Genetic Diversity and Combining Ability Analyses of Provitamin A Maize (*zea mays* L.) Inbred lines for Drought Stress Tolerance

By

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GENERAL ABSTRACT

White maize (*Zea mays* L.) grown in southern Africa, lacks adequate vitamin A content required by the human body, contributing to the prevalence of vitamin A deficiency (VAD) among rural people who largely rely on maize based diets. On the other hand, recurrent and episodic droughts in the region have contributed to low maize yields among smallholder farmers, the majority of whom rely on rain-fed agriculture. Thus, developing provitamin A maize that is tolerant to drought stress would significantly reduce VAD prevalence in the region and at the same time cushion maize farmers from the impacts of drought. The objectives of this study were therefore to: (i) determine the extent of genetic diversity among available provitamin A inbred lines using agro-morphological traits and single nucleotide polymorphism (SNP) markers, ii) screen the available provitamin A inbred lines for drought tolerance using morpho-physiological and biochemical traits, and (iii) assess the combining ability and gene action controlling grain yield and other secondary traits among the available provitamin A inbred lines and their hybrid combinations under optimum and drought stressed environments. The activities of this research study were conducted from 2016/17 to 2017/18 seasons in the laboratory, greenhouse and various field environments in South Africa and Zimbabwe.

Genetic diversity analysis of 48 provitamin A maize germplasm sourced from International Maize and Wheat improvement Centre (CIMMYT) and International Institute of Tropical Agriculture (IITA) was conducted using β-carotene content and eleven agro-morphological traits and 3046 single nucleotide polymorphism (SNP) markers. Inbred lines varied significantly for most of the traits studied. Grain yield averaged 1.8 t ha^{-1} ranging from 0.70 t ha^{-1} to 2.70 t ha^{-1}. Beta carotene, grain yield and anthesis-silking interval exhibited high heritability (H^2) and genetic advance as a percentage of the mean (GAM). Cluster analysis grouped the genotypes into three distinct clusters based on β-carotene content and morphological traits, and two distinct clusters based on SNP markers. Using SNP markers, the average genetic distance observed was 0.59 with an average of 1.615 effective alleles per locus and a mean polymorphic information content of 0.359. The average gene diversity was 0.363 and most of the variation (78%) was attributed to among individual genotypes and the remaining 22% was due to among population and within individual variation.

Fifty inbred lines were screened for drought tolerance in the greenhouse and field under both optimum and drought stress conditions using selection index (SI) involving morpho-physiological and biochemical traits. including; grain yield (GY), β-carotene content (BCC), anthesis-silking interval (ASI), number of ears per plant (EPP), plant height (PH), stomatal

conductance (G_s), leaf senescence (SEN), chlorophyll content (CC), leaf rolling (LR), and proline content (PC). Most of the genotypes that performed well under both optimum and drought conditions in terms of GY were ranked highly in the SI ranking. There were significant correlations ($p \leq 0.01$; $p \leq 0.05$) between GY and most of the traits measured under both optimum and drought stress environments. Proline content significantly increased to higher levels under drought conditions in tolerant genotypes indicating that it can be used for drought stress screening.

Sixty-four single cross hybrids generated from an 8 x 8 North Carolina design II scheme of provitamin A inbred lines were evaluated for combining ability, gene action and heterosis under drought-stressed and optimum environments. General combining ability attributable to males and females (GCA_m and GCA_f) and specific combining ability (SCA) were highly significant ($p \leq 0.001$) across the environments, suggesting the importance of both additive and non-additive gene action. Dominance variance was greater than additive variance indicating the preponderance of non-additive gene action. Single cross hybrids 34 (CLHP0352 X CLHP00322), 42 (CLHP00294 X CLHP00322), 4 (CLHP0312 X CLHP0310), 64 (CLHP0312 X CLHP0310), 55 (CLHP0364 X TZM113) and 46 (CLHP00294 X CML451) were identified as the best crosses by virtue of having high desirable SCA effects values for grain yield. Inbred lines 37 (TZM113), 19 (CLHP00294), 18 (CLHP0352), 19 (CLHP00294), 23 (CLHP0058), 11 (CLHP00432) and 20 (CLHP0364) were the best lines due to their desirable GCA effects.

