Loc x SCA	9.35**	2.75	1.11*	0.84	1.86
Residuals	2.80	5.39	0.64	1.12	0.80

MS = mean squares, GCA_f = line general combining ability, GCA_m = line general combining ability, SCA = specific combining ability, loc = location, * $p < 0.05$, ** $P < 0.01$

Table 4.9 General combining ability effects of lines for grain yield (t ha^{-1}) at five environments planted across seasons

Line	Optimum			Random drought stress			Managed drought stress			Combined drought and heat stress			Low N stress		
	Line mean	GCA	GCA rank	Line mean	GCA	GCA rank	Line mean	GCA	GCA rank	Line mean	GCA	GCA rank	Line mean	GCA	GCA rank
1	5.56	-0.55	8	3.35	-0.09	7	1.26	-0.05	6	2.55	-0.05	6	3.13	-0.30	9
2	5.03	-1.08	10	2.89	-0.56	10	1.20	-0.11	7	2.39	-0.21	7	2.87	-0.55	10
3	5.96	-0.15	5	2.98	-0.46	8	2.08	0.76	1	3.43	0.84	1	3.20	-0.22	8
4	5.27	-0.84	9	3.87	0.42	1	1.13	-0.18	8	2.75	0.16	5	3.37	-0.05	6
5	5.66	-0.45	7	2.94	-0.51	9	1.28	-0.04	4	2.08	-0.52	9	3.45	0.03	5
6	7.79	1.68	1	3.63	0.18	5	1.13	-0.18	9	2.16	-0.44	8	4.12	0.70	1
7	7.16	1.05	2	3.66	0.22	4	1.27	-0.04	5	2.98	0.38	3	3.88	0.45	2
8	6.93	0.82	3	3.79	0.35	2	1.79	0.47	2	3.31	0.72	2	3.48	0.06	4
9	5.97	-0.14	4	3.72	0.28	3	0.74	-0.57	10	1.45	-1.14	10	3.50	0.08	3
10	5.79	-0.32	6	3.62	0.18	6	1.34	0.03	3	2.85	0.26	4	3.22	-0.20	7

GCA = general combining ability, N = nitrogen, t ha^{-1} = ton per hectare

Table 4.10 General combining ability effects of testers for grain yield (t ha^{-1}) at five environments across seasons

Tester	Optimum			Random drought stress			Managed drought stress			Combined drought and heat stress			Low N stress		
	Tester mean	GCA	GCA rank	Tester mean	GCA	GCA rank	Tester mean	GCA	GCA rank	Tester mean	GCA	GCA rank	Tester mean	GCA	GCA rank
1	6.73	0.62	1	3.37	-0.07	2	1.32	0.00	2	2.83	0.23	2	3.75	0.33	1
2	5.47	-0.64	3	3.80	0.36	1	1.60	0.28	1	2.84	0.25	1	3.25	-0.17	3
3	6.14	0.03	2	3.17	-0.28	3	1.02	-0.29	3	2.11	-0.48	3	3.26	-0.16	2

GCA = general combining ability, N = nitrogen, t ha^{-1} = ton per hectare

SCA among the different crosses showed some variation, some combinations had positive and some negative SCA values. In 2014, the SCA values ranged from -0.849 to 0.925 and 14 crosses had positive SCA and 16 had negative SCA for grain yield. In 2015 the SCA values ranged from -1.227 to 1.085 and crosses with positive SCA were 16 and 14 crosses had negative SCA. Line 6 and tester 2 gave the highest SCA for grain yield, followed by line 8 and tester 1 cross in 2014. In 2015 line 10 and tester 1 had the highest and significant SCA, followed by a cross between line 3 and tester 3, which was not significant. A cross between line 8 and tester 1 which was ranked second in 2014, ranked third in 2015. Line 10 and tester 3 had a significant negative SCA in 2015 (Table 4.11).

The five best combiners in 2014 in each environment were 8 x 1, 6 x 2, 7 x 1, 1 x 1 and 4 x 2 optimum, 3 x 3, 10 x 1, 5 x 3, 4 x 1 and 7 x 3 random drought stress, 3 x 3, 1 x 3, 10 x 2, 7 x 3 and 8 x 2 managed drought stress, 8 x 1, 3 x 3, 3 x 1, 5 x 3 and 10 x 2 combined drought and heat stress, 7 x 2, 2 x 2, 5 x 1, 8 x 1 and 3 x 3 low N stress and 4 x 3, 1 x 1, 2 x 2, 6 x 2 and 6 x 1 low P stress (Table 4.12). In 2015 the best five combiners in each environment were 8 x 1, 6 x 2, 7 x 1, 1 x 1 and 4 x 2 optimum, 6 x 2, 3 x 3, 8 x 1, 9 x 3, 2 x 2 and 7 x 1 random drought stress, 3 x 3, 1 x 3, 10 x 1, 7 x 3 and 8 x 2 managed drought stress, 8 x 1, 3 x 3, 3 x 1, 5 x 3 and 10 x 1 combined drought and heat stress and 9 x 3, 6 x 1, 5 x 1, 10 x 2 and 8 x 1 low N stress (Table 4.13). The best combiners in combined analysis across years per environment were 8 x 1, 2 x 2, 1 x 1, 4 x 3 and 7 x 1 optimum, 3 x 3, 6 x 2, 5 x 3, 10 x 1 and 7 x1 random drought stress, 8 x 2, 9 x 2, 4 x 3, 8 x 3 and 3 x 1 managed drought stress, 8 x 1, 5 x 3, 3 x 3, 10 x 1 and 6 x 2 combined drought and heat stress and 10 x 1, 5 x 3, 8 x 1, 4 x 3 and 3 x 3 low N stress (Table 4.14).

Table 4.11 Specific combining ability estimates of provitamin A inbred lines across sites in 2014, 2015 and across years

Line x tester	2014		2015		Across years	
	SCA	rank	SCA	rank	SCA	rank
1 x 1	0.46	7	-0.08	17	0.18	10
1 x 2	0.13	13	0.14	10	0.13	12
1 x 3	-0.62	27	-0.11	19	-0.36	24
2 x 1	-0.58	26	-0.25	23	-0.42	26
2 x 2	0.65	3	0.63	4	0.64	3
2 x 3	-0.06	17	-0.30	24	-0.18	18
3 x 1	0.31	12	-0.63	28	-0.16	17
3 x 2	-0.85	29	-0.24	22	-0.53	30
3 x 3	0.57	4	0.87	2	0.72	2
4 x 1	-0.46	24	0.03	15	-0.22	20
4 x 2	0.54	5	-0.55	25	0.00	15
4 x 3	-0.07	18	0.52	6	0.23	9
5 x 1	0.40	10	-0.87	29	-0.26	21
5 x 2	-0.41	22	0.30	8	-0.05	16
5 x 3	0.02	14	0.61	5	0.32	7
6 x 1	-0.42	23	-0.13	21	-0.28	22
6 x 2	0.93	1	0.08	13	0.50	4
6 x 3	-0.47	25	0.05	14	-0.20	19
7 x 1	0.53	6	0.22	9	0.37	6
7 x 2	-0.40	21	-0.56	26	-0.47	28
7 x 3	-0.13	19	0.34	7	0.11	13
8 x 1	0.91	2	0.76	3	0.83	1
8 x 2	-0.88	30	-0.10	18	-0.48	29
8 x 3	-0.03	15	-0.61	27	-0.32	23
9 x 1	-0.84	28	-0.11	20	-0.41	25
9 x 2	0.44	8	0.13	11	0.26	8
9 x 3	0.40	9	0.01	16	0.17	11
10 x 1	-0.32	20	1.09*	1	0.39	5
10 x 2	-0.04	16	0.10	12	0.02	14
10 x 3	0.37	11	-1.23*	30	-0.43	27

SCA = specific combining ability, * $p < 0.05$

Table 4.12 Specific combining ability estimates of provitamin A elite lines and drought tolerant elite lines in six environments in 2014

Line x tester	Optimum	Random drought stress	Managed drought stress	Combined drought and heat stress	Low N stress	Low P stress
1 x 1	1.17	-0.31	-0.02	0.20	0.05	0.46
1 x 2	-1.16	0.26	-0.13	0.32	0.30	-0.42
1 x 3	-0.89	0.03	0.76	-0.47	-0.34	-0.03
2 x 1	-1.21	0.22	0.19	-0.18	-1.00	-0.56
2 x 2	-0.46	-0.08	0.10	0.18	0.62	0.34
2 x 3	-0.50	-0.15	-0.04	0.00	0.38	-0.34
3 x 1	-0.36	-0.98*	-0.07	0.42	-0.40	0.20
3 x2	-0.97	0.02	0.32	-0.17	-0.09	-0.26
3 x 3	0.18	0.95	0.83	0.56	0.49	0.13
4 x 1	-0.55	0.59	-0.94	0.00	0.17	-0.32
4 x 2	1.11	0.05	0.17	0.09	-0.33	-0.13
4 x 3	0.16	-0.65	-0.25	-0.16	0.16	0.64
5 x 1	0.35	-0.96	0.41	-0.41	0.58	0.02
5 x 2	-0.93	0.13	-0.27	-0.26	-0.24	-0.12
5 x 3	-0.27	0.81	-0.28	0.39	-0.34	0.00
6 x 1	0.25	-0.06	0.29	-0.16	0.34	0.31
6 x 2	1.52	0.05	-0.29	0.07	0.13	0.34
6 x 3	0.34	-0.01	-0.31	0.09	-0.47	-0.05
7 x 1	1.21	0.17	-0.70	0.14	0.11	0.19
7x 2	-0.50	-0.68	0.18	0.06	0.64	-0.04
7 x 3	0.64	0.49	0.44	-0.07	-0.75	-0.01
8 x 1	1.66	0.13	-0.41	0.85	0.54	0.21
8 x 2	-1.35	0.16	0.43	-0.31	-0.68	-0.39
8 x3	0.54	-0.31	0.08	-0.08	0.15	0.20
9 x 1	-1.23	0.16	0.18	-0.50	-0.04	0.00
9 x 2	1.10	0.39	-0.25	-0.32	-0.27	-0.08
9 x 3	0.78	-0.57	0.35	-0.20	0.31	0.04
10 x 1	-0.88	0.87	-0.61	0.07	-0.33	-0.51
10 x 2	-0.24	-0.34	0.66	0.34	-0.08	0.28
10 x 3	0.61	-0.60	-0.84	-0.48	0.41	-0.11

N = nitrogen, P = phosphorous * $p < 0.05$

Table 4.13 Specific combining ability estimates of provitamin A elite lines and drought tolerant elite lines in five environments in 2015

Line x tester	Optimum	Random drought stress	Managed drought stress	Combined drought and heat stress	Low N stress
1 x 1	1.17	-0.34	-0.02	0.20	-0.03
1 x 2	-1.16	0.31	-0.13	0.32	0.00
1 x 3	-0.89	-0.02	0.76	-0.47	-0.06
2 x 1	-0.88	-0.67	-0.61	0.07	-0.04
2 x 2	-0.46	0.62	0.10	0.18	0.02
2 x 3	-0.50	0.06	-0.04	0.00	0.01
3 x 1	-0.36	0.49	-0.07	0.42	-0.12
3 x2	-0.97	-1.09	0.32	-0.17	-0.09
3 x 3	0.18	0.69	0.83	0.56	0.03
4 x 1	-0.55	-0.15	-0.94	0.00	0.02
4 x 2	1.11	0.36	0.17	0.09	-0.07
4 x 3	0.16	-0.22	-0.25	-0.16	0.05
5 x 1	0.35	0.29	0.41	-0.41	0.11
5 x 2	-0.93	-0.38	-0.27	-0.26	-0.02
5 x 3	-0.27	0.09	-0.28	0.39	0.01
6 x 1	0.25	-0.11	0.29	-0.16	0.11
6 x 2	1.52	1.50	-0.29	0.07	0.06
6 x 3	0.34	-1.13	-0.31	0.09	0.00
7 x 1	1.21	0.62	-0.70	0.14	-0.02
7x 2	-0.50	-0.52	0.18	0.06	0.03
7 x 3	0.64	-0.10	0.44	-0.07	-0.13
8 x 1	1.66	0.64	-0.41	0.85	0.08
8 x 2	-1.35	-0.40	0.43	-0.31	-0.10
8 x3	0.54	-0.24	0.08	-0.08	0.05
9 x 1	-1.23	-0.84	0.18	-0.50	0.06
9 x 2	1.10	0.21	-0.25	-0.32	0.01
9 x 3	0.78	0.64	0.35	-0.20	0.12
10 x 1	-0.24	0.01	0.66	0.34	-0.02
10 x 2	0.61	-0.35	-0.84	-0.48	0.09
10 x 3	-1.21	0.34	0.19	-0.18	-0.19

N = nitrogen,

Table 4.14 Specific combining ability estimates of provitamin A elite inbred lines and drought tolerant elite inbreds across seasons

Line x tester	Optimum		Random drought stress		Managed drought stress		Combined drought and heat stress		Low N stress	
	SCA	Rank	SCA	Rank	SCA	Rank	SCA	Rank	SCA	Rank
1 x 1	1.17	3	-0.32	23	0.11	10	0.23	12	-0.31	24
1 x 2	-0.35	19	0.25	9	-0.01	19	0.38	9	0.25	9
1 x 3	-0.81	27	0.01	14	0.09	12	-0.98	28	0.07	11
2 x 1	-1.35	30	-0.21	20	-0.40	28	-0.39	22	-0.26	22
2 x 2	1.58	2	0.28	8	0.24	6	0.52	7	0.23	10
2 x3	-0.24	16	-0.05	15	-0.48	29	-0.01	17	0.03	13
3 x 1	-0.40	21	-0.24	21	0.28	5	0.19	13	-0.36	27
3 x 2	-0.32	18	-0.53	28	0.19	8	-0.95	27	-0.07	16
3 x 3	0.71	6	0.82	1	0.02	16	0.76	3	0.43	5
4 x 1	-0.66	25	0.22	10	0.11	11	-0.45	23	-0.12	18
4 x 2	-0.35	20	0.21	11	0.20	7	0.34	11	-0.34	26
4 x 3	1.00	4	-0.43	27	0.42	3	0.11	14	0.46	4
5 x 1	-0.14	15	-0.33	24	-0.61	30	-1.02	29	-0.32	25
5 x 2	-0.11	14	-0.12	18	0.03	15	0.10	15	-0.19	19
5 x 3	0.25	11	0.45	3	0.03	14	0.92	2	0.51	2
6 x 1	-0.28	17	-0.08	16	0.00	18	-0.68	25	-0.07	17
6 x 2	0.67	7	0.73	2	-0.30	24	0.70	5	0.38	8
6 x 3	-0.40	22	-0.56	29	-0.18	22	-0.02	18	-0.31	23
7 x 1	0.79	5	0.40	5	0.09	13	0.58	6	-0.42	28
7x 2	-0.72	26	-0.60	30	0.13	9	-0.59	24	0.39	7
7 x 3	-0.08	13	0.19	12	-0.37	27	0.01	16	0.03	14
8 x 1	1.83	1	0.39	6	-0.34	26	1.16	1	0.50	3
8 x 2	-1.20	29	-0.12	17	0.57	1	-0.78	26	-0.25	20
8 x 3	-0.64	24	-0.27	22	0.37	4	-0.38	21	-0.25	21
9 x 1	-1.10	28	-0.34	25	-0.30	25	-0.35	20	0.41	6
9 x 2	0.58	8	0.30	7	0.48	2	-0.08	19	-0.43	29
9 x 3	0.52	9	0.03	13	-0.01	20	0.44	8	0.03	15
10 x 1	0.14	12	0.45	4	0.02	17	0.72	4	0.95*	1
10 x 2	0.33	10	-0.36	26	-0.06	21	0.35	10	0.05	12
10 x 3	-0.48	23	-0.13	19	-0.24	23	-1.07	30	0.01*	30

N = nitrogen, * $p < 0.05$

4.4.9 Variance estimates

Broad sense heritability was very high (88.5%) under optimum conditions, 93.4% under managed drought stress, 76.2% under combined drought and heat stress and 92.2% for low P stress (Table 4.15). Narrow sense heritability for grain yield was high (above 50%) under optimum, managed drought stress, combined drought and heat stress and low P stress; however it was low (40.7%) under random drought stress (Table 4.15). Additive genetic variance was the major contributor followed by dominance variance to total variance under optimum and managed drought stress conditions. Testers contributed the least variance under optimum conditions. Under random drought stress conditions, environmental variance contributed the most to total variability, and dominance variance and line x tester variances were zero. Under combined drought and heat stress, dominance variance was the highest contributor to total genetic variability and tester variance the lowest contributor. Under low P stress, additive genetic variance was the most important. Additive genetic variance was higher than genotype variance across the environments. The differences between broad sense heritability and narrow sense heritability was 29.1% under optimum condition, 0% under random drought stress, 40.5% under managed drought stress, 22.7% under combined drought and heat stress and 17.5% under low P stress.

Table 4.15 Genetic variances, phenotypic variances and heritability estimates for line x tester crosses under different environments

	Optimum	Random drought stress	Managed drought stress	Combined drought and heat stress	Low P
Line variance	0.31	0.06	0.08	0.17	0.85
Tester variance	0.25	0.06	0.00	0.14	0.39
Line x Tester Variance	0.57	0.00	0.18	0.00	0.33
Genotype variance	1.15	0.08	0.24	0.44	1.41
Additive variance	4.61	0.33	0.96	1.74	5.63
Dominance variance	2.27	0.00	0.73	0.74	1.33
Environmental variance	0.89	0.49	0.12	0.78	0.59
Broad sense heritability	0.89	0.41	0.93	0.76	0.92
Narrow sense heritability	0.59	0.41	0.53	0.54	0.75

Mid-parent heterosis ranged from 72.0 to 407.0% under optimal conditions, -44.6 to 793.4% under managed drought stress and 154.0 to 745.3% under low N stress (Table 4.16). These high values were probably due to poor performance of inbred parents under stress conditions.