Overall, the study indicated the existence of sufficient genetic diversity in CIMMYT and IITA provitamin A inbred lines, which can be exploited through hybridization and selection. Inbred lines in different clusters were considered genetically divergent, therefore hybrids developed using selected parents from different clusters would be expected to exhibit high heterosis. The study selected twenty highly ranked inbred lines according to SI as parents for the hybridisation programme. Crosses with high positive SCA values for grain yield are recommended for further stability testing, while lines with high positive GCA values will be incorporated in the breeding programme as potential parents for further hybridisation programmes.

DECLARATION

I, Aleck Kondwakwenda, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
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Signed:

……..

Aleck Kondwakwenda

As the candidate's supervisors, we agree to the submission of this thesis:

……..

Dr. J. Sibiya (Supervisor)

……

Dr. R. Zengeni (Co-Supervisor)

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DEDICATION

I dedicate this thesis:

- In memory of my beloved uncle Simon Nyanungo who looked after me including paying my school fees from form three up to the end of my undergraduate studies. May his kind soul rest in peace.
- To my children Mukundinashe, Elgibo and Adonai who are still in the Spirit.

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CHAPTER 1
INTRODUCTION

1.1 Background

Maize (*Zea mays* L.) is a strategic crop used in combating food and nutrition challenges in sub-Saharan Africa (SSA), particularly in southern Africa where it is widely produced and consumed (Cairns et al., 2012). It is a staple food for more than 300 million people in SSA, a significant proportion of whom are suffering from hunger and malnutrition (Nuss and Tanumihardjo, 2010). However, non-biofortified maize (white endosperm maize), which is the most commonly produced and consumed maize type, lacks vitamin A among other micronutrients. This has contributed to the high prevalence of vitamin A deficiency (VAD) related sicknesses in the region among consumers who largely rely on maize based diets without other complementary food sources (WHO, 2009).

Vitamin A deficiency can cause blindness, depressed immune response and stunted growth mainly in children and women (Stevens et al., 2015). This is because vitamin A is an important micronutrient responsible for controlling several biological processes including vision, growth and immunity. Over 190 million cases of preschool-age children and 19 million pregnant women are reported to be affected by VAD triggered infections in Africa and South Asia (Schmaelzle et al., 2014).

Several strategies have been put forward as efforts to curb VAD among people who largely rely on maize based diets. These include promoting diet diversification with vitamin A food sources included, medical based direct vitamin A supplementation and food fortification. Diet diversification has proved to be less efficient especially in rural areas because of financial constraints and is greatly affected by crop seasonality (Nuss and Tanumihardjo, 2010). Poor infrastructure in developing countries has limited the widespread coverage of direct vitamin supplementation programmes with rural areas mostly affected because of poor accessibility (WHO, 2009). Food fortification can be categorised into exogenous and endogenous. Exogenous vitamin A fortification involves addition of vitamin A premixes to food products while endogenous, which is also called biofortification is the genetic enhancement of provitamin A content in crops through crop breeding and/or biotechnology (Nuss and Tanumihardjo, 2010). Although exogenous fortification has been successful in the developed world, biofortification of is more sustainable, cost effective and practical solution for VAD in

rural developing countries where the bulk of the people rely on what they grow in their fields for food (Bouis et al., 2011).

Despite the large-scale production and consumption of maize in SSA, yields in this region have remained low mainly due to drought and other biotic and abiotic factors (Messmer et al., 2009). The impact of climate change exacerbated droughts are predicted to be worse in SSA than in any other region because the majority of farmers rely on rain fed agriculture (Cairns et al., 2013). Drylands make up about 43% of SSA's land surface, accounting for about 75% of arable land (Cairns et al., 2013). The high population growth rate in SSA further intensifies competition for water between people and crops (Edmeades, 2013).

Drought stress affects maize and other crops at almost all growth stages, but flowering and grain filling stages are the most susceptible with yield losses of over 90% reported when drought coincides with these growth stages (Lu et al., 2011). Different maize genotypes vary in terms of their agronomic, morphological, physiological, and biochemical responses to drought stress (Shakeel et al., 2011). This attribute makes genetic improvement for drought tolerance a possible task as selections can be effectively conducted. Genetic improvement of maize for drought tolerance through breeding and biotechnology is a sustainable means of mitigating the impacts of drought stress to maize productivity (Almeida et al., 2014). It has a potential to close 20-25% yield gaps between drought-affected and optimal conditions (Edmeades, 2013).

1.2 Rationale for research focus

Although tremendous progress has been made to develop provitamin A maize via biofortification in other African countries (Andersson et al., 2017), South Africa has been lagging behind in terms of maize biofortification as indicated by shortage of provitamin A maize cultivars on the market. The bulk of vitamin A enriched maize flour and other maize products consumed in South Africa are exogenously enriched and sold mainly in urban areas. In rural areas where most farmers do not buy maize flour and certain maize products but rather process self-grown maize into flour using grinding mills. As a result, most of rural maize consumers are missing out on the exogenously incorporated vitamin A, which explains the disparities in VAD prevalence between rural and urban communities (WHO, 2017). According to Faber and Wenhold (2007), 33.3% of preschool going children are vitamin A deficient in South Africa with KwaZulu-Natal and Limpopo provinces having the highest prevalences of 14% and 16%, respectively (Figure 1.1).

Moreover, among the few provitamin A maize cultivars developed in South Africa, none has been primarily improved for drought tolerance. Therefore, there is need to develop more maize cultivars with both drought tolerance and high provitamin A content to provide cheap and sustainable sources of vitamin A to resource limited maize consumers and at the same time cushioning dryland farmers from the prevailing drought conditions.

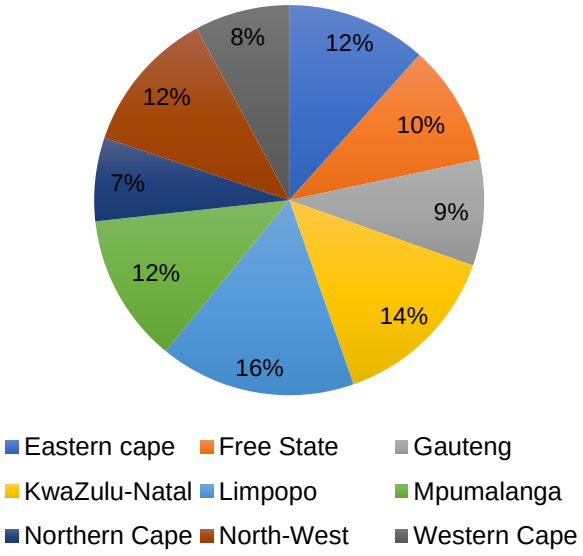

Figure 1:1: Percentage of children (≤ 5 years old) with vitamin A deficiency in South Africa at provincial level (Faber and Wenhold, 2007).

Improving drought tolerance in provitamin A maize cultivars could help to increase its adaptability in the farmers' fields during drought periods. This in turn can help in promoting the adoption of provitamin A maize by farmers given that its uptake has been very slow in Africa. Many consumers, especially in east and southern Africa prefer white maize over nutritious provitamin A yellow maize for different reasons as reviewed by several authors (Muzhingi et al., 2008; Pillay et al., 2011). Furthermore, development of more improved maize cultivars would attract more seed producers to the seed industry, including small-scale producers. This would increase seed supply over demand, which would result in seed prices decreasing. This, in turn can also result in increased adoption of improved maize cultivars by farmers since higher cost of hybrid maize seeds has been noted as one of the reasons why most subsistence farmers are not adopting improved cultivars (Sibiya et al., 2013). Development of improved maize lines can also enable breeders to develop open pollinated varieties (OPVs) and synthetic varieties which are affordable to farmers.