Table 4.16 Heterosis estimates of provitamin A and drought tolerant elite inbreds under optimum, managed drought stress and low N stress conditions

	Optimum				Managed drought stress				Low N stress			
Entry	GYD	P1	P2	MPH	GYD	P1	P2	MPH	GYD	P1	P2	MPH
1	7.38	2.63	1.71	240.09	1.43	1.22	0.35	82.17	3.14	0.43	0.89	375.76
2	4.65	2.21	1.51	150.00	0.68	0.43	0.39	65.85	2.93	0.30	0.72	474.51
3	6.56	1.49	1.92	284.75	1.45	0.16	0.22	663.16	3.27	0.75	0.45	445.00
4	6.18	3.74	1.71	126.79	0.85	0.25	0.35	183.33	3.58	0.53	0.89	404.23
5	6.79	2.52	1.51	236.97	1.04	0.34	0.39	184.93	3.46	1.91	0.72	163.12
6	10.2	2.52	1.92	359.46	0.77	0.35	0.22	170.18	4.39	0.59	0.45	744.23
7	8.14	2.85	1.71	257.02	1.17	0.40	0.35	212.00	3.79	1.55	0.89	210.66
8	10.4	3.49	1.51	316.00	0.96	0.49	0.39	118.18	4.32	1.17	0.72	357.14
9	6.69	2.62	1.92	194.71	0.61	0.81	0.22	18.45	4.30	0.85	0.45	561.54
10	7.76	3.07	1.71	224.69	1.61	0.37	0.35	347.22	4.50	1.18	0.89	334.78
11	5.27	2.63	1.51	154.59	2.29	1.22	0.39	184.47	3.20	0.43	0.72	456.52
12	6.57	2.21	1.92	218.16	2.09	0.43	0.22	543.08	2.92	0.30	0.45	678.67
13	5.32	1.49	1.71	232.50	1.63	0.16	0.35	539.22	2.96	0.75	0.89	260.98
14	5.04	3.74	1.51	92.00	1.32	0.25	0.39	312.50	2.85	0.53	0.72	356.00
15	5.30	2.52	1.92	138.74	0.98	0.34	0.22	250.00	3.08	1.91	0.45	161.02
16	8.48	2.52	1.71	300.95	0.86	0.35	0.35	145.71	4.33	0.59	0.89	485.14
17	5.21	2.85	1.51	138.99	1.16	0.40	0.39	193.67	4.09	1.55	0.72	260.35
18	4.72	3.49	1.92	74.49	1.77	0.49	0.22	398.59	3.06	1.17	0.45	277.78
19	5.05	2.62	1.71	133.26	1.13	0.81	0.35	94.83	2.89	0.85	0.89	232.18
20	6.16	3.07	1.51	169.00	2.09	0.37	0.39	450.00	3.10	1.18	0.72	226.32
21	3.91	2.63	1.92	71.87	0.56	1.22	0.22	-22.22	3.04	0.43	0.45	590.91
22	4.45	2.21	1.71	127.04	0.40	0.43	0.35	2.56	2.75	0.30	0.89	362.18
23	7.60	1.49	1.51	406.67	2.47	0.16	0.39	798.18	3.48	0.75	0.72	373.47
24	7.76	3.74	1.92	174.20	1.14	0.25	0.22	385.11	3.67	0.53	0.45	648.98
25	6.26	2.52	1.71	195.98	0.59	0.34	0.35	71.01	3.80	1.91	0.89	171.43
26	9.10	2.52	1.51	351.61	1.39	0.35	0.39	275.68	3.36	0.59	0.72	412.98
27	6.82	2.85	1.92	185.95	0.78	0.40	0.22	151.61	3.75	1.55	0.45	275.00
28	7.15	3.49	1.71	175.00	1.65	0.49	0.35	292.86	2.85	1.17	0.89	176.70
29	7.24	2.62	1.51	250.61	0.43	0.81	0.39	-28.33	3.37	0.85	0.72	329.30
30	7.31	3.07	1.92	192.99	0.16	0.37	0.22	-45.76	2.07	1.18	0.45	153.99

P1 = line, P2 = tester, MPH = mid-parent heterosis, GYD = grain yield,

4.5 Discussion

Qi et al. (2010) and Alamerew and Warsi (2015) reported that both GCA and SCA were important for expression of maize grain yield. Positive GCA is important for grain yield, because it means the line or tester contributes positively to grain yield and negative GCA is undesirable. Bayisa et al. (2008) observed predominantly non-additive gene action for grain yield while de Souza et al. (2009) reported significant GCA for grain yield in maize.

In this study highly significant GCA for both lines and testers were evident in some environments. Significant GCA effects were seen under all production conditions, but variances due to SCA were higher than those for line and tester GCA combined, suggesting strong non-additive gene action. Pixley and Bjarnason (1991) reported that maize grain yield was controlled mostly by additive gene action. Lines 6, 7, and 8 with overall positive GCA were identified which should be considered for breeding for high yields for high potential areas. This contrasted findings by Makumbi (2005) who found no significant GCA and SCA for grain yield under optimum growing conditions.

It is important for a breeder to identify the best lines and testers and the best hybrid combination under the proposed production conditions. Significant location x GCA interaction means that parental inbred lines for hybrid breeding for specific environments must be selected under those specific environments. Machado et al. (2009) and Alamerew and Warsi (2015) showed significance of environment x GCA and environment x SCA interaction, which means both GCA and SCA effects for grain yield were different in different environments in both season, however, across seasons it was not significant. The significant differences between location and line x tester variances in this study implies that the specific hybrid combinations were not stable across different environments. Bänziger et al. (2000), Aguiar et al. (2003), Mohammadi and Haghparast (2010), Tiawari et al. (2011) and Alamerew and Warsi (2015) also reported significant GEI on maize grain yield and other agronomic traits. The larger magnitude of location x GCA when compared with line GCA and tester GCA suggests that interaction effects were important for the expression of grain yield. The environment is very important in the expression of maize grain yield and other traits of agronomic importance (Bänziger, 2000). Hence for greater response to selection, the environment component of variation should be taken into account.

Hybrid performance can be predicted on the basis of SCA of progenies (Mutengwa et al., 2012). SCA values have been reported to be a major determinant of heterosis as well as hybrid performance and in the choice of hybrid development progeny (Hallauer and Miranda, 1995; Falconer and Mackay, 1996). Crosses between line 2 and tester 2, line 3 and tester 3 line 8 and tester 1, which showed high SCA across sites in both 2014 and 2015, should be selected for further evaluation and used as parents for development of

three way or double cross hybrids and/or released as cultivars important for all growing conditions in Zimbabwe.

Narrow sense heritability is the proportion of additive genetic variance to total phenotypic variance. It reflects the flexible component of variance through selection, leading to increased magnitude of quantitative traits (Chakraborty et al., 2010). Narrow sense heritability was high under optimal conditions, managed drought stress, combined drought and heat stress and low P stress, indicating the predominance of additive genetic variance in the expression of grain yield under these conditions. Broad sense heritability under random drought stress was equal to narrow sense heritability. This suggests that dominance and epitasis interaction was of little importance. High heritability indicates that selection for grain yield will be effective under all the environments except under random drought stress. Narrow sense heritability for grain yield was high, hence additive genetic variance was the major contributor followed by dominance variance to total variance under optimum and managed drought stress conditions.

Because of its outcrossing nature, maize exhibits heterosis, and superior combinations can be identified and used as hybrids. Heterosis under optimum conditions compared very well with values obtained by Qi et al. (2010) who observed values ranging from 28.28 to 491.96%. Under stress conditions, heterosis values were very high because of the relatively poor performance of inbred lines under these stress conditions. Ziyomo (2004) also observed similar results when evaluating maize inbred lines and their corresponding hybrids under low N stress and optimal conditions. Very high heterosis values were observed under low N stress. This study indicated the importance of heterosis in maize grain yield expression. The germplasm was diverse having originated from divergent gene pools. The lines were from Mexico, a temperate region, bred for high provitamin A carotenoids and the testers were developed for tolerance to drought stress in the Southern Africa region. There was consistence between heterosis and maize grain yield, as hybrids exhibiting high heterosis also had higher grain yields.

4.6 Conclusions

Lines and testers with positive and negative GCA values were observed in every environment. Line and tester combinations were not consistent across sites, suggesting you cannot use the same parents when breeding hybrids to be used in different environments. Lines and testers with positive GCA values must be considered as parents when one is breeding for higher yields in the environment they show positive values. Both line and tester GCA were highly significant across environments and seasons, suggesting both contributed significantly to grain yield. Both GCA and SCA were important for expression of grain yield in all the environments. Significant location x GCA interaction means that parents to be used for making hybrids for specific environments must be selected in that specific environment. Lines 6, 7 and 8 and tester 1 and 2 that showed desirable GCA under optimum conditions, should be considered when breeding for higher potential areas. The parents to consider when breeding for random drought stress, were lines 4, 8, 9, 7, 10, 6 and tester 2. The ideal parents for managed drought stress were lines 3, 8 and 10 and tester 1 and 2, for a combination of drought and heat stress lines 3, 8, 7, 10 and 4 were desirable. Hybrids that perform well under low N stress should contain lines 6, 7, 9, 8, and 5 as parents. Lines 6, 4, 7, 3, 8 and 1 and tester 3 had positive GCA values under low P stress conditions, suggesting they can be desirable parents when breeding for varieties that do well under these conditions. No tester was suitable to use as a parent when breeding for a combination of drought and heat stress and low N stress. Crosses showed some variation for SCA, some had negative values and some had positive values. SCA among the different crosses was also not consistent across environments and across seasons. Crosses with positive SCA must be considered for further evaluation. Heritability for grain yield was very high. Additive genetic variance was more important for expression of maize grain yield under optimum, managed drought stress and low P stress. Under combined drought and heat stress, dominance variance was most important. These results suggest it is possible to breed provitamin A hybrids adapted to SSA that are high yielding.

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CHAPTER 5

GENOTYPE X ENVIRONMENT INTERACTION AND STABILITY ANALYSES FOR GRAIN YIELD IN SINGLE CROSS HYBRIDS PRODUCED FROM CIMMYT PROVITAMIN A AND DROUGHT TOLERANT ELITE INBRED LINES UNDER ABIOTIC STRESS AND OPTIMAL CONDITIONS

5.1 Abstract

The development of stable, high yielding provitamin A maize hybrids for resource poor farmers will go a long way in alleviating the problem of vitamin A malnutrition among vulnerable populations. These resource poor farmers grow maize in diverse, adverse climatic and soil fertility conditions which reduce attainable yields, leading to food insecurity. The success of maize breeding and recommendation in diverse environments is reduced by genotype by environment interaction (GEI), which slows down the progress of selection by reducing the relationship between genotype and phenotype. The understanding of GEI affecting a trait enables breeders to identify locations which are efficient in distinguishing ideal genotypes across sites as well as environments which are good representatives of the target regions of interest. The objective of this study was to estimate GEI of provitamin A hybrids using AMMI model and GGE biplot under the prevailing smallholder farmer growing stress and under optimal conditions so as to come up with provitamin A hybrids suitable for cultivation under these conditions. Both AMMI and GGE showed that GEI was a very important source of maize grain yield variability. G31 ranked first in terms of grain yield and was identified as the ideal genotype by the GGE biplot. Ranking of the other genotypes in relation to the ideal genotype was G34 > G8 > G16 > G6 > G7. Provitamin A genotypes G8, G6, G7, G16, G23 and G27 produced moderately high yields, above the average yield, and also exhibited some stability. These genotypes must be considered for further screening and released for commercial cultivation. The environments grouped together in one mega-environment and environments E6, E4, E5, E1 and E2 were more representative of the mega-environment and were ideal environments for selecting superior genotypes. Environment E7 was the most discriminating of the genotypes, followed by E10, E1 and E2, in that order. Environment E2 was the most ideal, followed by environment E10 and E1, in that order.

The highly significant correlation coefficients between the environments show that it is possible to select in one environment and the genotypes will perform similar in the other environments. Environments E2, E5, E7 and E10 were highly significantly correlated with the optimum environment E1, so if funds are limiting one can select genotypes at E1 and they will be suitable for the other four environments. The study showed the importance of understanding GEI as it provides valid observations that enhance selection of new genotypes that are stable in diversified environmental conditions prevailing in a region.

5.2 Introduction

In Zimbabwe, maize production accounts for 80% of the total cereal crop (FAOSTAT, 2014) and the country is considered to be an important maize producer and the people rely heavily on it as the major source of carbohydrates (Mashingaidze, 2004). Because of popularity of maize, all smallholder farmers living in all five different agro-ecological regions of the country produce the crop, even those situated in the most arid regions, which receive erratic rainfall and also have infertile soils (Mashingaidze and Mataruka, 1992). Maize production area in the country stands at 1.52 million ha after the land reform programme of the year 2000 and is mostly done by smallholder and communal farmers with a yield estimate of 0.82 t ha^{-1} (ZIMSTAT, 2015). Normal annual production ranges from 1.8 to 2.1 million ton against a national requirement of 1.8 million ton for human and animal consumption and strategic national reserves of 300 000 ton.

Low yields in smallholder production systems can be explained by the fact that the producers are mainly located in dry regions with highly infertile soils and they add very little, if any, external inputs. These farmers are also faced with production constrains like lack of inputs and use of varieties that are not pest and disease resistant (Mashingaidze, 2004). According to Bänziger et al. (2000) maize yield stability, pest and disease resistance and tolerance to drought and high soil fertility generally produce yield improvements of 30-50%. Therefore there is a need to develop stress tolerant provitamin A maize varieties, especially for the smallholder stress prone environments. The national maize breeding programme in Zimbabwe has been trying to solve some of the production constraints highlighted, through the development of maize varieties with drought, low N

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and disease tolerance (Mhike et al., 2011), however little has been done in terms of low P and heat stress tolerance.

The success of maize cultivar breeding and recommendation in diverse environments is reduced by GEI. It has been noted that genotypes tested in different locations or years often have significant fluctuations in yield due to the response of genotypes to environmental factors such as climate, soil fertility, pests and disease pathogens (Kang, 2004). These variations in yield are usually referred to as GEI and they occur frequently in experiments. GEI is common under abiotic stress conditions and makes breeding progress difficult due to the fact that it complicates the demonstration of superiority of any genotype across many environments. According to Voltas et al. (2002), GEI weakens the association between phenotype and genotype, hence reducing genetic progress in breeding programmes. According to de Souza et al. (2009), GEI is important for plant breeding because it affects the genetic gain, recommendation and selection of genotypes with wider adaptability. GEI may originate from environmental variation in the timing and severity of abiotic or biotic stress (Bänziger and Cooper, 2001; Setimela et al., 2005). The most important type of GEI is crossover interaction, as it implies that the choice of the best genotype is determined by the environment (Ferreira et al., 2006; Malosetti et al., 2013).

High maize yield can be achieved through effective choice of cultivars aiming to capitalise on GEI under different abiotic stress production conditions (Fritsche-Neto et al., 2010). However in analysing multi-environmental data, most researchers are interested in main effects. GEI is ignored as noise or confounding factor (Yan and Tinker, 2006). GEI has been studied in maize by many researchers (Fan et al., 2007; Muungani et al., 2007; Setimela et al., 2010; Kamutondo et al., 2013) putting much emphasis on confirming its effects in cultivar selection and recommendation. All these studies were reporting on white and yellow maize and little research has been done on provitamin A maize.

GEI has been studied using the AMMI model (Gauch, 1992) and GGE biplots (Yan and Kang, 2002). The AMMI model has been widely used because of its ability to explain GEI and because of easy interpretation and identification of genotypes for specific and wide adaptation, which is important in measuring the genetic gain of plant breeding programmes (Gauch, 1992; Kvitschal et al., 2009; Najafian et al., 2010). It clarifies GEI

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by summarising patterns and relationships of genotypes and environments. AMMI gives valuable information on cultivars, environmental stratification and GEI. GEI is an important aspect of both plant breeding programmes and the breeding of new crop cultivars. AMMI analysis gives estimates of total GEI effects of each genotype and also partitions it into interaction effects due to individual environments (Balapure et al., 2016). This will enable a plant breeder to effectively predict the yielding potential of a genotype and how it is influenced by the prevailing environment. Low GEI of a genotype indicates its stability over a range of environments (Yan and Kang, 2002; Balapure et al., 2016). A genotype showing high positive interaction in an environment is adapted to that specific environment. AMMI analysis permits estimation of interaction effect of the genotype in each environment and is important for analysing GEI in yield data of multi-location variety trials.

The GGE biplot methodology or analysis that was developed by Yan et al. in 2000, is another very important tool for graphical analysis of multi-environment trials data. GGE denotes genotypic main effect (G) plus the interaction of the genotype and the environment (GEI). These have been considered to be the two main sources of variation that are important for assessment of genotype performance across different locations. According to Fan et al. (2007) the GGE biplot analysis methodology is a very important tool for categorizing sites that lead to optimum cultivar performance and efficient utilization of limited resources available for most of the breeding and other testing programmes. The GGE biplot is a visual display of the G + GE effects of multi-environmental data where groups of locations with similar cultivar responses are presented and it identifies the highest yielding varieties for each group. It also shows the relationship between genotypes and environments for selected traits graphically and allows visual assessment of GEI patterns of multi-locational or multi-environment data (Yan and Hunt, 2002).

The objectives of this study were (i) to identify high yielding stable provitamin A hybrids suitable for cultivation under diverse abiotic stress conditions and (ii) to identify hybrids adapted for each of the specific growing conditions, (iii) determine the correlations between the environments, (iv) to identify ideal and mega-environments and (v) to rank locations based on discriminating ability and representativeness.

5.3 Materials and methods

The study was conducted at the sites and environments described in Chapter 3 Section 3.3. The same genotypes presented in Appendix 2 were analysed.

5.3.1 Statstical analysis

Both AMMI ANOVA and GGE analysis were implemented in GenStat® 17th Edition statistical software (VSN International, 2015) to determine if there is a significant GEI effect for grain yield. The AMMI model, which combines ANOVA with principal component analysis (PCA), was used to study GEI. GEI was partitioned into sources of variation (i) additive main effects for genotype and environment and (ii) non-additive main effects due to GEI. The following model for AMMI was used

$$Y_{ger} = \mu + \alpha g + \beta e + \varepsilon \lambda n Y g n \delta e n + \rho g e + \text{E} g e r$$

where:

Yger =Yield of genotype *g* in environment *e* for replicate *r*

μ = Grand mean

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

βe = Environment mean deviation

n = Number of PCA axes retained in the model

λn = Singular value for PCA axis n

Ygn = Genotype eigenvector values for PCA axis n

$\delta e n$ = Environment eigenvector values for PCA axis n

$\rho g e$ = Residuals

Eger = Error term (Gauch, 1992)

The AMMI stability value (ASV) was calculated using the formula described by Purchase et al. (2000).

$$ASV = \sqrt{\left[\frac{SS_{IPCA1}}{SS_{IPCA2}}(IPCA1_{score})\right]^2 + IPCA2_{score}^2}$$

where:

ASV = AMMI Stability Value

SS_{IPCA1} = sum of squares of interaction principal component analysis 1

SS_{IPCA2} = sum of squares of interaction principal component 2

IPCA1 = the interaction principal component analysis 1 and

IPCA2 = interaction principal component analysis 2.

Yield Stability Index (YSI) was calculated by summing the ranks from ASV and mean grain yield as described by Farshadfar (2008).

$$YSI_i = RASV_i + RGY_i$$

where:

RASV$_i$ = rank of AMMI stability value of the ith genotype and

RGY$_i$ = rank of mean grain yield of the ith genotype

AMMI biplots for grain yield was generated using genotypic and environmental scores of the first two AMMI components (Rea et al., 2011).

GGE biplot analysis was conducted using the GGE biplot in GenStat[®] 17th Edition statistical software (VSN International, 2015). The model for a GGE biplot (Yan and Hunt, 2002) based on single value decomposition of the first two principal components (PC) was used:

$$Y_{ij} = \mu - \beta_j - \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij}$$

where:

Yij= the measured mean (DBH) of genotype i in environment j

μ = grand mean

βj= main effect of environment j,

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

$\lambda 1$ and $\lambda 2$ = singular values (SV) for the first and second principal component (PC1 and PC2) respectively

$\xi i1$ and $\xi i2$ = eigenvectors of genotype I for PC1 and PC2 respectively

$\eta j1$ and $\eta j2$ = eigenvectors of environment j for PC1 and PC2 respectively

εij= residual associated with genotype i in environment j).

5.4 Results

5.4.1 AMMI analysis of variance

The AMMI ANOVA is presented in Table 5.1. The AMMI analysis of maize grain yield of 30 provitamin A maize hybrids and five checks tested across 10 environments showed

that environmental effects accounted for 72.21% of total sums of squares. Genotype and GEI accounted for only 11.46% and 16.35% of variation, respectively. The genotypes, environments and GEI were highly significant (P < 0.01). GEI was highly significant and variation due to GEI was partitioned among the first three principal component axes (IPCAs), which were significant. The first two IPCAs were highly significant (p < 0.01) and the third IPCA was significant (p < 0.05) for grain yield. The first three IPCAs explained a total of 71.83% of variation due to GEI. ICPA1 of the GEI captured 46.68% of the GEI sums of squares. IPCA2 and IPCA3 explained a further 14.09% and 11.06% of variation, respectively. GEI variance was higher than genotype variance. Overall, AMMI explained 75.19% of the total sums of squares for yield.

Table 5.1 Additive main effects and multiplicative interaction analysis of variance for grain yield (t ha^{-1}) of provitamin A hybrids across environments

Source	DF	SS	MS	Explained total SS (%)	Explained GE SS (%)
Total	1399	9934	7.10		
Treatment	349	7469	21.40**		
Genotypes	34	856	25.17**	11.46	
Environments	9	5393	599.19**	72.21	
Block	10	30	3.03	0.04	
Interactions	306	1221	3.99**	16.35	
IPCA 1	42	570	13.57**		46.68
IPCA 2	40	172	4.30**		14.09
IPCA 3	38	135	3.56*		11.06
Residuals	186	343	1.84		28.08
Error	1040	2434	2.34		

DF= Degrees of freedom, SS = sum of squares, MS = mean squares, GE = genotype by environment interaction, IPCA = interaction principal component axis

The IPCA scores of a genotype in the AMMI model indicates how stable a genotype is across environments. IPCA scores with large magnitude (negative or positive) show genotypes adapted to a specific environment. IPCA scores close to zero show the most stable and adapted genotypes across the environments (Purchase, 2000). Variable IPCA scores with similar sign or close to zero reveal a non-crossover GEI or a proportionate genotype response (Mohammadi et al., 2007).

Purchase et al. (2000) developed the ASV, which is a quantitative stability value used to rank stable genotypes through the AMMI model. They considered ASV to be the most appropriate method of describing the stability of genotypes. Genotype ASV ranged from 0.169 to 2.584. G3 had the lowest ASV and hence is the most stable genotype, while G31 had the largest ASV, meaning it is unstable across environments. YSI is also recommended as a measure of stability. Genotypes with the lowest value are desirable genotypes with high mean yield and stability (Farshadfar et al., 2011). Genotypes G25, G30 and G5 were the most stable and G31 was the least stable genotype.

Table 5.2 shows the first three IPCA scores for genotype. The genotypes showed large variation for grain yield. Average grain yield per genotype across environments ranged from 2.36 to 6.27 t ha^{-1} with a mean of 3.85 t ha^{-1}. Five provitamin A hybrids yielded higher than 4 t ha^{-1} and these were G8 (5.23 t ha^{-1}), G7 (4.66 t ha^{-1}), G16 (4.65 t ha^{-1}), G6 (4.29 t ha^{-1}), and G4 (4.26 t ha^{-1}). All the check hybrids had grain yield higher than 4 t ha^{-1}. These were G31 (6.27 t ha^{-1}), G34 (5.55 t ha^{-1}), G 32 (4.40 t ha^{-1}), G33 (4.40 t ha^{-1}) and G35 (4.19 t ha^{-1}). Genotype 31, which is a check cultivar, ranked first overall across environments. G2, G21, G22 and G30 yielded less than 3 t ha^{-1} and were the lowest yielding. Mean grain yield per environment ranged from 1.14 to 6.98 t ha^{-1} (Table 5.3). Environment E1, which is Harare optimum, gave the highest mean yield and environment E2 (managed drought stress at Chiredzi Research Station) gave the lowest grain yield.

Table 5.2 IPCA scores, AMMI stability value and yield stability index for 30 provitamin A hybrids and five checks based on mean grain yield at 10 sites for two years

Entry code	Grain yield (t ha^{-1})	IPCA1	IPCA2	IPCA3	Mean IPCA (1-3)	ASV	YSI
G1	4.00	-0.28	0.04	-0.01	-0.08	0.51	36
G2	2.36	0.84	0.11	0.58	0.51	1.54	32
G3	3.88	0.09	-0.04	0.96	0.34	0.17	22
G4	3.33	0.38	0.22	-0.38	0.07	0.73	22
G5	3.33	-0.10	0.14	0.40	0.15	0.23	14
G6	4.29	-1.16	0.15	0.24	-0.26	2.12	61
G7	4.66	-0.39	-0.51	-0.03	-0.31	0.88	50
G8	5.23	-0.58	-0.98	0.06	-0.50	1.44	61
G9	3.34	-0.07	0.29	0.31	0.18	0.32	16
G10	3.97	0.22	-1.01	0.57	-0.07	1.09	46
G11	3.54	0.72	-0.50	0.18	0.13	1.41	39
G12	3.80	0.30	-0.62	0.32	0.00	0.83	35
G13	3.17	0.70	0.47	0.27	0.48	1.36	31
G14	3.18	0.95	0.77	-0.33	0.46	1.90	38
G15	3.27	0.54	-0.16	0.15	0.18	1.00	29
G16	4.65	-0.40	0.27	-0.18	-0.10	0.78	46
G17	3.73	0.45	-0.19	-0.50	-0.08	0.84	34
G18	3.67	0.73	-0.68	-0.17	-0.04	1.50	44
G19	3.71	0.50	-0.14	-0.93	-0.19	0.92	35
G20	3.72	0.69	0.30	-0.34	0.22	1.29	41
G21	2.87	0.23	0.36	-0.20	0.13	0.55	16
G22	2.61	0.49	-0.08	0.02	0.14	0.90	21
G23	4.26	0.02	0.21	0.44	0.22	0.21	31
G24	3.95	-0.57	0.59	0.47	0.16	1.20	46
G25	3.47	0.04	0.17	-0.18	0.01	0.19	13
G26	3.84	-0.71	0.67	0.48	0.15	1.46	49
G27	3.99	-0.20	-0.06	-0.33	-0.20	0.37	33
G28	3.75	-0.14	0.22	0.18	0.09	0.34	25
G29	3.58	-0.32	0.19	-0.43	-0.19	0.61	26
G30	2.80	0.07	0.40	0.14	0.20	0.42	13
G31	6.27	-1.41	-0.25	-0.77	-0.81	2.58	70
G32	4.40	0.10	-0.05	-0.53	-0.16	0.19	32
G33	4.40	-0.30	-0.75	-0.06	-0.37	0.93	51
G34	5.55	-1.36	0.10	0.01	-0.42	2.48	68
G35	4.19	-0.05	0.33	-0.42	-0.05	0.34	34
Mean	3.85	0.00	0.00	0.00	0.00	0.96	36
Minimum	2.36	-1.41	-1.01	-0.93	-0.81	0.17	13
Maximum	6.27	0.95	0.77	0.96	0.51	2.58	70

IPCA = interaction principal component axis, ASV = AMMI stability value, YSI = Yield Stability Index, G = genotype

Table 5.3 IPCA scores for the ten sites for two years

Environment code	Mean grain (t ha^{-1})	IPCA1	IPCA2	IPCA3	Mean IPCA (1-3)
E1	6.98	-2.01	0.03	0.43	-0.52
E2	1.14	1.23	0.38	0.70	0.77
E3	2.65	0.98	-0.32	0.64	0.43
E4	5.80	-0.80	-1.51	0.35	-0.65
E5	2.42	0.79	0.77	-0.99	0.19
E6	3.59	0.21	0.15	0.33	0.23
E7	2.87	0.72	-0.43	-1.54	-0.42
E8	1.49	1.02	0.14	0.83	0.66
E9	5.88	-0.70	-0.81	-0.67	-0.73
E10	5.69	-1.44	1.61	-0.10	0.02
Mean	3.85	0.000	0.001	-0.002	-0.002
Minimum	1.14	-2.01	-0.81	-1.54	-0.73
Maximum	6.98	1.23	1.61	0.83	0.77

IPCA = interaction principal component axis, E1 = optimum at CIMMYT Harare, E2 = managed drought stress at Chiredzi Research Station, E3 = combined drought and heat stress at Chiredzi Research Station, E4 = random drought stress at Kadoma Research Station, E5 = random drought stress at Kaguvi Training Centre, E6 = low N stress at CIMMYT Harare, E7 = random drought stress at Rattray Arnold Research Station, E8 = managed drought stress at Save Valley Experimental Station, E9 = optimum at Rio Tinto Agricultural College, E10 = low P stress at CIMMYT Harare.

The top four yielding genotypes based on AMMI selections per environment are shown in Table 5.4. Genotype 31 was highest yielding in five environments. Only provitamin A hybrids ranked top in environment E2 (managed drought stress at Chiredzi Research Station) and E3 (combined drought and heat stress at Chiredzi Research Station). A provitamin A hybrid G8, appeared in the top four ranking genotypes in six environments. G6 appears in three environments and G7 and G3 in two environments. Environments E1 (Harare optimum), E4 (Kadoma Research Station) and E9 (Rio Tinto Agricultural College) with negative mean IPCA scores had the highest average grain yield.

Table 5.4 The four top yielding genotypes based on AMMI selections per environment

Environment rank	Environment	Mean yield (t ha^{-1})	Score	1	2	3	4
3	E2	1.14	1.23	G23	G11	G20	G12
9	E8	1.49	1.02	G3	G8	G31	G15
4	E3	2.65	0.98	G8	G3	G10	G7
6	E5	2.42	0.79	G35	G19	G31	G32
8	E7	2.87	0.72	G31	G8	G20	G32
7	E6	3.59	0.21	G34	G33	G10	G6
10	E9	5.88	-0.70	G31	G7	G34	G8
5	E4	5.80	-0.80	G31	G34	G33	G8
2	E10	5.69	-1.44	G31	G34	G24	G6
1	E1	6.98	-2.01	G31	G34	G8	G6

E1 = optimum at CIMMYT Harare, E2 = managed drought stress at Chiredzi Research Station, E3 = combined drought and heat stress at Chiredzi Research Station, E4 = random drought stress at Kadoma Research Station, E5 = random drought stress at Kaguvi Training Centre, E6 = low N stress at CIMMYT Harare, E7 = random drought stress at Rattrey Arnold Research Station, E8 = managed drought stress at Save Valley Experimental Station, E9 = optimum at Rio Tinto Agricultural College, E10 = low P stress at CIMMYT Harare.

The AMMI1 model allows the development of a biplot using the first principle component and the mean yields, making it easy to evaluate the genotypes, environments and their interactions. Results for the AMMI1 biplot are presented in Figure 5.1. Environments E10 (Harare low P stress), E9 (Rio Tinto Agricultural College) and E4 (Kadoma Research Station) were high yielding, but unstable. Environments E2 (managed drought stress Chiredzi Research Station) and E8 (Save Valley Experimental Station) were the lowest yielding but stable. Genotypes G31 and G34 were the highest yielding but unstable. In the AMMI2 biplot, genotypes were scattered across the whole biplot (Figure 5.2). E1 (Harare optimum), E4 (Kadoma Research Station) and E10 (Harare low P stress) had long vectors so they showed significant interaction. Most environments had short vectors, hence they were stable. E6 (Harare low N stress) was the most stable environment. Genotype G14 was the most adapted; however the highest yielding genotype G31 was the most unstable.
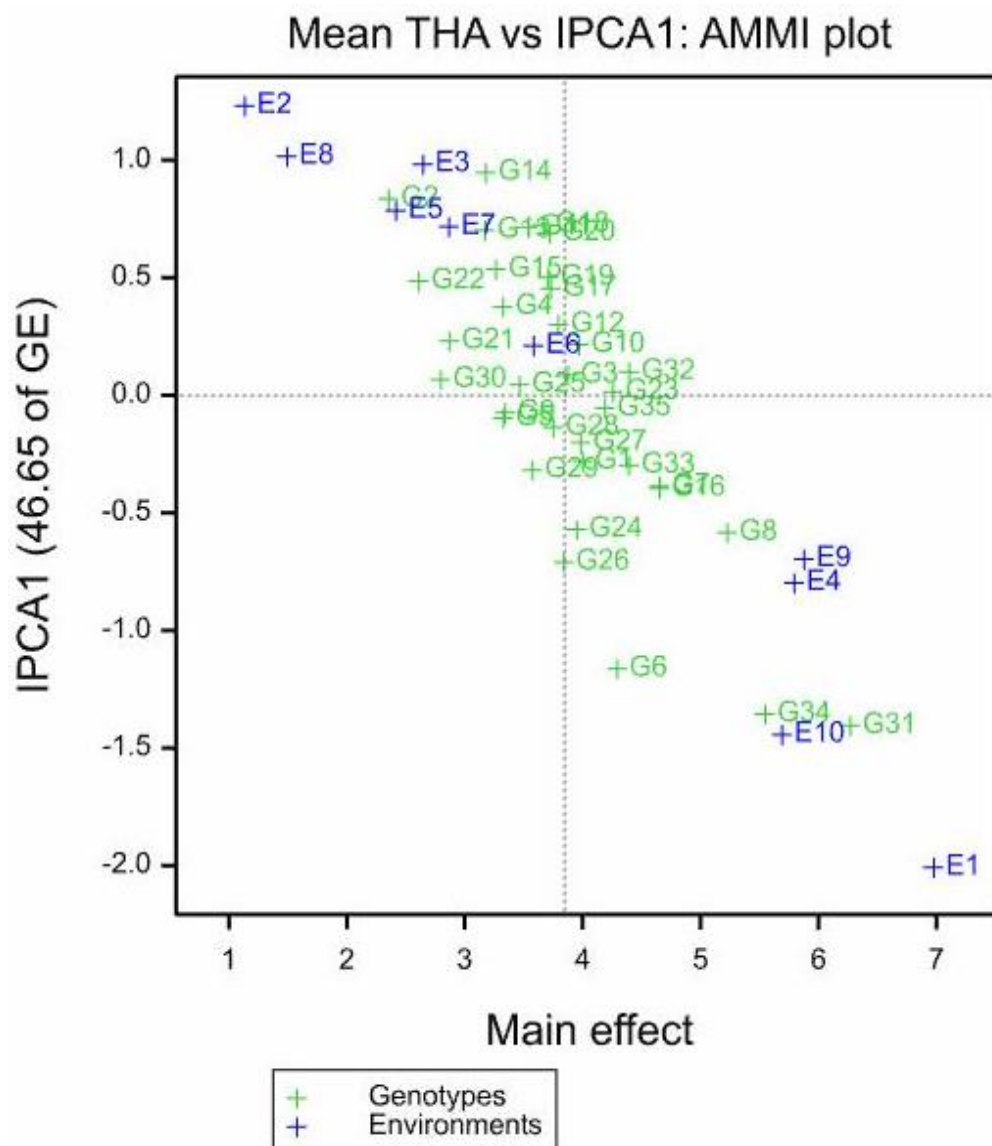
Figure 5.1 AMMI1 biplot for grain yield for genotypes and environments across two years, 2014 and 2015.
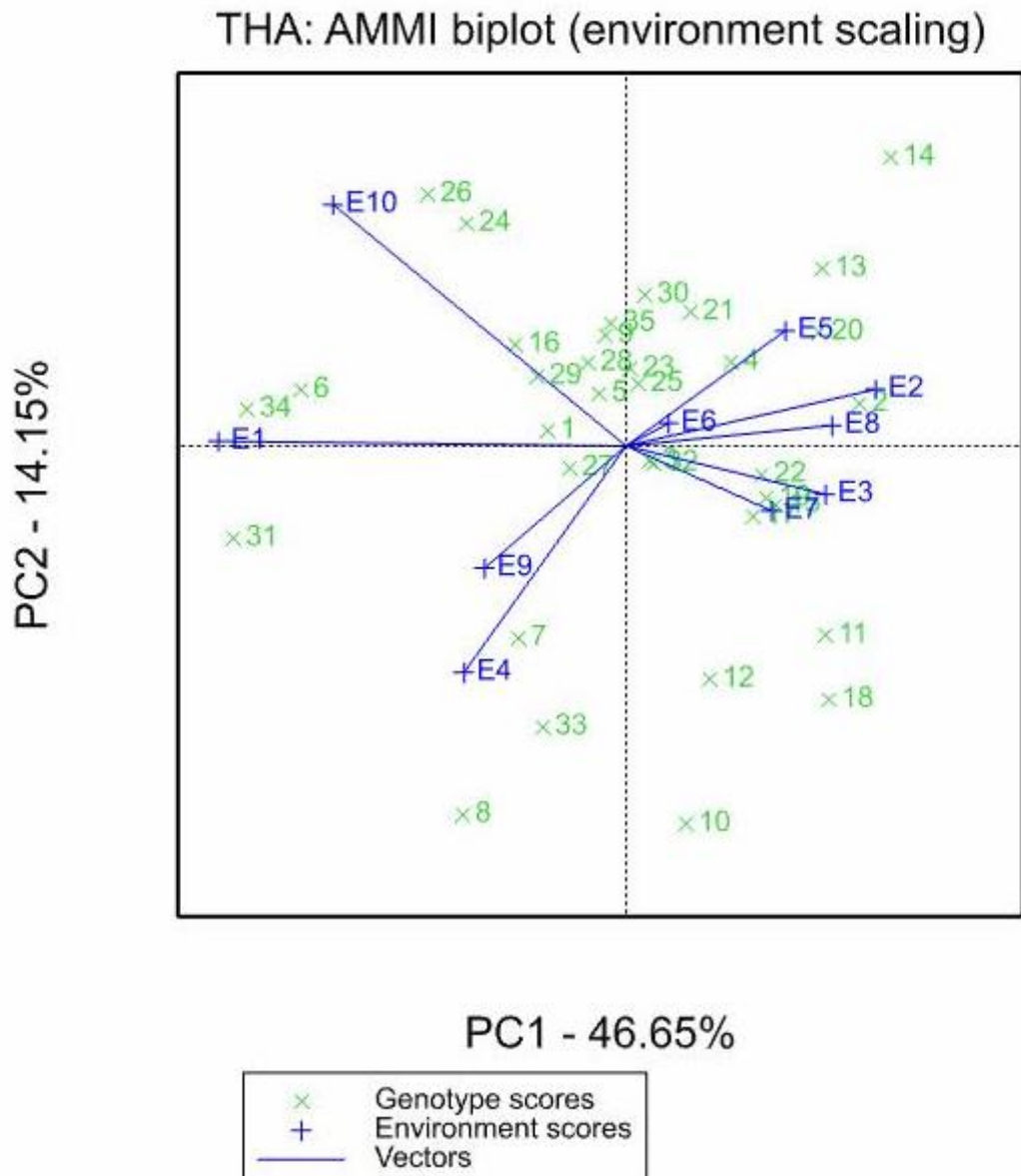
E1 = optimum at CIMMYT Harare, E2 = managed drought stress at Chiredzi Research Station, E3 = combined drought and heat stress at Chiredzi Research Station, E4 = random drought stress at Kadoma Research Station, E5 = random drought stress at Kaguvi Training Centre, E6 = low N stress at CIMMYT Harare, E7 = random drought stress at Rattray Arnold Research Station, E8 = managed drought stress at Save Valley Experimental Station, E9 = optimum at Rio Tinto Agricultural College, E10 = low P stress at CIMMYT Harare.

Figure 5.2 AMMI2 biplot for maize grain yield of the first two GEI principal components axes of 35 genotypes and 10 environments across two years, 2014 and 2015.

E1 = optimum at CIMMYT Harare, E2 = managed drought stress at Chiredzi Research Station, E3 = combined drought and heat stress at Chiredzi Research Station, E4 = random drought stress at Kadoma Research Station, E5 = random drought stress at Kaguvi Training Centre, E6 = low N stress at CIMMYT Harare, E7 = random drought stress at Rattrey Arnold Research Station, E8 = managed drought stress at Save Valley Experimental Station, E9 = optimum at Rio Tinto Agricultural College, E10 = low P stress at CIMMYT Harare.

5.4.2 GGE biplot analysis

Environments in this analysis constituted of the test sites/locations. For low P stress only data for the 2014 season was included. The GGE biplot (genotype-focused) was generated in order to identify the positioning of genotypes. The GGE biplot analysis of grain yield for 30 provitamin A hybrids and five checks across all environments explained 69.03% of genotype main effects and GEI with primary IPC1 and secondary IPC2 scores contributing 57.23 and 11.80% respectively (Figure 5.3).

5.4.2.1 Mega-environment analysis

For any plant breeding programme to be successful and make meaningful progress, it is important to understand and select suitable test locations, among other many important factors that affect plant growth in general (Yan et al., 2011). An efficient test location is discriminating, so that differences among genotypes can be easily detected using few replications, and is representative of the target environments for the cultivars to be released. The representation of the target environments should be repeatable so that genotypes selected in each year will have superior performance in future years (Yan et al., 2011). Because of this, knowledge of target environments for breeding for specifically adapted genotypes is of paramount importance as well, as it also requires a subdivision of the target locations into mega-environments. The polygon view of the GGE biplot is the best way for identifying the winning genotypes and visualising the interaction patterns between genotypes and environments in multi-trial data (Yan and Kang, 2003). This is of paramount importance in estimating existence of possible mega-environments (Yan and Tinker, 2006; Akter et al., 2015).