Drought tolerance is a complex quantitative trait that is influenced by numerous genes (Derera et al., 2007). Therefore, integrated approaches should be employed when breeding for drought tolerance to increase the probability of success. In conventional breeding, direct selection for high grain yield is inefficient because of low genotypic variance and heritability under drought stress conditions (Ge et al., 2011; Almeida et al., 2014). To counteract this challenge, plant breeders select for secondary traits in the form of agronomic, morphological, physiological and biochemical traits (Betrán et al., 2003; Reynolds and Langridge, 2016). Repetitive use of similar secondary traits for selection from the same population for a longer time may reduce variation for those traits within the population (Monneveux et al., 2008). Therefore, plant breeders are challenged to constantly use new and diverse secondary traits for selection of traits such as drought tolerance. The use of biochemical traits such as proline and abscisic acid (ABA) in selecting maize for drought tolerance has not been explored despite wide utilisation in water use efficient studies of other crops such as wheat and cowpea (Moayedi et al., 2011; Zegaoui et al., 2017). Heritability levels of some of the secondary traits such as stomatal conductance under drought stress is not known.

To increase, chances of success in any breeding programme, it is essential to carry out genetic diversity assessment as a pre-breeding step to ascertain the presence of adequate genetic variation among the available materials. Information about the genetic diversity and population structure in advanced maize inbred lines is of fundamental importance in designing an efficient hybrid breeding programme. This is because, to effectively exploit heterosis in hybrid maize development, crosses should be made between genetically divergent inbred lines (Govindaraj et al., 2015). Diversity analysis of germplasm can be carried out at phenotypic or molecular levels (Beyene et al., 2005; Prasanna, 2012). The use of molecular markers has revolutionised the science of plant breeding through increased selection efficiency and speeding the breeding process. Since their advent, the application of single nucleotide polymorphism (SNP) markers in plant breeding has been on the rise due to their high relative abundance, polymorphism and easy automation procedures (Rafalski, 2002). Furthermore, the discovery of SNPs in maize and other few crops is relatively direct because of high levels of intraspecific nucleotide diversity, and the availability of many genes and expressed sequence tags (ESTs) (Ganal et al., 2009).

In addition, information on combining ability and variance components of the available materials is also fundamental in breeding as it helps breeders to make important decisions about their materials (Badu-Apraku et al., 2013). This is because combining ability information

indicates the type of gene action influencing the traits of interest in the available genotypes. General combining ability (GCA) and specific combining ability (SCA) effects are associated with additive and non-additive gene action, respectively (Worku et al., 2008). In this regard, materials that are good general combiners can be used to improve the population whilst good specific combiners can be used for hybridisation programmes. The information can also be used in forming and describing heterotic patterns for the maize breeding programme (Kassa. et al., 2012).

Given this background, it is important to integrate both drought tolerance and enhanced vitamin A content in maize to ensure food and nutrition security in the face of recurrent droughts and widespread VAD in SSA. Therefore, this study sought to characterise provitamin A inbred lines sourced from the International Maize and Wheat Improvement Centre (CIMMYT) and International Institute of Tropical Agriculture (IITA) for drought tolerance. The study was guided by the following objectives:

1.3 Overall objective

The overall objective of this study was to assess the genetic diversity and combining ability effects of selected tropical provitamin A inbred lines and their hybrid combinations for grain yield and various secondary traits for drought tolerance improvement, targeting production in some of the southern Africa's maize mega environments.

1.3.1 Specific objectives

The specific objectives of the study were to:

i. Determine the extent of genetic diversity among available provitamin A inbred lines at phenotypic level using agro-morphological traits.
ii. Determine the level of genetic diversity among the available provitamin A inbred lines at molecular level using SNPs markers.
iii. Identify provitamin A inbred lines that are drought tolerant using morpho-physiological and biochemical traits.
iv. Assess the combining ability effects and gene action controlling grain yield and other secondary traits among the available provitamin A inbred lines and their hybrid combinations under optimum and drought stressed environments.