Dividing of environments into different mega-environments and assigning different genotypes in different mega-environments is the best way to exploit GEI (Mostafavi et al., 2012). To achieve this, a polygon was constructed with genotypes G13, G26, G31, G33, G10, G18 and G2 as markers (Figure 5.3). These genotypes were the best or the poorest in some or all of the environments, since they had the largest distance from the origin of the biplot (Yan and Kang, 2003; Yan and Tinker, 2006). The genotype at the vertex of the polygon performs best in the environment falling within the sectors (Yan, 2002; Yan and Tinker, 2006). Genotypes located near the biplot origin are less responsive to the change of environments (Mohammadi and Amri, 2012).
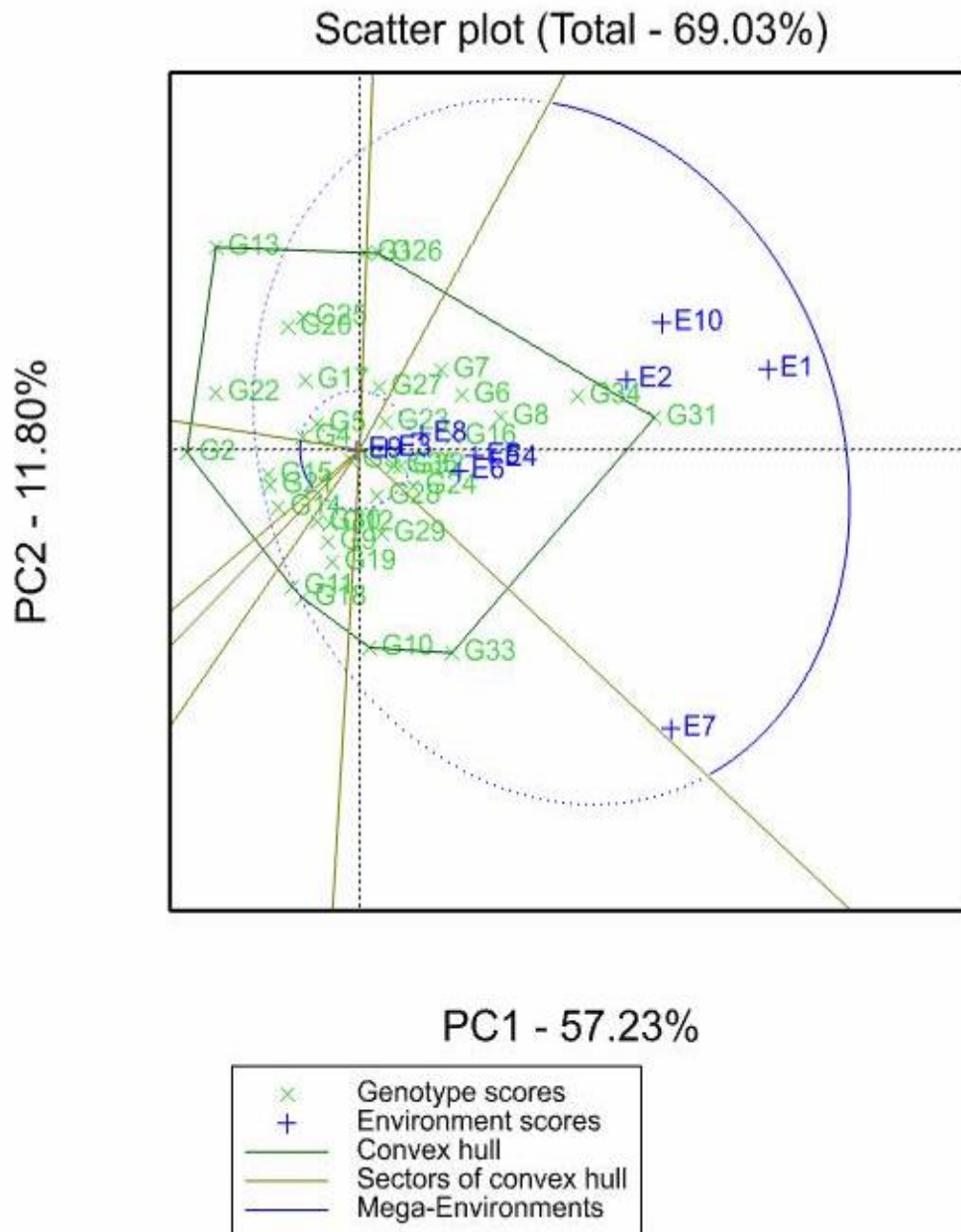
Figure 5.3 GGE biplot showing mega-environments for 10 test environments planted with 30 provitamin A maize hybrids and five checks across two consecutive years.

E1 = optimum at CIMMYT Harare, E2 = managed drought stress at Chiredzi Research Station, E3 = combined drought and heat stress at Chiredzi Research Station, E4 = random drought stress at Kadoma Research Station, E5 = random drought stress at Kaguvi Training Centre, E6 = low N stress at CIMMYT Harare, E7 = random drought stress at Rattrey Arnold Research Station, E8 = managed drought stress at Save Valley Experimental Station, E = optimum at Rio Tinto Agricultural College, E10 = low P stress at CIMMYT Harare.

Eight perpendicular lines were then drawn from the origin and extended beyond the polygon, dividing the biplot into eight sectors representing different mega-environments. The environments fell into one big sector meaning that the environments clustered into one mega-environment. Environments that fall in the same mega-environment have the same effects on genotype performance, and hence should be considered as a homogenous group (Ramburan and Zhou, 2011). Genotypes G31, G34, G8, G6, G7, G16, G27, G23 and G24 were some of the hybrids that fell in the same sector with the environments meaning they were stable in all the environments. Genotype G31 was the highest yielding hybrid followed by G34.These were followed by provitamin A genotypes G8, G6, G16 and G7, however, they were not very different from some of the genotypes in the sector.

5.4.2.2 Correlations between environments

The reason for evaluating environments is to find environments that effectively identify superior genotypes for the mega-environment (Yan et al., 2007). In GGE biplots, lines connecting environment markers to the biplot origin are called environment vectors. The relationship between two environments is approximated by the cosine of the angle between their vectors (Mohammadi and Amri, 2011; Farshadfar et al., 2012). An acute angle means positive correlation, an obtuse angel a negative correlation and a right angle no correlation (Yan and Kang, 2003). The vector view also approximates the standard deviation within each environment, which is a measure of their discriminating power (Dehghani et al., 2006). A short vector means the environment is not related to the other environments. All the environments were positively correlated, as demonstrated by acute angles. This means data from one environment can be used to predict the performance of the genotype in other environments since there is no crossover GEI between the environments. Absence of crossover GEI means there will be no change in genotype ranking from one environment to the other. To test the discrimination power of the environments, a scatter biplot based on genotype singular value partitioning was constructed (Figure 5.4). Environments E10 (Harare low P stress), E1 (Harare optimum) and E7 (Rattrey Arnold Research Station) had longer vectors, meaning they were good in discriminating the genotypes, hence selecting in these environments will be very effective. Environments E3 (Chiredzi combined drought and heat stress), E5 (Kaguvi Training Centre) and E9 (Rio Tinto Agricultural College) had very short vectors, hence they had poor discriminating ability among the genotypes, hence selection in the environments might not be effective. These environments should not be used for selecting

maize genotypes because they provide very little information and including them will be a waste of funds.
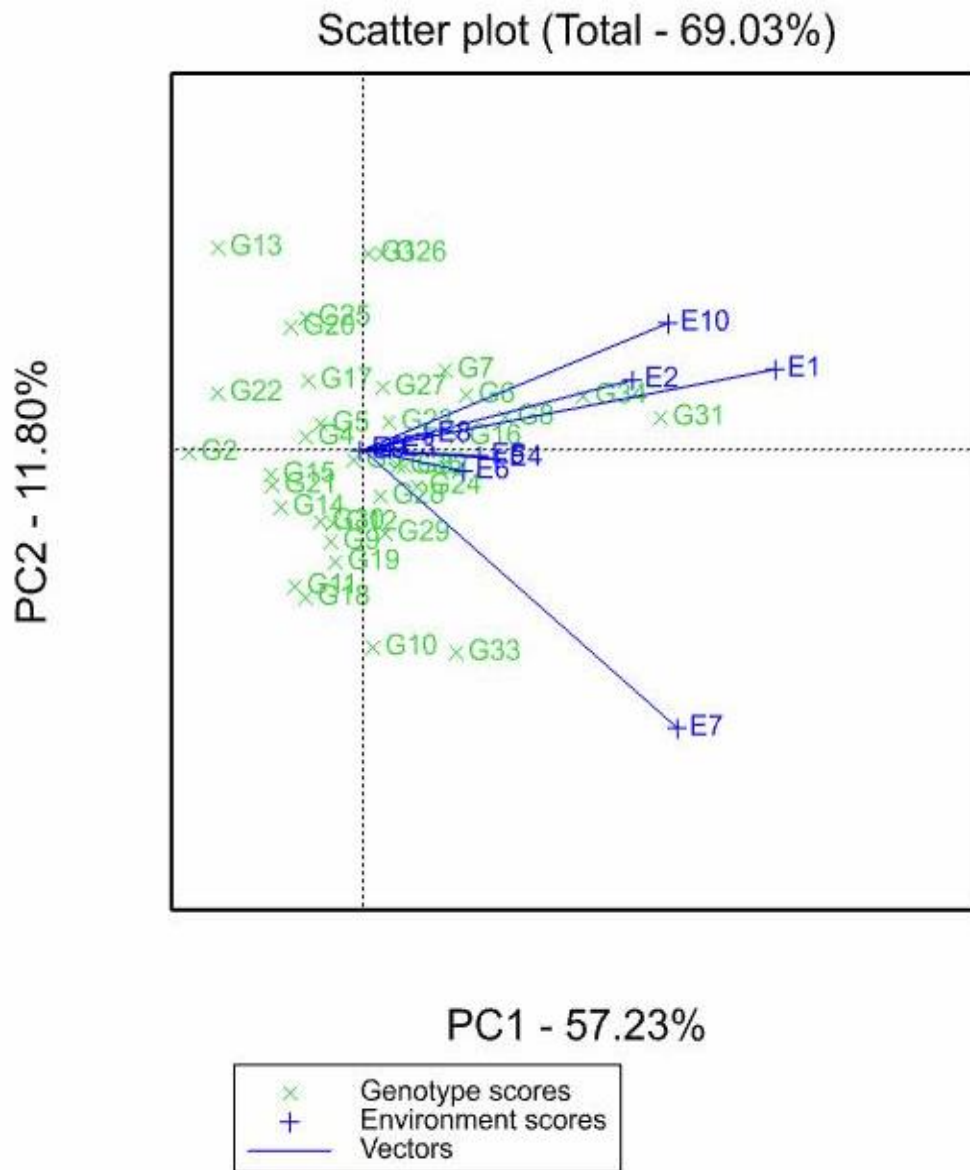
Figure 5.4 GGE biplot showing the correlations among 30 provitamin A maize hybrids and five checks tested in 10 environments across two consecutive years.

E1 = optimum at CIMMYT Harare, E2 = managed drought stress at Chiredzi Research Station, E3 = combined drought and heat stress at Chiredzi Research Station, E4 = random drought stress at Kadoma Research Station, E5 = random drought stress at Kaguvi Training Centre, E6 = low N stress at CIMMYT Harare, E7 = random drought stress at Rattrey Arnold Research Station, E8 = managed drought stress at Save Valley Experimental Station, E9 = optimum at Rio Tinto Agricultural College, E10 = low P stress at CIMMYT Harare.

Environment E1 (Harare optimum) was highly significantly correlated (P < 0.01) with E2 (Chiredzi managed drought stresss), E5 (Kaguvi Training Centre), E7 (Rattrey Arnold Research Station), E10 (Harare low P stress) and significantly correlated with E8 (Save Valley Experimental Station) (Table 5.5). Environment E2 (Chiredzi managed drought stress) was highly significantly correlated with E5 (Kaguvi Training Centre), E10 (Harare low P stress) and E4 (Kadoma Research Station) and significantly correlated with E6, E7 and E8. E3 was highly significantly correlated with E8 (Save Valley Experimental Station) and E9 (Rio Tinto Agricultural College). E4 (Kadoma Research Station) had a highly significant relationship with E8 (Save Valley Experimental Station) and a significant relationship with E6 (Harare low N stress) and E9 (Rio Tinto Agricultural College). E5 (Kaguvi Training Centre) had a highly significant relationship with E7 (Rattrey Arnold Research Station) and E10 (Harare low P stress) and a significant relationship with E6 (Harare low N stress) and E8 (Save Valley Experimental Station). E6 (Harare low N stress) had a significant relationship with E7 (Rattrey Arnold Research Station) and E8 (Save Valley Experimental Station). E7 (Rattrey Arnold Research Station) and E10 (Harare low P) had a significant relationship and E8 (Save Valley Experimental Station) and E9 (Rio Tinto Agricultural College) had a highly significant relationship.

**Table 5.5** Environmental correlations based on grain yield

	E1	E2	E3	E4	E5	E6	E7	E8	E9
E2	0.62***								
E3	0.05	0.16							
E4	0.10	0.37**	0.20						
E5	0.68***	0.59***	0.10	0.18					
E6	0.16	0.27*	0.03	0.39*	0.33*				
E7	0.59***	0.39*	0.001	0.15	0.49**	0.27*			
E8	0.28*	0.36*	0.54***	0.53***	0.29*	0.19*	0.12		
E9	0.02	0.02	0.47**	0.40*	0.29	0.19	0.04	0.58***	
E10	0.73***	0.63***	0.08	0.10	0.49**	0.07	0.37*	0.11	0.07

$* p < 0.05$, $** P < 0.01$, $*** P < 0.001$, E1 = optimum at CIMMYT Harare, E2 = managed drought stress at Chiredzi Research Station, E3 = combined drought and heat stress at Chiredzi Research Station, E4 = random drought stress at Kadoma Research Station, E5 = random drought stress at Kaguvi Training Centre, E6 = low N stress at CIMMYT Harare, E7 = random drought stress at Rattrey Arnold Research Station, E8 = managed drought stress at Save Valley Experimental Station, E9 = optimum at Rio Tinto Agricultural College, E10 = low P stress at CIMMYT Harare.

5.4.2.3 Mean performance and stability of genotypes and environments

Genotype evaluation is meaningful only for a specific mega-environment. GGE biplot software has the ability to evaluate genotypes relative to an ideal genotype. According to Yan and Kang (2003), Yan et al. (2007), Mohammadi and Amri (2012), Farshadfar et al. (2012) and Monstafavi et al. (2012), an ideal variety should have the highest mean performance and be absolutely stable. Even though that genotype does not exist in real life, it can be still used as a reference genotype when evaluating genotypes (Mitrovic et al., 2012). Using the ideal genotype as the centre, concentric circles are drawn to help visualize the distance between each genotype and the ideal genotype. G31, located at the centre of the concentric circle, can be said to have both high mean yield and high stability and hence is the ideal genotype. Ranking of the other genotypes in relation to the ideal genotype was G34 > G8 > G16 > G6 > G7 (Figure 5.5). Genotypes G13, G2 and G22 were the least ideal because they were very far from the ideal genotype.

Ranking of 30 provitamin A hybrids and five checks based on their mean yield and stability performance across 10 environments is shown in Figure 5.5. The line which passes through the origin of the biplot is called the average environment coordinate, which is defined by the average principal component 1 and 2 scores of all the environments (Yan and Kang, 2003; Kaya et al., 2006; Mohammadi and Amri, 2012). Any direction away from the origin of the biplot on the axis indicates greater GEI and hence reduced genotype stability (Akter et al., 2015). The average environment coordinate separates genotypes with below-average means from those with above average means. Genotypes on the positive side have above average mean yield, while those on the negative side have mean yield below the average. The ideal genotypes are positioned close to the biplot origin and have shorter vectors from the average environment coordinates. G31 and G34 with the highest yield and stability performance, can be considered as genotypes with high yield and stability performance (Mohammadi and Amri, 2012). Based on the comparison biplot (Figure 5.5) G31 was the ideal genotype, since it was closest to the centre of the concentric circles, followed by G34. Among the provitamin A hybrids, G8 was the most ideal. Most hybrids were relatively stable considering their close proximity to the average environment coordinate. More than 50% of the genotypes fell on the positive side of the average environment coordinate, meaning they had above average yield.
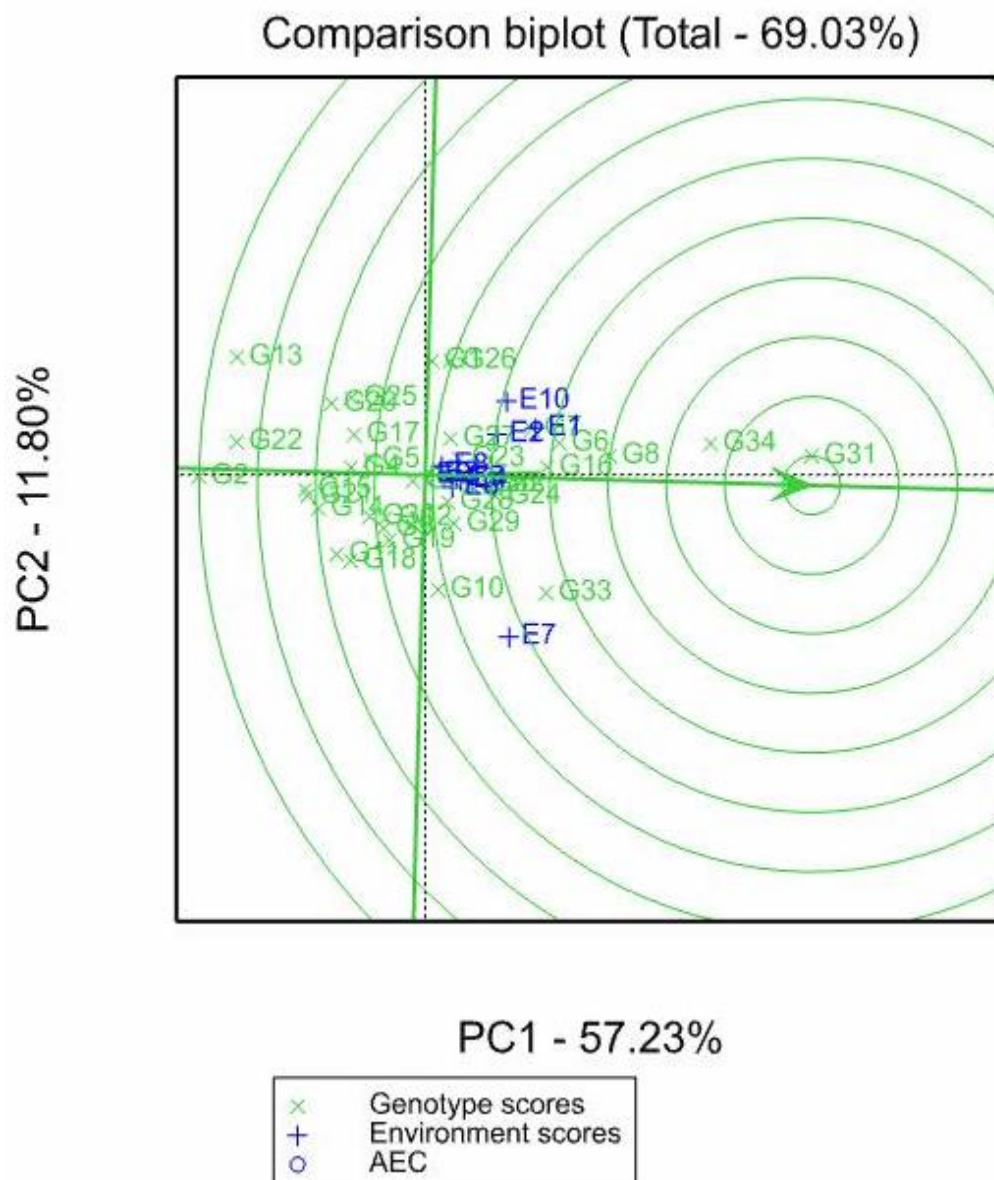
Figure 5.5 GGE biplot showing yield performance and stability of 30 provitamin A hybrids and five checks tested over ten sites for grain yield.

E1 = optimum at CIMMYT Harare, E2 = managed drought stress at Chiredzi Research Station, E3 = combined drought and heat stress at Chiredzi Research Station, E4 = random drought stress at Kadoma Research Station, E5 = random drought stress at Kaguvi Training Centre, E6 = low N stress at CIMMYT Harare, E7 = random drought stress at Rattrey Arnold Research Station, E8 = managed drought stress at Save Valley Experimental Station, E9 = optimum at Rio Tinto Agricultural College, E10 = low P stress at CIMMYT Harare.
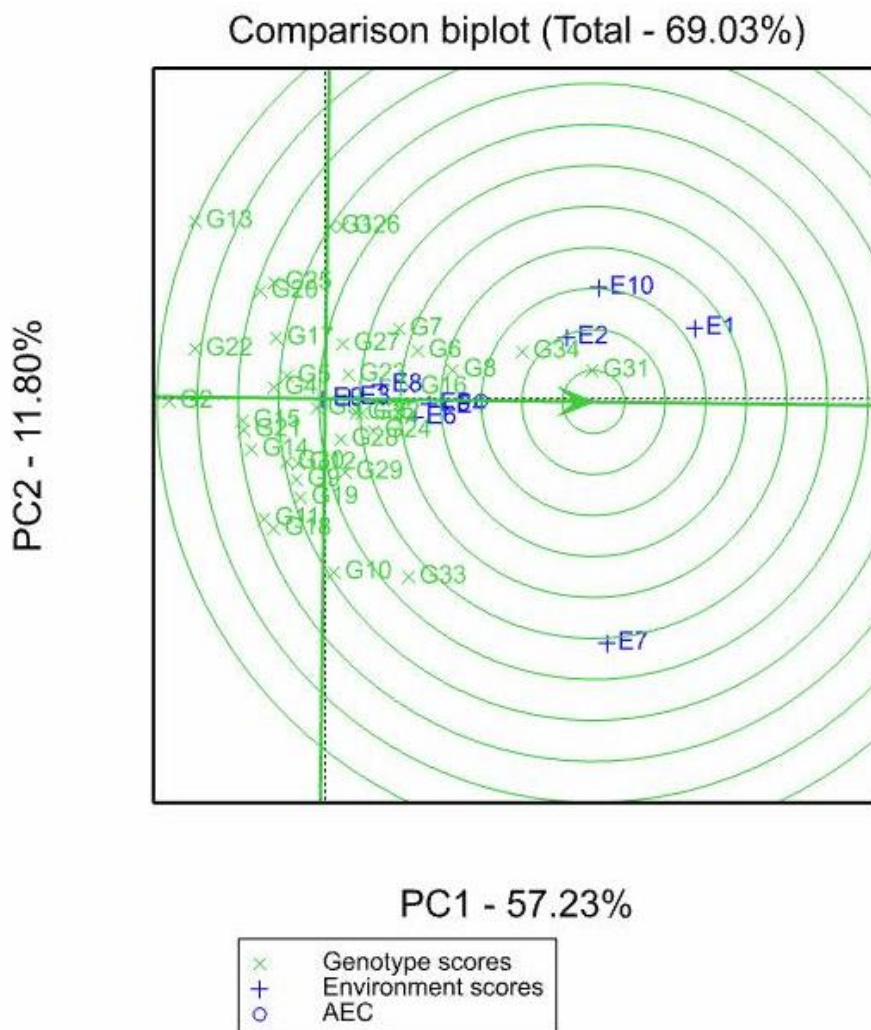
Figure 5.6 GGE biplot showing discriminating ability and representativeness of 10 test environments planted with 30 provitamin A maize hybrids and five checks averaged over two years for grain yield.

E1 = optimum at CIMMYT Harare, E2 = managed drought stress at Chiredzi Research Station, E3 = combined drought and heat stress at Chiredzi Research Station, E4 = random drought stress at Kadoma Research Station, E5 = random drought stress at Kaguvi Training Centre, E6 = low N stress at CIMMYT Harare, E7 = random drought stress at Rattrey Arnold Research Station, E8 = managed drought stress at Save Valley Experimental Station, E9 = optimum at Rio Tinto Agricultural College, E10 = low P stress at CIMMYT Harare.

All the environments had higher than average performance (Figure 5.6). E7 (Rattrey Arnold Research Station) was the most unstable environment followed by E1 (Harare optimum) and E10 (Harare low N stress). E2 (drought stress at Chiredzi Research Station) was the ideal environment and can be said to be the most stable.

5.5 Discussion

Zimbabwe has diverse agro-climatic conditions and Vincent and Thomas (1961) and Mugandani et al. (2012) grouped the country into five natural agro-ecological regions based on rainfall amount and agricultural potential. The locations used in this study fall into three of the five natural ecological regions, which are agro-ecological region II, III and V. Natural region II and III are the major maize growing regions of the country and natural region V represent the most arid region. The 30 provitamin A hybrids and five check hybrids were grown at 10 sites under different environmental conditions, which include two optimal management, one low N stress, one low P stress, three random drought stress (rainfed), two managed drought stress and one combined managed drought and heat stress. The large sums of squares for environments shown in this study indicated that the environments were diverse, with large differences among environmental means causing most of the observed variation in grain yield. This was expected, since the genotypes were exposed to different stress conditions. The very large effect of environments indicated that the test environments were highly variable, as a result, their potential for maize production differed significantly. According to Klomsa-ard et al. (2013) variability in production conditions and systems results in differences in crop productivity between different test environments. Pests and diseases and variability in rainfall, soil and photoperiod are important factors responsible for environmental variability in crop production systems (Ferreira et al., 2006). Variations due to environmental conditions are classified into predictable, which include climate, soil type, day length and cultural practices and unpredictable, which include rainfall, radiation and temperature (Allard and Bradshaw, 1964). Of these, unpredictable factors contribute much to GEI. In this study, differences in sites and management system were the major contributors to this large environmental effect. Yan and Rajcan (2002) reported that environmental variance explained more variance than other variances in their study on soyabean yield. Rezene (2014) observed environmental variance as high as 89.6%. Generally, environment main effect accounts for 80% or more of the total yield variation (Yan, 2002). Muungani et al. (2007) and Malosetti et al. (2013) reported 88.6% and 79.9% for maize, respectively, Rakshit et al. (2012) reported 76.30% for sorghum, Farshadfar et al. (2013) reported 86% for wheat, and Nowosad et al. (2016) reported 69.82% for rapeseed. In a study on maize, seasonal variability and management systems were the major causes of large environmental effects (Issa, 2009).

GEI variance was higher than genotype variance. GEI was highly significant and had a large effect on genotype performance in different environments. This justifies the need to conduct hybrid evaluation trials at more than one testing site. The significant effects of GEI reflected the differential response of genotypes in various environments (Mohammadi et al., 2015). Both AMMI and GGE biplots showed significant GEI. Since GEI was significant there was need to calculate grain yield stability across environments (Lin et al., 1986). Although grain yield is influenced by the genotype and environment component and GEI (in this case environmental component contributing 72.21% of total variance), only genotype and GEI components are important when evaluating hybrids in multi-location trials (Yan and Kang, 2002). The magnitude of GEI variance was more than 140% higher than genotypic variance. Ahmadi et al. (2012) reported that it is common for multiple location trials to have a mixture of crossover and non-crossover types of GEI. GEI makes it difficult to select best performing and stable cultivars (Mohammadi et al., 2015). The magnitude of GEI affects the genetic background that controls the physiological processes governing expression and hence stability of grain yields in diverse environments. This makes selection difficult and reduces the usefulness of hybrids, since the relationship between genotype and phenotype is reduced (Voltas et al., 2002). The results of this study corroborate those of Bertero et al. (2004), Sabaghnia et al. (2013) and Mohammadi et al. (2015) who also found large GEI variance compared to genotypic variance. However Muungani et al. (2007), Kamutando et al. (2013) and Malosetti et al. (2013) reported contrasting results. The large environmental variance and GEI variance means it is possible to select hybrids suitable for specific environments (Yan and Kang, 2002).

The highly significant effect of genotype indicated that there was a considerable amount of phenotypic variability between the hybrids. Because of outcrossing, maize exhibits a lot of genetic variability. Heterosis in maize arises when parents originating from two diverse populations are crossed because this maximises heterozogosity. The variability between the genotypes means hybrids that perform better than others can be selected for further advancements and released as cultivars. Genetic variability is ideal for making progress during selection of superior varieties (Zhou et al., 2011).

If the first two PCs explain more than 60% of total variability, and the combined G + GEI effects account for more than 10% of the variation, then the biplot adequately

approximates the variability in the GEI data (Yang et al., 2009; Yan and Holland, 2010; Rakshit et al., 2012). In this study the first two principal components of the GGE biplots explained 69.03% and that for AMMI explained 60.80% of the total GEI, and the combined G + GEI effects accounted for 16.35% for AMMI of the total variability. This means both AMMI and GGE biplots in this study gave effective graphical representation of the variability present in the data and, as such, their interpretation is meaningful. The first two PCs of GGE biplots explained more GEI (69.03%) than the AMMI, which explained only 60.80%. This supports Yan et al. (2007)'s assertion that the GGE biplot explains more G+GEI than the AMMI method and is, therefore, a more accurate presentation of the GGE of the data. According to Malosetti et al. (2013) GGE biplots approximate overall G+GEI while AMMI analysis approximate only the GEI part of the phenotype. Yet G and GEI must be considered simultaneously because they are the two sources of variation in variety evaluation (Yan and Hunt, 2001). GGE is more logical and biological compared to AMMI in explaining PC1 score, which represents genotypic effect rather than additive main effect (Yan, 2002). Statistical and biological reasons for preferring GGE over AMMI in assessing complex GEI are explained by Crossa et al. (2010). Rodriguez et al. (2010) used both multivariate analyses (AMMI and GGE), and found that they were similar in showing variety performance across environments, and the difference being that the GGE captured more GEI than the AMMI.

G6, G7, G8, G16, G19, G27 and G29 provitamin A hybrids had the lowest mean IPCA scores and some of them were among the top yielding across environments. These seven provitamin A hybrids can be said to be stable. Genotypes in this study also had both positive and negative IPCA scores. Genotypes that had large positive IPCA scores in one environment also had large negative interaction with other environments, meaning there was disproportionate genotype response (Mohammadi et al., 2007), which might be the main source of variation for any crossover interaction (Farshadfar et al., 2011). Based on ASV the top five stable genotypes were G3, G25, G32, G23 and G5 and the least stable genotypes were G31, G34, G6, G14 and G2. The most stable and high yielding genotypes like G3 and G23 should be considered for further evaluation and release as provitamin A stable hybrids to cultivate across diverse environments.

The environments fell in only one sector and genotypes fell in eight sectors. Genotypes G31 and G34 were the best yielding and were most suitable for environments E2

(managed drought stress at Chiredzi Research Station), E10 (Harare low P stress) and E1 (Harare optimum). Kamutando et al. (2013) also reported the existence of one mega-environment for maize in GEI studies in Zimbabwe. G31, G34, G8, G6, G7, G16, G27, G23 and G24 were some of the hybrids that fell in the same sector with the environments, meaning they were adapted to all the environments. The most adapted provitamin A genotypes were G8, G6 and G7, however, they were not very different from some of the other genotypes in the sector. Provitamin A genotypes G8, G16 and G6 were the highest yielding and stable among the provitamin A genotypes, though they were lower yielding and less stable when compared to checks G31 and G34. Other stable genotypes were G26, G23, G27 and G7; these genotypes had yield performance greater than overall mean yield. Provitamin A genotypes G8, G16, G6 can be candidates for commercial release targeting all the given environments.

Yan et al. (2007) defined an ideal test environment as one that should be both discriminating of the genotypes and representative of the mega-environment. All environments were positively correlated, because all angles among them were smaller than 90°. Environments with longer vectors are more discriminating of the genotype. Environment E7 (Rattrey Arnold Research Station) was the most discriminating of the genotypes, followed by E10 (Harare low P stress), E1 (Harare optimum) and E2 (managed drought stress at Chiredzi Research Station) in that order, and the other environments had very small vectors, meaning that genotypes might have performed similarly in them and are of no value in giving information about the genotype variability. Environments E7 and E10, with large angles, were the least representative of the mega-environment (Yan et al., 2007). These two environments cannot be used for selecting best genotypes, but they are important in screening unstable and poorly adapted genotypes. Environments E6 (Harare low N stress), E4 (Kadoma Research Station), E5 (Kaguvi Training Centre), E1 (Harare optimum) and E2 (managed drought stress at Chiredzi Research Station) were more representative of the mega-environment and were ideal environments for selecting superior genotypes. These environments can be considered if there are budgetary constraints, since they can effectively screen the genotypes suitable for the mega-environment. Environments E3 (combined drought and heat stress) and E9 (Rio Tinto Agricultural College) provided very little information about the genotypes and therefore should not be used as test environments.

A test environment should have the power to discriminate genotypic variability and be representative of the mega-environment (Yan, 2002; Yan and Kang, 2003). The ideal environment is defined and visualised by the small circle with an arrow pointing to it (Akter et al., 2015). Environment E2 (managed drought stress at Chiredzi Research Station) was the most ideal, followed by environment E10 (Harare low P stress) and E1 (Harare optimum) in that order. Environment E7 (Rattrey Arnold Research Station) was the most discriminating environment. E2 (managed drought stress at Chiredzi Research Station) was more stable and suitable for all the genotypes.

Spearman's rank correlation coefficients between environments revealed significant correlations between most environments. This indicates that it is possible to select in one environment and the genotypes will perform similarly in the other correlated environments. If funds are limiting, one can use only one of the correlated environments and screen genotypes suitable for all the correlated environments. This will significantly reduce the cost. Genotypes that perform well in E1 (Harare optimum) should also have good performance at E2 (managed drought stress at Chiredzi Research Station), E5 (Kaguvi Training Centre), E7 (Rattrey Anorld Research Station) and E10 (Harare low P stress).

5.6 Conclusions

Variability in crop growing conditions because of variation in climatic and edaphic conditions across seasons and regions, causes maize yield performance to be variable. Maize grain yield is affected by the genotype, which involves its genes and the environment, which involves soil and climatic factors, and also by the interaction between the genotype and the environment. Ideal cultivars should be both high yielding and stable, hence it is important to understand the GEI affecting maize yields under different abiotic stress conditions. The objective of this study was to identify high yielding, stable provitamin A hybrids, suitable for cultivation under diverse abiotic stress conditions and to identify hybrids adapted for each specific growing environment. Both AMMI and GGE biplot showed that GEI was a very important aspect influencing maize yields under diverse environments. The large environmental variance and GEI variance means it is possible to select hybrids suitable for specific environments.

Both AMMI and GGE identified G31 as the ideal genotype. Provitamin A genotypes G6, G7, G8, G16, G19, G27 and G29 had above average grain yield and stability. G3 had the lowest ASV and hence is the most stable genotype, however, it was low yielding. Genotypes G31 and G34 were the best yielding and were most suitable for environments E2 (managed drought stress at Chiredzi Research Station), E10 (Harare low P stress) and E1 (Harare optimum). G31, G34, G8, G6, G7, G16, G27, G23 and G24 were some of the hybrids that fell in the same environment sector, meaning they were adapted to all the environments. Provitamin A genotypes G8, G16 and G6 were the highest yielding and stable among the provitamin A genotypes, though they were lower yielding and stable when compared to checks G31 and G34. Other stable genotypes with above average grain yield were G26, G23, G27 and G7. Provitamin A genotypes G8, G16, G6 can be candidates for commercial release, targeting all the given environments. Environment E7 (Rattrey Arnold Research Station) was the most discriminating of the genotypes followed by E10 (Harare low P stress), E1 (Harare optimum) and E2 (managed drought stress at Chiredzi Research Station) in that order and genotypes performed similarly in these environments. However environments E7 (Rattrey Arnold Research Station) and E10 (Harare low P stress) were the least representative of the mega-environment. These two environments cannot be used for selecting best genotypes, but they are important in screening unstable and specifically adapted genotypes. Environments E6 (Harare low N stress), E4 (Kadoma Research Station), E5 (Kaguvi Training Centre), E1 (Harare optimum) and E2 (managed drought stress at Chiredzi Research Station) were more representative of the mega-environment and were ideal environments for selecting superior genotypes. Environments E3 (combined drought and heat stress at Chiredzi Research Station) and E9 (Rio Tinto Agricultural College) provided very little information about the genotypes and therefore should not be used as test environments. G31 was identified as the ideal genotype. Ranking of the other genotypes in relation to the ideal genotype was G34 > G8 > G16 > G6 > G7. Genotypes G13, G2 and G22 were the least ideal. Environment E2 was the most ideal followed by environment E10 (Harare low P stress) and E1 (Harare optimum), in that order. E2 (managed drought stress at Chiredzi Research Station) was more stable and suitable for all the genotypes. The significant correlation coefficients between the environments indicate that it is possible to select in one environment and the genotypes will perform the same in the other correlated environments. If funds are limiting, one can use only one of the correlated environments and screen genotypes suitable for all the correlated environments, this will significantly

reduce the cost. Genotypes that perform well in E1 (Harare optimum) should also have good performance at E2 (managed drought stress at Chiredzi Research Station), E5 (Kaguvi Training Centre), E7 (Rattry Arnold Research Station) and E10 (Harare low P stress).

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CHAPTER 6

ANALYSIS OF β-CAROTENE CONCENTRATION IN PROVITAMIN A MAIZE UNDER ABIOTIC STRESS AND OPTIMUM CONDITIONS

6.1 Abstract

White maize is the major staple food crop for most of the people living in SSA. However, it is deficient in vitamin A carotenoids and a large number of people living in the region suffer from vitamin A malnutrition. To address this problem, breeding programmes in many developing countries have incorporated biofortification of staple crops in their programmes. In this study 30 provitamin A hybrids and five checks were evaluated under optimum and abiotic stress conditions and β-carotene concentration was determined by high-performance liquid chromatography. GEI effect, combining ability, phenotypic and genotypic variance components and heritability for β-carotene were determined. Hybrid, environment, year and GEI effect for β-carotene were highly significant ($P < 0.01$). Beta-carotene concentration was higher under optimum than under stress conditions. The GGE biplot grouped hybrids into one mega-environment and one minor mega environment and it identified hybrid 29 as the ideal genotype for β-carotene content. Additive genetic variance constituted the highest percentage of total variation, followed by dominance variance. Broad sense heritability for β-carotene was very high, 97% and 90% under optimum and 70% and 94% under managed drought stress in 2014 and 2015, respectively. Narrow sense heritability across seasons was 54%. Best performing genotypes were identified and these can be evaluated further and released as cultivars or used in development of three-way cross hybrids and synthetics.

6.2 Introduction

Vitamin A is important in human nutrition for the normal functioning of the visual system, growth and development of infants, a strong immune system and reproduction (Stephensen, 2001; WHO, 2009). Unfortunately, more than 250 million people worldwide are at risk of visual impairment and blindness because of vitamin A malnutrition (African Union, 2005; Menkir et al., 2008). The main cause of vitamin A malnutrition in humans is lack of the required amount of vitamin A in the diet, resulting in low body stores and failure to meet physiological needs. This is a major problem during nutritionally demanding periods of life, such as infancy, childhood, pregnancy and lactation

(MOHCW, 1999). The problem of vitamin A malnutrition in SSA is exacerbated by over-dependence on cereal-based diets, mostly porridges prepared from white maize flour (van der Merwe et al., 2001; Egounlety et al., 2002; Nuss et al., 2012).

Supplementation, food fortification and diet diversification recommended as strategies to combat the problem are unsustainable and beyond the reach of poor households (Frossard et al., 2000; Bouis, 2003; Pfeiffer and McClafferty, 2007). Therefore there is a need to look for other affordable and sustainable options to address the pandemic. Research has shown that daily vitamin A requirements can be satisfied by plant-based carotenoids through several servings of fruits, vegetables or cereals like orange maize, hence this can be one of the viable options. Biofortified staple food crops like orange maize provides a sustainable option to address the problem among the poor (Bouis, 2003; Welch and Graham, 2004). The option is sustainable, especially in the SSA region because the people are already subsisting on maize (Harvest Plus, 2003). Fruits are seasonal and the poor households living in urban areas have no land to cultivate vegetables, leaving biofortified staple foods as the most viable option to improve nutritional status. Biofortified orange maize can be grown and consumed by resource poor households even without any government support and donor funding (Bouis, 2003). This will provide a sustainable means of reaching poor people living in very remote areas with no access to fortified foods that may be available in urban setups.

Orange maize contains provitamin A carotenoids such as α-carotene, β-carotene and β-cryptoxanthin (Kurilich and Juvik, 1999; Harjes et al., 2008; Menkir et al., 2008) which have provitamin A activity when they are cleaved in the intestinal lumen to produce vitamin A (Aluru et al., 2008). Replacement of white maize with provitamin A rich orange maize varieties might have a significant and positive impact on vitamin A status for people living in SSA who subsist on white maize (Tanumihardjo et al., 2008; Nuss et al., 2012). Howe and Tanumihardjo (2006a) showed that provitamin A in orange maize is as bioavailable as β-carotene supplements.

Maize in the SSA region is mostly produced by smallholder farmers in marginal areas under low input production systems (Mtambanengwe and Mapfumo, 2005). This leads to maize being exposed to biotic and abiotic stresses that give rise to GEI on cultivar performance. The key abiotic stresses common in the SSA region include drought, heat,

low N, low pH and low P (Edmeades et al., 2011). The understanding of GEI, stability parameters, and genetic correlations for provitamin A carotenoids is important for an informed choice of appropriate breeding strategies for high provitamin A maize. Kang (2004) defined GEI as the differential response of cultivars to fluctuating growing conditions. GEI complicates evaluation and identification of valuable genotypes, resulting in reduced genetic gain of targeted traits (Shafii and Price, 1998). Understanding of GEI also allows making of informed choices regarding sites and management of breeding trials for improved nutrient levels.

Systematic evaluation of GEI effects for a given trait is useful for understanding varietal stability and hence strategic deployment of varieties (Acquaah, 2012; Esuma et al., 2016). Rosello et al. (2011), while studying GEI in tomatoes, reported that β-carotene accumulation has a large genetic component. Manrique and Hermann (2000) reported that β-carotene in sweet potato cultivars is stable across varying environments. Ndirigwe (2005) observed significant GEI for β-carotene levels in sweet potatoes grown in diverse environments. Menkir and Maziya-Dixon (2004) reported that β-carotene is influenced more by genotype than by the environment. They observed no significant GEI, suggesting that the relative performance of the varieties for β-carotene were consistent across test environments. They also reported no significant correlation between β-carotene content and maize grain yield. Wong et al. (1998) reported that β-carotene in temperate maize germplasm is heritable and is also stable across years, showing no GEI. However very little information on stability of β-carotene content in tropical maize germplasm under diverse growing conditions is available.

The importance of provitamin A maize varieties as source of vitamin A in a breeding programme depends on the stability of expression of these compounds across diverse growing conditions. Since limited information is available on the effect of different growing conditions and its interaction with the genotype on provitamin A carotenoids content in SSA, this study seeks to bridge that gap. It seeks to examine the influence of genotype, environment and their interaction on provitamin A carotenoids content in single cross hybrids developed by crossing CIMMYT elite provitamin A inbred lines with CIMMYT elite drought tolerant inbred lines as testers under low N stress, managed drought stress and optimum growing conditions.

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6.3 Materials and methods

The genotypes were grown at the sites and environments as described in Chapter 3 Section 3.3. Provitamin A analysis was done for Harare optimum, Harare low N stress and Save Valley Experimental station (drought stress), only (for both seasons), because of the cost of the laboratory analyses. Grain yield and grain texture were also measured as described in Chapter 3.

6.3.1 Beta-carotene determination

Beta-carotene determination was done following a method modified from that described by Kurilich and Juvik (1999) and Howe and Tanumihardjo (2006b), described in Galicia et al. (2009). After harvesting, grain was kept in a refrigerator at 4°C. Thirty grains were randomly selected from each genotype and ground into fine powder using a Yellowline A10 analytical grinder and the powder was placed in a brown envelope and immediately sealed. The milled flour was kept in a freezer at -20°C. The samples were analysed on the same day they were milled. The β-carotene was extracted from the samples with yellow light and analysed by HPLC just after extraction.

The β-carotene extraction procedure was as follows. For each sample 600 mg of maize flour was weighed and placed in 15 ml falcon tubes previously wrapped with aluminium paper. Six mm of 0.1% butylhydroxytoluene (BHT) in ethanol was then added and the sample vortexed thoroughly before being incubated at 85°C in a water bath for 5 minutes. Approximately 120 µl 80% potassium hydroxide (KOH) was added to the sample and vortexed thoroughly and then incubated at 85°C in a water bath for 10 minutes. The falcon tubes were transferred to ice under the fume hood and 3 ml of cold deionised water was added to each sample followed by 3 ml hexane. The samples were then vortexed thoroughly before being centrifuged at 3000 rpm for 10 minutes. After centrifuging, the upper phase was transferred to a new falcon tube, also wrapped with aluminium paper and immediately covered to avoid contact of the upper phase with air. The new tubes were kept on ice. The remaining aqueous layer was extracted twice more with hexane.

After extraction, 3 ml of deionised water was added to the combined hexane fractions and the sample vortexed thoroughly and then centrifuged at 3000 rpm for 10 minutes. The upper phase was then transferred to a new falcon tube wrapped with aluminium paper. The hexane was then dried by putting the tubes under nitrogen. This was also done in the

fume hood. The tubes were immediately covered after the hexane had dried. The samples were resuspended in 200 µl of acetonitrile:methanol:methylene chloride (45:20:35) just before being injected into the HPLC machine. HPLC separation and quantification of carotenoids was done using a Shimadzu Co HPLC with an YMC Carotenoid 5u, 4x2 Gd Cat pre-column and YMC Carotenoid S-5 4,6x 150 mm column. Acetonitrile: methanol: methylene chloride (75:20:5) containing 0.05% triethylamine (TEA) and 0.1% BHT was used as the mobile phase. The flow rate was set at 1.8 ml min^{-1} and analysis per sample lasted 40 minutes. Chromatographs were generated at 450 nm. Identification and estimation of carotenoids was then done using standards which were purchased from SIGMA. The β-carotene content was then calculated using a standard calibration curve.

6.3.2 Statistical analysis

AMMI and GGE biplot analysis was done on provitamin A carotenoid data as explained in Chapter 5 Section 5.3.1. The R programme for line x tester analysis was used to calculate the GCA and SCA effects according to the method of Singh and Chaudhary (1977) as explained in Chapter 4 Section 4.3.1. Heritability, genotypic and phenotypic variances and correlations were calculated per year, per environment, across environments and across environments and years as explained in Chapter 4 Section 4.3.1. Pearson's rank correlation coefficients were calculated between β-carotene and grain yield and grain texture to determine these relationships under different environments (described in Table 3.2) using GenStat (16th Edition).

6.4 Results

6.4.1 Performance and ranking of hybrids for grain yield and β-carotene concentration

Hybrid, environment, and year and hybrid x environment interaction effects were highly significant ($P < 0.01$) and environment x year and hybrid x environment x year effects were significant ($p < 0.05$) (Table 6.1) for β-carotene concentration. Hybrid x year effect was not significant.

Table 6.1 Analysis of variance for 30 provitamin A hybrids and five checks for β-carotene concentration

Source of variation	DF	SS	MS
Hybrid	34	717.82	21.11**
Environment	2	1948.89	974.45**
Year	1	24.78	24.78**
Hybrid x Environment	68	456.35	6.711**
Hybrid x Year	34	97.72	2.87
Environment x Year	2	25.68	12.84*
Hybrid x Environment x Year	68	258.09	3.80*
Residual	210	478.02	2.28
Total	419	4007.35	9.56

* $P<0.05$, ** $P<0.01$

Hybrids had significantly higher ($p < 0.05$) β-carotene concentration when cultivated under optimum conditions than under managed drought stress or low N stress conditions (Table 6.2). Genotypes, however, produced similar β-carotene concentration when cultivated under managed drought stress and low N stress. Under optimum conditions β-carotene concentration varied from 0.00 to 12.55 µg g^{-1} and the average was 7.39 µg g^{-1}. Under managed drought stress and low N stress it ranged from 0.00 to 4.08 µg g^{-1} averaging 2.86 µg g^{-1} and 0.00 to 4.43 µg g^{-1} and the average was 2.77 µg g^{-1}, respectively. Genotypes 8, 6, 7, 10 and 23, which were among the top 10 in grain yield production and had high β-carotene concentration, higher than 7 µg g^{-1} under optimum conditions, should be considered for further evaluation and released as cultivars under optimum conditions.

Table 6.2 Mean performance of 30 provitamin A hybrids and five checks for grain yield and β-carotene concentration over two seasons

Rank	Across environment			Optimum			Low N stress			Managed drought stress		
	Grain yield (t ha⁻¹)	Entry	Beta-carotene (μg g⁻¹)	Grain yield (t ha⁻¹)	Entry	Beta-carotene (μg g⁻¹)	Grain yield (t ha⁻¹)	Entry	Beta-carotene (μg g⁻¹)	Grain yield (t ha⁻¹)	Entry	Beta-carotene (μg g⁻¹)
1	7.01	31	0.00	12.87	31	0.00	5.74	34	4.27	4.76	31	0.00
2	6.19	34	4.69	10.94	34	7.84	4.86	33	3.23	4.61	7	3.29
3	6.02	8	4.82	10.41	8	9.10	4.50	10	3.01	4.22	8	2.91
4	5.37	16	3.27	10.16	6	7.70	4.39	6	2.06	4.05	16	2.35
5	5.36	7	5.45	9.1	26	4.79	4.33	16	3.17	3.85	19	3.18
6	5.04	6	4.22	8.57	16	4.29	4.32	8	2.45	3.73	3	4.07
7	4.86	33	4.18	8.14	7	9.48	4.26	31	0.00	3.67	34	1.97
8	4.74	26	3.43	7.76	24	5.08	4.24	9	2.83	3.60	27	3.20
9	4.62	32	0.00	7.62	10	9.17	4.24	32	0.00	3.59	1	2.28
10	4.49	1	3.55	7.60	23	7.84	4.09	17	2.89	3.53	33	2.88
11	4.48	10	5.2	7.49	32	0.00	3.80	25	2.97	3.40	14	2.99
12	4.47	23	4.52	7.40	33	6.44	3.79	7	3.59	3.22	28	3.19
13	4.45	3	4.79	7.38	1	6.51	3.75	27	3.76	3.20	30	3.25
14	4.42	27	5.40	7.24	29	11.3	3.72	35	4.43	3.09	23	2.95
15	4.27	35	4.98	7.15	28	9.46	3.67	24	3.43	3.03	17	3.51
16	4.20	28	5.32	7.03	35	8.25	3.66	26	3.08	2.99	5	4.08
17	4.20	5	5.96	6.85	9	7.23	3.58	4	3.14	2.98	35	2.25
18	4.18	24	4.01	6.82	27	9.25	3.48	23	2.77	2.96	32	0.00
19	4.15	29	5.84	6.79	5	11.15	3.46	5	2.65	2.91	2	2.98
20	3.99	9	4.57	6.57	12	5.84	3.37	29	2.03	2.77	29	4.18
21	3.95	17	5.05	6.56	3	7.99	3.20	11	2.28	2.75	18	2.94
22	3.91	12	3.75	6.37	30	6.69	3.17	3	2.32	2.69	15	3.80
23	3.81	19	5.02	6.26	25	12.55	3.14	1	1.85	2.60	26	2.41
24	3.78	25	6.24	6.16	20	6.12	3.10	20	2.14	2.38	20	2.43
25	3.77	30	4.15	6.00	4	8.57	3.08	15	3.21	2.37	12	3.09
26	3.69	20	3.56	5.36	13	9.16	3.07	28	3.32	2.30	24	3.51
27	3.61	4	4.66	5.34	15	7.85	3.06	18	2.57	2.26	25	3.22
28	3.58	15	4.96	5.27	11	5.44	3.04	21	2.34	2.23	21	2.24
29	3.45	2	5.19	5.21	17	8.74	2.96	13	2.57	2.23	22	3.11
30	3.45	14	4.18	5.05	19	8.31	2.95	2	2.83	2.13	10	3.41
31	3.43	18	3.57	5.04	14	6.69	2.92	12	2.31	2.07	4	2.28
32	3.32	13	4.83	4.72	18	5.21	2.89	19	4.4	2.06	13	3.04
33	3.32	11	3.47	4.65	2	9.76	2.85	14	2.85	1.96	11	2.70
34	2.96	22	4.86	4.45	22	7.68	2.75	22	3.78	1.92	6	2.91
35	2.93	21	3.89	3.91	21	7.08	2.07	30	2.52	1.75	9	3.66
Mean	5.28		4.33	6.98		7.39	3.59		2.77	2.97		2.86
Min	3.47		0.00	3.91		0.00	2.07		0.00	1.75		0.00
Max	8.57		6.24	12.87		12.55	5.74		4.43	4.76		4.08
P-value			0.001	0.001		0.001	0.001		0.001	0.004		0.001
LSD	0.87		1.21	2.33		3.23	1.12		1.38	1.14		1.12
CV%	29.90		34.75	23.63		30.97	29.47		35.35	53.90		27.84

6.4.2 AMMI analysis of variance

Table 6.3 shows the effects of hybrids, environments and their interactions on β-carotene concentration. Environmental effects accounted for 56.83% of total sum of squares. Genotype and GEI accounted for 20.07% and 23.10% of variation, respectively. The effects of hybrids, environments and their interactions were highly significant (p<0.01). Only the first two IPCAs were highly significant (p<0.01) for β-carotene concentration. Environments were the largest source of variation (56.83%). GEI effects were larger than hybrid effects. The first two IPCAs accounted for 83.51% of total variation of GEI observed, which was confirmed by the significant GEI effect. ICPA1 of the GEI captured 49.81% of the sum of squares for GEI and IPCA2 explained a further 33.69 % of variation. All in all, the AMMI model explained 87.98% of the total sums of squares.

Table 6.3 AMMI analysis of variance for β-carotene concentration of provitamin A maize hybrids tested over three sites in two years

Source	DF	SS	MS	Explained total SS (%)	Explained GEI SS (%)
Total	419	4027.10	9.61		
Treatment	209	3542.90	16.95**		
Genotypes	34	711.00	20.91**	20.07	
Environments	5	2013.50	402.70**	56.83	
Block	6	122.30	20.39**	3.45	
Interactions	170	818.30	4.81**	23.10	
IPCA 1	38	407.60	10.73**	11.50	49.81
IPCA 2	36	275.70	7.66**	7.87	33.69
Residuals	-28	0.00	0.00		
Error	204	361.90	1.77		

** P<0.01, DF = Degrees of freedom, SS = sum of squares, MS = mean squares, IPCA = interaction principle component axis, GEI = genotype by environment interaction

6.4.3 Combining ability for β-carotene

Results for combining ability analysis of provitamin A and drought lines are presented in Table 6.4. Across years, environment effect was highly significant (P < 0.01) and GCA_f, GCA_m, environment x GCA_f and environment x GCA_m were significant (p < 0.05). Environment x SCA and SCA were not significant. In 2014 and 2015, environment and GCA_f were highly significant (P < 0.01) and SCA was significant (p < 0.05) in 2014.

Table 6.4 Analysis of variance for combining ability of provitamin A and drought tolerant elite lines

Source	Degrees of freedom	2014 Mean squares	2015 Mean squares	Across years Mean squares
Replication	1	1.36	23.41	6.73
Environment	2	389.09**	582.26**	961.00**
GCA_f	9	8.46**	11.22**	16.85*
GCA_m	2	2.10	18.50*	14.63*
Environment x GCA_f	18	8.14**	2.23	6.12*
Environment x GCA_m	4	2.93	18.32**	12.85*
SCA	18	3.11*	2.42	2.96
Environment x SCA	36	3.84	3.82	4.08

* P<0.05, ** P<0.01

6.4.4 Identifying superior genotypes and mega environments

The vertex genotypes were 31, 32, 4, 10, 25 and 14 (Figure 6.1). The GGE biplot also indicated mega-environments (a detailed explanation of mega-environment is given in Chapter 5). Two mega environments were suggested from this analysis. The first mega environment contained environment optimum 15 only and the second mega environment had the remaining five environments. The first mega environment had genotypes 4 and 10 as the best performers.
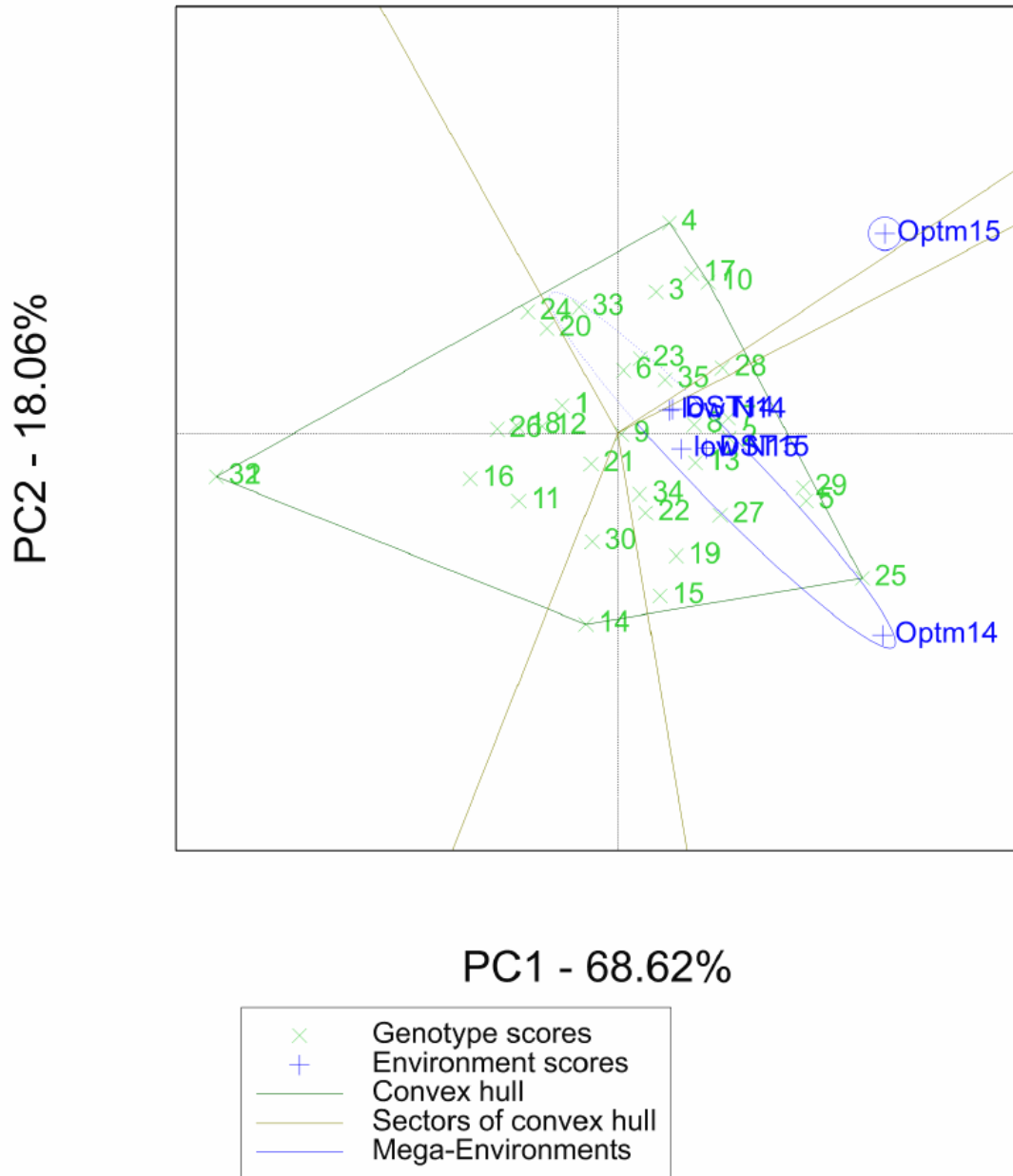
Figure 6.1 Polygon view of the GGE biplot based on symmetrical scaling for which-won-where pattern of genotypes and environments for β-carotene

Opt14 = Harare optimum 2014, Opt15 = Harare optimum 2015, LowN14 = Low N stress 2014, LowN15 = Low N stress 2015, DST14 = Save valley experimental station drought stress 2014, DST15 = Save valley experimental station drought stress 2015

The second mega environment had genotype 25 as the best performer. Data from this trial suggests that environment Optm15 (Harare optimum in 2015) is the best for breeding maize rich in β-carotene. Most of the hybrids were located near the origin of the biplot, suggesting they were less responsive to the change of environments.

The ideal genotype was 29 followed by genotype 5 (Figure 6.2). The most discriminating environment was Optm15 (Harare optimum in 2015) followed by Optm14 (Harare optimum in 2014).
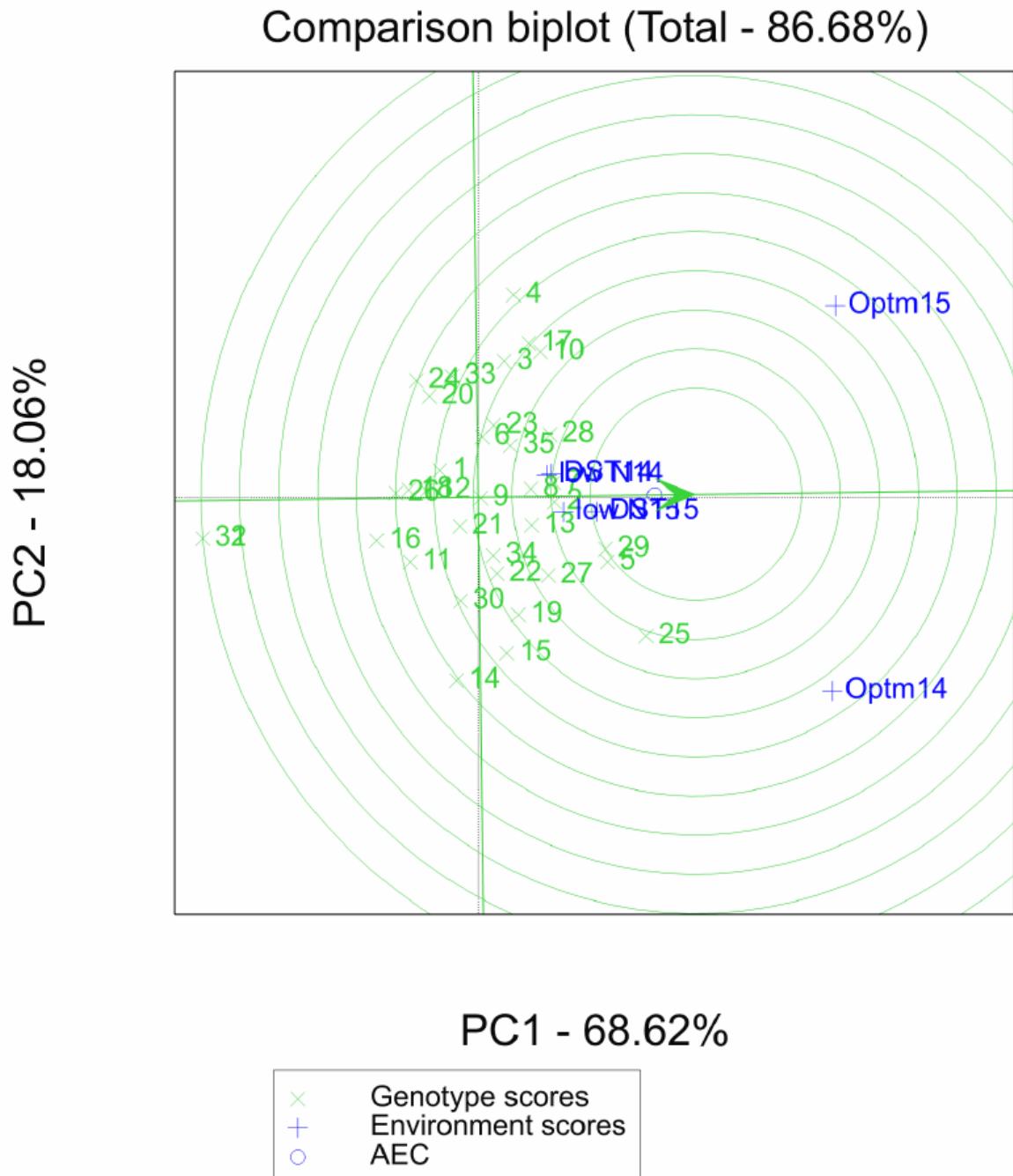
Figure 6.2 GGE biplot showing β-carotene production performance and stability of 30 provitamin A hybrids and five checks tested over ten sites for grain yield
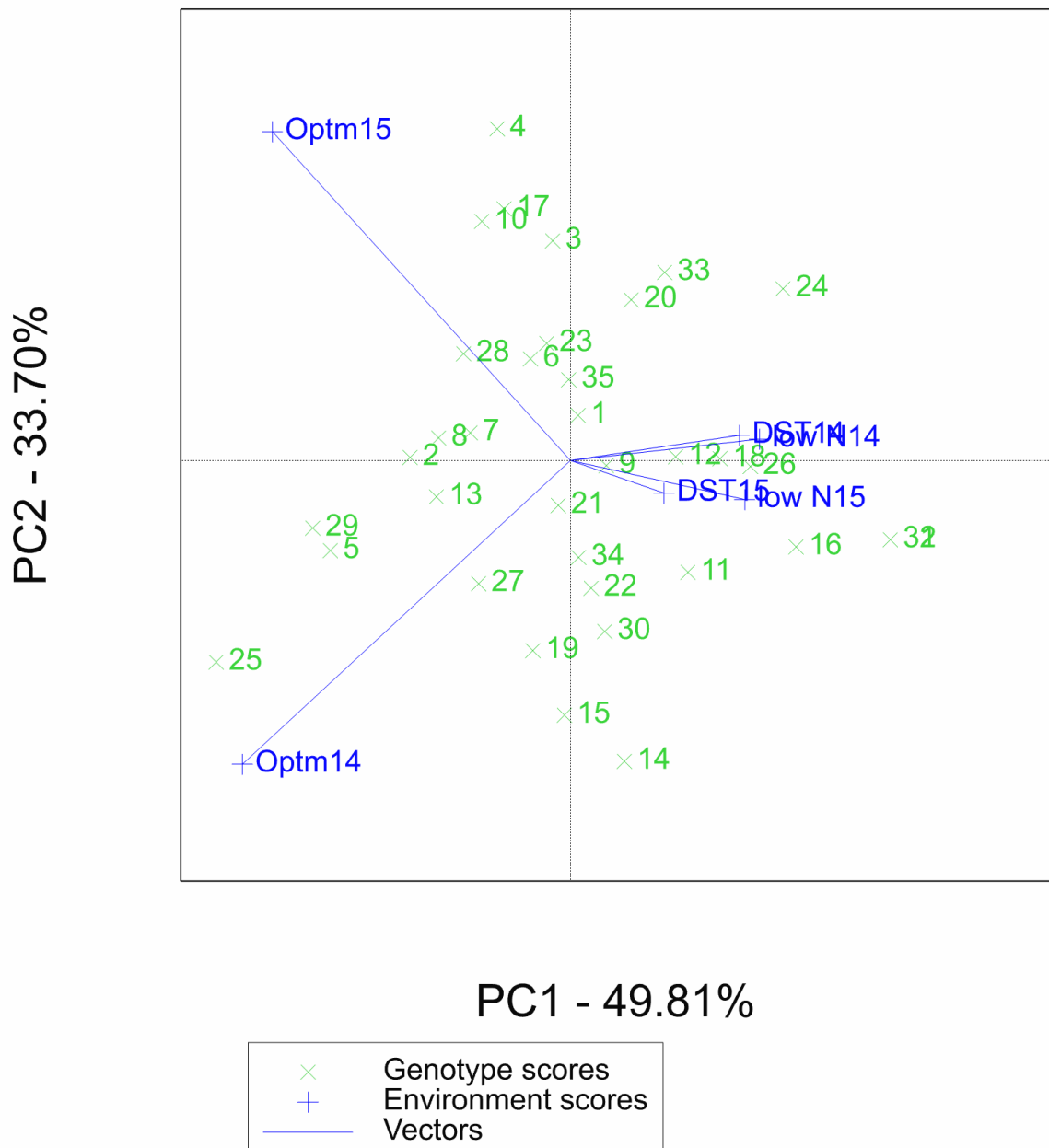
Opt14 = Harare optimum 2014, Opt15 = Harare optimum 2015, LowN14 = Low N stress 2014, LowN15 = Low N stress 2015, DST14 = Save valley experimental station drought stress 2014, DST15 = Save valley experimental station drought stress 2015.

Figure 6.3 Correlations between test environments based on grain β-carotene concentration

Opt14 = Harare optimum 2014, Opt15 = Harare optimum 2015, LowN14 = Low N stress 2014, LowN15 = Low N stress 2015, DST14 = Save Valley Experimental Station drought stress 2014, DST15 = Save Valley Experimental Station drought stress 2015

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Table 6.5 Correlations between test environments based on grain β-carotene concentration

	E1	E2	E3	E4	E5
E2	0.32*				
E3	0.12	0.19			
E4	0.31*	0.07	0.30*		
E5	0.12	0.12	0.06	0.11	
E6	0.46**	0.27*	0.21	0.21	0.12

* $P<0.05$, ** $P<0.0$, E1 = Harare optimum 2014, E2 = Harare optimum 2015, E3 = Low N stress 2014, E4 = Low N stress 2015, E5 = Save Valley Experimental Station drought stress 2014, E6 = Save Valley Experimental Station drought stress 2015.

6.4.5 Correlations between test environments

The correlations between environments are presented in Figure 6.3. The lines that connect the origin of the biplot in the vector view of the GGE-biplot and the markers of the environments are called environment vectors. The angle between the vectors of two environments is related to the correlation coefficient between them. All stress environments were positively correlated because all angles among them were less than 90°. They were also negatively correlated with all the optimum environments. From results presented in Table 6.5, E1 (Harare optimum in 2014) was highly significantly correlated with E6 (managed drought in 2015) and significantly correlated with E2 (optimum in 2015) and E4 (low N stress in 2015). E2 (optimum 2014) was significantly correlated to E6 (managed drought stress 2015). E3 (low N stress in 2014) was significantly correlated with E4 (low N stress in 2015)

6.4.6 Correlations among β-carotene, yield and seed texture

Beta-carotene concentration in maize grain showed highly significant positive correlations ($P < 0.01$) with grain yield (Table 6.6), but no relationship with grain texture.

Table 6.6 Correlations between β-carotene, grain texture and yield of 30 provitamin A hybrids and five checks grown under optimum and stress conditions

	β-carotene	Texture
Texture	0.06	
Grain yield	0.48**	0.20*

** p≤ 0.01, * p≤ 0.05

6.4.7 Estimates of variance components and heritability under optimum, low nitrogen stress and managed drought stress

The estimates of variance components and heritability under optimum, low N stress and managed drought stress are presented in Table 6.7. Additive genetic variance constituted the highest percentage of total variance accounting for 42.22%, 46.31% and 48.78% of total variance in 2014, 2015 and across seasons, respectively (data not shown). Dominance variance also contributed a high percentage to total variance. Environment contributed marginally to total variation under optimum conditions but more under both stress conditions. Heritability was very high in all three environments. Broad sense heritability was 97%, 0% and 70% under optimum, low N stress and managed drought stress respectively in 2014. In 2015, it was 90%, 66% and 94% under optimum, low N stress and managed drought stress, respectively. Narrow sense heritability varied significantly at 61%, 0%, 35% and 62%, 37%, 70% under optimum, low N stress and managed drought stress in 2014 and 2015 respectively. Across seasons and environments, broad sense heritability and narrow sense heritability were equal (54%).

Table 6.7 Estimates of variance components and heritability under optimum, low nitrogen stress and managed drought stress

	2014			2015			Across seasons			Across season and environments
	Optimum	Low N stress	Managed drought stress	Optimum	Low N stress	Managed drought stress	Optimum	Low N stress	Managed drought stress	
Line	2.50	0.00	0.00	0.44	0.02	0.48	1.37	0.02	0.00	0.30
Tester	0.00	0.00	0.00	2.21	0.02	0.03	0.42	0.00	0.00	0.01
Line x tester	3.23	0.00	0.12	1.55	0.13	0.23	0.43	0.10	0.00	0.00
Genotype	5.56	0.00	0.12	3.48	0.16	0.70	1.90	0.12	0.00	0.23
Additive	22.25	0.00	0.00	13.92	0.65	2.81	7.61	0.46	0.00	0.92
Dominance	12.92	0.00	0.00	6.19	0.54	0.94	1.73	0.40	0.00	0.00
Environmental	1.06	0.36	0.41	2.27	0.59	0.25	2.14	0.23	0.37	0.64
Broad sense heritability	0.97	0.00	0.70	0.90	0.66	0.94	0.81	0.79	0.00	0.54
Narrow sense heritability	0.61	0.00	0.35	0.62	0.37	0.70	0.66	0.42	0.00	0.54

6.5 Discussion

Provitamin A hybrids had β-carotene concentration in the expected range for first generation medium to high provitamin A maize genotypes (3-8 µg g^{-1}). The HarvestPlus target is 15 µg g^{-1} (Pfeiffer and McClafferty, 2007; Pixley et al., 2011; Halilu et al., 2016). The hybrids in this study were generated by crossing elite provitamin A lines with elite drought tolerant yellow lines. This is the reason why the β-carotene concentration was average. Egesel et al. (2003) reported that crosses between yellow inbreds with high and low carotene concentration showed that the pollen parent had considerable effect on carotenoid content of the subsequent hybrid when they studied gene dosage effects on carotenoid concentration in maize grain. Grogan and Blessin (1968) also observed that provitamin A levels in F$_1$ hybrid seed tended to be little lower than mid-parent value, indicating some dominance for low provitamin A.

To understand gene action affecting β-carotene concentration in maize grain, combining ability was evaluated. Egesel et al. (2003) reported significant GCA and SCA effects. In this study GCA was significant, suggesting that additive gene action was more important for determining β-carotene concentration in maize grain. SCA was not significant, suggesting that non additive gene action was not important in expression of β-carotene genes. Babu et al. (2012) reported that gene action for *Lcy-5ʹ*TE and *CrtRB1-3ʹ*TE associated with total provitamin A in maize grain was partially dominant and partially recessive, suggesting that it is not totally additive. Significant GEI for β-carotene was seen in this study, despite high heritability estimates and large additive gene effects. This was probably due to the fact that the environments were vastly different, varying from optimum to drought and low N stressed conditions, where β-carotene was much higher under optimal conditions. Suwarno et al. (2014) reported significant GCA and weak SCA effects and concluded that provitamin A concentration in maize grains is controlled mainly by additive gene action. However, results from this study agree with those of Rios et al. (2009) who reported significant GEI for provitamin A carotenoids in maize. Provitamin A concentration in maize grain is controlled by a few (~2) major genes, and is simply inherited (Egesel et al., 2003; Menkir and Maziya-Dixon, 2004; Gruneberg et al., 2005).

High additive variance was seen, emphasising the importance of additive gene action in expression of β-carotene. The fact that β-carotene is controlled predominantly by additive gene action was further supported by high heritability values.

The AMMI analysis for β-carotene indicated that almost 50% of the variation in GEI sum of squares was captured by IPCA1 and more than 33% was captured by IPCA2. The rest of the IPCAs were not significant, suggesting that they mostly captured random error.

Indirect selection can be done if the same trait is measured on the same hybrids in different environments if the environments are correlated (Kaya et al., 2006). All the stress environments were positively correlated, suggesting that if resources are limiting one can select in only one environment and the genotypes should perform similarly in other stress environments.

According to Kaya et al. (2006) an ideal genotype should have the highest mean performance and be absolutely stable for the trait under consideration. Such a genotype is defined by having the greatest vector length of the high yielding genotypes and with zero GEI as shown by an arrow pointing to it. Although such a genotype does not exist in real life, it can be used as a reference for genotype evaluation. Genotypes that are located closer to the ideal genotypes are stable. Hybrid 29 was the ideal genotype followed by hybrid 5. Most hybrids fell on the positive side of the average environment coordinate, hence they had above average performance on grain β-carotene concentration (more detail on ideal genotypes can be read in Chapter 5 Section 5.4.2.3). Only 11 genotypes had below average β-carotene concentration in their grain and they fell on the negative side of the average environment coordinate.

The highly significant positive correlation of β-carotene concentration with grain yield, suggests that it is possible to select for both traits in a breeding programme. Halilu et al. (2016) also reported similar results for grain yield and total carotenoids and concluded that there is scope to concurrently improve grain yield and the provitamin A carotenoids for biofortification. However, Egesel et al. (2003) and Suwarno et al. (2014) reported no correlation between grain yield and total provitamin A concentration.

The significant GEI suggests that genotype ranking for β-carotene was different in various environments. The results corroborate findings by Halilu et al. (2016) who reported a highly significant effect of environment and also agree with Egesel et al. (2003) who observed significant location effects for all carotenoids except zeaxanthin in yellow maize. On the contrary, Esuma et al. (2016) reported no significant effect of environment and GEI on total carotenoids in cassava. The results were also in contrast to findings reported by Menkir et al. (2008) who observed that location and GEI had no significant influence on carotenoid concentrations in maize grain. Menkir and Maziya-Dixon (2004) reported that GEI for β-carotene was not significant, when they studied 17 genotypes in three locations. In this study, environment had highly significant (P < 0.01) effects on carotenoid concentration. In spite of this, high additive gene action and high heritability were shown for β-carotene concentration under the different production conditions. High levels of β-carotene was produced when hybrids were cultivated under optimum conditions and there were no significant differences in β-carotene concentration when hybrids were cultivated under managed drought stress and low N stress. The differences observed may be due to the stress imposed, suggesting that water availability and good soil nutrition is important for maximum expression of genes controlling β-carotene concentration. This is a cause of concern, since the targeted poor households inflicted by vitamin A malnutrition produce maize under erratic rainfed conditions and on inherently infertile soils. Because of over cultivation without N fertilisation of soils in remote areas, they are deficient in soil N. The poor households cannot afford supplementary irrigation and fertilizer inputs because of lack of funds.

The significant differences of genotypes for β-carotene concentration mean that it is possible to develop hybrids with high β-carotene concentration. These results corroborate the findings of Egesel et al. (2003), Menkir and Maziya-Dixon (2004), Chander et al. (2008) and Menkir et al. (2008) who proposed that there is scope for genetic improvement of provitamin A carotenoids in maize because of genetic diversity exhibited by maize genotypes.

6.6 Conclusions

Efforts to address vitamin A malnutrition through biofortification of staple food crops is underway in breeding programmes in many countries. Thirty provitamin A hybrids with five checks were evaluated under abiotic stress and optimum conditions. Beta-carotene

concentration in maize grain was affected by environment and hybrid and their interaction. AMMI and GGE models were effective in explaining GEI variation. Beta-carotene concentration was high under optimum conditions when compared to stress conditions. Hybrids expressed variability for β-carotene that enables improvement of β-carotene in maize cultivars. The estimation of heritability showed that the variation of grain β-carotene concentration was controlled by genetic attributes of the genotypes and the environment had a much smaller influence than genotype on variation of this trait.

Beta-carotene concentration in maize grain is predominantly influenced by additive and dominance gene action. It is possible to develop hybrids that would have high levels of β-carotene production in a given environment. Beta-carotene concentration in the maize grain was positively correlated with grain yield and was also highly heritable. The first two principal components accounted for a total of 96.45% of total variation Hybrids 17, 18, 20, 21, 22, 23, 26, 27, 28 and 30 were good performers and hybrids 1, 2 and 35 were poor performers. The significant differences of genotypes for β-carotene concentration mean that it is possible to develop hybrids with high β-carotene concentration. The results reported in this study gives hope that it is possible to develop hybrids with high β-carotene concentration to address vitamin A malnutrition among most vulnerable people in SSA, without any yield penalty when compared to white maize.

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CHAPTER 7

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Maize is the most important cereal crop grown in the SSA region, including Zimbabwe, providing about 34% of the region's food calories, and 15% of the world's food-crop protein and hence is very important for household food security. Zimbabweans view maize as synonymous with food as almost every meal taken by the majority of the people in the country contains a component of maize. Area devoted to maize production ranks first among all the crops for both smallholder and commercial farmers. All smallholder farmers produce maize, even those situated in the most marginal areas of the country. However, realised yield in the smallholder sector is very low because of poor soil fertility; worsened by credit unworthiness to borrow funds to buy fertilisers; erratic and inadequate rainfall; use of poorly adapted varieties; late planting and poor pest and disease management.

Overreliance on white maize, which is nutritionally poor and contains no vitamin A, as staple, exposes the people in the region to various forms of malnutrition. For instance the region is burdened with high prevalence of vitamin A malnutrition. Vitamin A malnutrition leads to visual impairment and even blindness and a weak immune system, which contributes to predisposition to diseases such as anaemia, diarrhoea, measles, malaria and respiratory infection. Stunted growth among children and poor intellectual development may also result from vitamin A malnutrition. Various strategies such as food diversification, food fortification and supplementation have been used to fight the problem of vitamin A malnutrition, but there are a lot of challenges affecting sustainability and success of the strategies. This calls for a need to develop other options that are sustainable and are compatible with the resource poor rural households.

Unlike white maize, orange maize contains provitamin A carotenoids like β-carotene, α-carotene and β-cryptoxanthin, which are converted in the intestinal lumen to produce vitamin A. It is generally agreed that cultivation and consumption of orange maize by resource poor households in the SSA region can militate against vitamin A malnutrition in the region. Orange maize germplasm with high provitamin A carotenoids is found in the temperate germplasm pool, while information on the performance of the germplasm in the

tropics is not available. To benefit SSA, agronomic performance and stability of the germplasm in the region must be well understood. Information on breeding behaviour of provitamin A germplasm from the temperate gene pool in the tropics should be understood to guide breeders before initiating robust breeding programmes for provitamin A rich orange maize in the region.

Provitamin A elite maize inbred lines were imported from CIMMYT-Mexico in 2012 and used as parents to develop single cross hybrids by crossing them with elite drought tolerant yellow maize lines from CIMMYT-Zimbabwe's drought tolerant maize for Africa (DTMA) programme. The 30 single cross hybrids with adequate seed for replicated trials were evaluated for agronomic performance in Zimbabwe under six different environmental conditions; optimum conditions, random drought stress, managed drought stress, a combination of drought and heat stress, low N stress and low P stress.

Provitamin A hybrids showed some variability for grain yield with some producing yields similar to commercial hybrids already on the market. For example under optimum conditions, entries 8, 7, 6, 16 and 26 performed better than three check hybrids 33, 32 and 35. Entry 23 and 3, both provitamin A hybrids, ranked first and second when genotypes were evaluated under managed drought stress conditions. Sixteen provitamin A hybrids performed better than four checks (entries 32, 33, 34 and 35) under managed drought stress. Entry 8 ranked in the top 10 hybrids in all the environments. Entries 23, 8, 11, 3, 20 and 12 experimental provitamin A hybrids appeared under the top 10 genotypes under managed drought stress and under combined drought and heat stress. These hybrids need to be further evaluated and released as cultivars under those conditions. Since farmers largely consider yield when selecting varieties, this gives hope that the provitamin A hybrids will be accepted in the region. It was also encouraging to observe that most of these hybrids had moderate texture (another trait considered by farmers when selecting varieties to grow), which should make them acceptable to farmers, because of ease of processing and storage.

The hybrids ranked differently across environments and seasons because of the existence of significant GEI. This leads to the need to carry out GEI and stability analysis to see the best and stable hybrids across all environments or for specific environments. Both AMMI and GGE showed that GEI was a very important source of maize grain yield variability.

Entry 31 ranked first in terms of grain yield and was identified as the ideal genotype by the GGE biplot. Ranking of the other genotypes in relation to the ideal genotype was 34 > 8 > 16 > G6 > 7. Provitamin A hybrids entries 8, 6, 7, 16, 23 and 27 were stable and high yielding. These hybrids must be considered for further screening and can be released for commercial cultivation or used as parents to develop three-way cross / double cross / synthetic / open pollinated varieties.

It was also important to find the best tests environments and environments that were correlated with each other to reduce duplication and loss of resources when evaluating hybrids. All the environments were grouped together in one mega-environment and environments E6 (Harare low N stress), E4 (combined drought and heat stress at Chiredzi Research Station), E5 (Kaguvi Training Centre), E1 (Harare optimum) and E2 (managed drought stress at Chiredzi Research Station) were more representative of the mega-environment and were ideal environments for selecting superior genotypes. Environment E7 (Rattrey Arnold Research Station) was the most discriminating of the genotypes. Environment E2 (managed drought stress at Chiredzi Research Station) was the most ideal. The highly significant correlation coefficients between the environments show that it is possible to select in one environment and the genotypes will perform similarly in the other environments.

Combining ability and heterosis of provitamin A elite parental inbred lines and drought tolerant elite inbred testers for grain yield was estimated under optimal and abiotic stress conditions. Highly significant ($P < 0.01$) effects for both GCA and SCA were observed, meaning that both additive and non-additive gene actions were important in expression of grain yield under all the tested production conditions. However, non-additive gene action was important in yield under the stress conditions, which was reflected in high amounts of heterosis. There was a positive relationship between grain yield and SCA. Heterosis was very high, suggesting that it is possible to develop high yielding hybrids using the parents in this study. Narrow sense heritability was high (> 50%) under optimal conditions, managed drought stress, combined and drought and heat stress and low P stress, again indicating the importance of additive gene action in the expression of grain yield under the tested conditions and that selection for grain yield would be effective.

In both 2014 and 2015 lines 6, 7, and 8 had positive GCA values under optimum conditions, 4, 8, 9, 7, 10 and 6 under random drought stress, 3, 8 and 10 under managed drought stress, 3, 8, 7, 10 and 4 under combined drought and heat stress, 6, 7, 9, 8 and 5, under low N stress and 6, 4, 7, 3, 8 and 1 under low P stress. These lines should be considered for breeding for higher yields under these specific conditions. Lines 6, 7 and 8 can be used for breeding hybrids suitable for all environments except for managed stress conditions. Testers 1 and 2 were ideal for breeding for optimum conditions, managed drought stress, tester 2 for random drought stress and tester 3 for low P stress. All the testers were not suitable for low N stress. Across all environments and seasons, line 8 contributed positively to grain yield, line 3 was favourable under managed drought stress and combined drought and heat stress, lines 6, 7, 8 and 9 were desirable under low N, 6, 7 and 8 under optimum conditions, 4, 6, 7, 8, and 10 under random drought stress, and 3, 8 and 10 under managed drought. The best line by tester combinations in each environment should be taken for further evaluation and used as parents for development of three way or double cross hybrids and/or released as cultivars important for all these growing conditions.

It was also important to determine the β-carotene levels of the hybrids and its stability across environments and seasons, and also to understand gene action under abiotic stress conditions. Hybrids had β-carotene concentration in the expected range for first generation medium to high provitamin A maize genotypes (3-8 µg g^{-1}). This is very encouraging, since it is giving hope that it is possible to develop hybrids with acceptable levels of β-carotene to combat vitamin A malnutrition among vulnerable people subsisting on maize.

To understand gene action affecting β-carotene concentration in maize grain, combining ability was evaluated. GCA was significant, suggesting that additive gene action was more important than dominance for determining β-carotene concentration in maize grain. This was supported by high additive variance observed. Heritability was also very high, suggesting the importance of the genotype in the expression of β-carotene genes. SCA was not significant, suggesting that non-additive gene action was not important in expression of β-carotene genes. These observations means selection of genotypes with high β-carotene concentration is possible, giving hope for breeding maize with high β-carotene levels.

From ANOVA it was seen that β-carotene concentration in maize grain was affected by environments and hybrids and their interaction. Beta-carotene concentration was higher under optimum conditions compared to stress conditions. Hybrids expressed variability for β-carotene that should allow improvement of β-carotene in maize cultivars. Beta-carotene concentration in maize grain was positively correlated with grain yield, indicating that selection for high β-carotene will not have any yield penalty, but may even have the opposite effect.

The results reported in this study confirms the possibility of develop high yielding hybrids with high β-carotene concentration to address vitamin A malnutrition among most vulnerable people in SSA. Since the breeding of orange maize is novel in SSA as compared to development of white and yellow varieties, it is acknowledged that a lot of effort is still required to develop orange maize varieties in Zimbabwe. Efforts should focus on eradicating any yield difference between orange, white and yellow varieties, and for β-carotene concentration to meet the Harvest Plus minimum target of 15 μg g^{-1}.

Appendices

Appendix 1 Provitamin A elite germplasm used for developing hybrids

Stock ID	Name	Pedigree or name	Heterotic Group	ProA Levels (ug/g)
		Lines		
HP730-5	CLHP0072	([[[K64R/G16SR]-39-1/[K64R/G16SR]-20-2]-5-1-2-B*4/CML390]-B-38-1-B-7-#/[BETASYN]BC1-1-1-1-#/CML305)-4-2-1-B-B-B-B	B	6-8
HP730-7	CLHP0074	([[[K64R/G16SR]-39-1/[K64R/G16SR]-20-2]-5-1-2-B*4/CML390]-B-38-1-B-7-#/[BETASYN]BC1-1-1-1-#/CML305)-5-2-1-B-B-B-B	B	6-8
HP730-9	CLHP0076	([[[NAW5867/P30SR]-40-1/[NAW5867/P30SR]-114-2]-16-2-2-B-2-B/CML395-6]-B-20-1-B-3-#/[BETASYN]BC1-3-1-1-#/CML300)-3-3-3-B-B-B	B	6-8
HP730-48	CLHP0008	CML488/[BETASYN]BC1-15-5-B-B-B-B-B-B	B	6-8
HP730-50	CLHP0002	CML489/[BETASYN]BC1-2-#-B-B-B-B-B-B-B-B	B	6-8
HP730-53	CLHP0020	KUI carotenoid syn-FS17-3-2-B-B-B-B-B-B-B-B	B	6-8
HP730-54	CLHP0022	KUI carotenoid syn-FS25-3-2-B-B-B-B-B-B-B-B	B	6-8
HP730-56	CLHP0003	MAS[206/312]-23-2-1-1-B-B-B/[BETASYN]BC1-10-3-#-B-B-B-B-B-B-B-B	A	6-8
HP730-57	CLHP0005	MAS[206/312]-23-2-1-1-B-B-B/[BETASYN]BC1-11-3-1-#-B-B-B-B-B-B-B-B	A	6-8
HP730-63	CML300	CML300	A/B	6-8
HP730-64	CML304	CML304	A/B	6-8
HP730-66	CML496	CML496	A	6-8
HP730-75	CML297	CML297	A/B	6-8
HP857-7		(KUI carotenoid syn-FS25-3-2-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-2(MAS:L4H1)-1-B-B-B-B	Unknown	>15
HP857-8		(KUI carotenoid syn-FS17-3-2-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-1(MAS:L4H1)-2-B-B-B-B	Unknown	>15
HP857-9		(KUI carotenoid syn-FS17-3-2-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-1(MAS:L4H1)-5-B-B-B-B	Unknown	>15
HP857-10		(KUI carotenoid syn-FS17-3-2-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-4(MAS:L4H1)-2-B-B-B-B	Unknown	>15
HP857-15		(KUI carotenoid syn-FS11-1-1-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-2(MAS:L4H1)-1-B-B-B-B	Unknown	>15
HP857-17		(KUI carotenoid syn-FS11-1-1-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-2(MAS:L4H1)-3-B-B-B-B	Unknown	>15
HP857-18		(KUI carotenoid syn-FS11-1-1-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-2(MAS:L4H1)-4-B-B-B-B	Unknown	>15
HP857-22		(KUI carotenoid syn-FS17-3-2-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-1(MAS:L4H1)-1-B-B-B-B	Unknown	>15
		Testers		
		[[CML202/[CML202/CML442//[DTP2WC4H255-1-2-2-B/[[NAW5867/P30-SR]-111-2/[NAW5867/P30-SR]-25-1]-8-1-1-B-1]-1-2-2-B]-1-1-1-1-BB/CML223]/CML489]-4-B-1-1-1-1-B	B	
		CLA91-B-B/CML312	B	
		CLA106	B	
		CLQ-RCYQ40	A	
		DTPYC9-F46-1-2-1-1	A	

Appendix 2 Single cross provitamin A hybrids produced and five standard checks

Entry	Sock I.D	Hybrids
1	PM2-1	([[[K64R/G16SR]-39-1/[K64R/G16SR]-20-2]-5-1-2-B*4/CML390]-B-38-1-B-7-#/[BETASYN]BC1-1-1-1-#/CML305)-4-2-1-B-B-B-B-B//[[CML202/[CML202/CML442//[DTP2WC4H255-1-2-2-B/[[NAW5867/P30-SR]-111-2/[NAW5867/P30-SR]-25-1]-8-1-1-B-1]-1-2-2-B]-1-1-1-1-BB/CML223]/CML489]-4-B-1-1-1-B-B
2	PM2-2	([[[K64R/G16SR]-39-1/[K64R/G16SR]-20-2]-5-1-2-B*4/CML390]-B-38-1-B-7-#/[BETASYN]BC1-1-1-1-#/CML305)-5-2-1-B-B-B-B-B//[[CML202/[CML202/CML442//[DTP2WC4H255-1-2-2-B/[[NAW5867/P30-SR]-111-2/[NAW5867/P30-SR]-25-1]-8-1-1-B-1]-1-2-2-B]-1-1-1-1-BB/CML223]/CML489]-4-B-1-1-1-B-B
3	PM2-3	CML488/[BETASYN]BC1-15-5-B-B-B-B-B-B-B//[[CML202/[CML202/CML442//[DTP2WC4H255-1-2-2-B/[[NAW5867/P30-SR]-111-2/[NAW5867/P30-SR]-25-1]-8-1-1-B-1]-1-2-2-B]-1-1-1-1-BB/CML223]/CML489]-4-B-1-1-1-B-B
4	PM2-4	CML489/[BETASYN]BC1-2-#-B-B-B-B-B-B-B-B-B//[[CML202/[CML202/CML442//[DTP2WC4H255-1-2-2-B/[[NAW5867/P30-SR]-111-2/[NAW5867/P30-SR]-25-1]-8-1-1-B-1]-1-2-2-B]-1-1-1-1-BB/CML223]/CML489]-4-B-1-1-1-B-B
5	PM2-5	KUI carotenoid syn-FS25-3-2-B-B-B-B-B-B-B-B-B-B//[[CML202/[CML202/CML442//[DTP2WC4H255-1-2-2-B/[[NAW5867/P30-SR]-111-2/[NAW5867/P30-SR]-25-1]-8-1-1-B-1]-1-2-2-B]-1-1-1-1-BB/CML223]/CML489]-4-B-1-1-1-B-B
6	PM2-6	MAS[206/312]-23-2-1-1-B-B-B/[BETASYN]BC1-10-3-#-B-B-B-B-B-B-B-B-B//[[CML202/[CML202/CML442//[DTP2WC4H255-1-2-2-B/[[NAW5867/P30-SR]-111-2/[NAW5867/P30-SR]-25-1]-8-1-1-B-1]-1-2-2-B]-1-1-1-1-BB/CML223]/CML489]-4-B-1-1-1-B-B
7	PM2-7	CML304-B//[[CML202/[CML202/CML442//[DTP2WC4H255-1-2-2-B/[[NAW5867/P30-SR]-111-2/[NAW5867/P30-SR]-25-1]-8-1-1-B-1]-1-2-2-B]-1-1-1-1-BB/CML223]/CML489]-4-B-1-1-1-B-B
8	PM2-8	CML496-B//[[CML202/[CML202/CML442//[DTP2WC4H255-1-2-2-B/[[NAW5867/P30-SR]-111-2/[NAW5867/P30-SR]-25-1]-8-1-1-B-1]-1-2-2-B]-1-1-1-1-BB/CML223]/CML489]-4-B-1-1-1-B-B
9	PM2-9	(KUI carotenoid syn-FS11-1-1-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-2(MAS:L4H1)-3-B-B-B-B-B//[[CML202/[CML202/CML442//[DTP2WC4H255-1-2-2-B/[[NAW5867/P30-SR]-111-2/[NAW5867/P30-SR]-25-1]-8-1-1-B-1]-1-2-2-B]-1-1-1-1-BB/CML223]/CML489]-4-B-1-1-1-B-B
10	PM2-10	DTPYC9-F46-1-2-1-1-B//[[CML202/[CML202/CML442//[DTP2WC4H255-1-2-2-B/[[NAW5867/P30-SR]-111-2/[NAW5867/P30-SR]-25-1]-8-1-1-B-1]-1-2-2-B]-1-1-1-1-BB/CML223]/CML489]-4-B-1-1-1-B-B
11	PM2-11	([[[K64R/G16SR]-39-1/[K64R/G16SR]-20-2]-5-1-2-B*4/CML390]-B-38-1-B-7-#/[BETASYN]BC1-1-1-1-#/CML305)-4-2-1-B-B-B-B-B//CLA106-B
12	PM2-12	([[[K64R/G16SR]-39-1/[K64R/G16SR]-20-2]-5-1-2-B*4/CML390]-B-38-1-B-7-#/[BETASYN]BC1-1-1-1-#/CML305)-5-2-1-B-B-B-B-B//[[CML202/[CML202/CML442//CLA106-B
13	PM2-13	CML488/[BETASYN]BC1-15-5-B-B-B-B-B-B-B//[[CML202/[CML202/CML442//CLA106-B
14	PM2-14	CML489/[BETASYN]BC1-2-#-B-B-B-B-B-B-B-B-B//[[CML202/[CML202/CML442////CLA106-B
15	PM2-15	KUI carotenoid syn-FS25-3-2-B-B-B-B-B-B-B-B-B-B//[[CML202/[CML202/CML442//CLA106-B
16	PM2-16	MAS[206/312]-23-2-1-1-B-B-B/[BETASYN]BC1-10-3-#-B-B-B-B-B-B-B-B-B//CLA106-B
17	PM2-17	CML304-B//CLA106-B

18	PM2-18	CML496-B//CLA106-B
19	PM2-19	(KUI carotenoid syn-FS11-1-1-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-2(MAS:L4H1)-3-B-B-B-B-B//CLA106-B
20	PM2-20	DTPYC9-F46-1-2-1-1-B//CLA106-B
21	PM2-21	([[[K64R/G16SR]-39-1/[K64R/G16SR]-20-2]-5-1-2-B*4/CML390]-B-38-1-B-7-#/[BETASYN]BC1-1-1-1-#/CML305)-4-2-1-B-B-B-B-B//CLQ-RCYQ40
22	PM2-22	([[[K64R/G16SR]-39-1/[K64R/G16SR]-20-2]-5-1-2-B*4/CML390]-B-38-1-B-7-#/[BETASYN]BC1-1-1-1-#/CML305)-5-2-1-B-B-B-B-B//CLQ-RCYQ40
23	PM2-23	CML488/[BETASYN]BC1-15-5-B-B-B-B-B-B-B//CLQ-RCYQ40
24	PM2-24	CML489/[BETASYN]BC1-2-#-B-B-B-B-B-B-B-B-B//CLQ-RCYQ40
25	PM2-25	KUI carotenoid syn-FS25-3-2-B-B-B-B-B-B-B-B-B-B//CLQ-RCYQ40
26	PM2-26	MAS[206/312]-23-2-1-1-B-B-B/[BETASYN]BC1-10-3-#-B-B-B-B-B-B-B-B-B//CLQ-RCYQ40
27	PM2-27	CML304-B//CLQ-RCYQ40
28	PM2-28	CML496-B//CLQ-RCYQ40
29	PM2-29	(KUI carotenoid syn-FS11-1-1-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-2(MAS:L4H1)-3-B-B-B-B-B//CLQ-RCYQ40
30	PM2-30	DTPYC9-F46-1-2-1-1-B//CLQ-RCYQ40
		Checks
31	PM2-44	SC727
32	PM2-45	SC633
33	PM2-46	SC402
34	PM2-47	SC608
35	PM2-48	HP1005