1.4 Hypotheses tested

The following hypotheses were tested;

i. There is significant phenotypic and genetic diversity among the available provitamin A inbred lines.
ii. The available provitamin A inbred lines respond differently under drought and non-drought conditions.
iii. Grain yield and secondary traits of the available inbred lines and their hybrid combinations are controlled by both additive and non-additive gene action under optimum and drought stressed conditions.

1.5 Outline of thesis

The objectives stated above were addressed and achieved in various chapters which constitute this thesis. Overlaps of content and references is expected between some chapters. The chapters are divided as follows:

1. Chapter 1: Introduction to thesis. The importance of provitamin A maize biofortification in sub-Saharan Africa are outlined.
2. Chapter 2: Literature review focusing on provitamin A maize biofortification in sub-Saharan Africa and maize drought tolerance breeding. Pre-breeding and actual breeding activities applicable to drought tolerance breeding are reviewed.
3. Chapter 3: Diversity analysis of provitamin A maize inbred lines using agro-morphological traits and β-carotene content.
4. Chapter 4: Diversity analysis of provitamin A maize inbred lines using single nucleotide polymorphism markers.
5. Chapter 5: Screening of provitamin A maize inbred lines for drought tolerance using β-carotene content, morpho-physiological and biochemical traits.
6. Chapter 6: Combining ability analysis of provitamin A maize genotypes under water stress and non-stress environments.
7. Chapter 7: General overview and implications of the study to food security, provitamin A maize biofortification and production.

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CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

This review of literature focuses on provitamin A biofortification and breeding for drought tolerance. It gives an insight on vitamin A deficiency (VAD) prevalence in sub-Saharan Africa, possible interventions to solve VAD, genetics and breeding approaches applicable to provitamin A biofortification and the progress achieved in terms of provitamin A varieties released to date. For drought tolerance, aspects on the effect of drought on maize, secondary traits that can be utilised in breeding for drought tolerance in maize and the progress made so far in tropical and sub-tropical environments in terms of yield genetic gain under drought stress conditions are discussed. These topics create an important frame of reference for the research study.

2.2 Provitamin A maize biofortification in sub-Saharan Africa[1]

2.2.1 Importance of provitamin A biofortification

Food and nutrition insecurities are the primary challenges in most developing countries especially in sub-Saharan Africa (SSA). Increasing maize productivity has been identified as one of the strategies to curb food insecurity in SSA. This is because maize; (i) is widely produced and consumed in this region, (ii) has higher yield potential and (iii) is more responsive to management than other cereals crops grown in SSA like sorghum and millet (Badu-Apraku et al., 2011). Maize accounts for 30-60% of total caloric intake in SSA, where rural households under subsistence farming (Cairns et al., 2013) mostly produce the crop. Therefore, it is a model crop for productivity and nutritional improvements as part of efforts to curb food and nutrition insecurities in SSA.

However, white maize which is popularly consumed in many African countries has serious micronutrients deficiencies which hinders its suitability to provide solutions for both food and nutrition insecurities for the region. White maize has a starchy endosperm, which provides huge quantities of energy to the human diet but has low micronutrients content (Nuss and

[1] Accepted for publication in Maydica journal devoted to maize and allied species on the 6th of November 2018.

Tanumihardjo, 2010). This has been implicated in the prevalence of 'hidden hunger' in maize consuming SSA nations (Muthayya et al., 2013; FAO et al., 2017). White maize by virtue of its white colour has very low and undetectable carotenoids, which makes it a poor source of vitamin A (Wurtzel et al., 2012). This, in combination with a generally low-provitamin A diet, has resulted in high cases of Vitamin A deficiency (VAD) related illnesses in most maize consuming nations in SSA.

In contrast to white maize, yellow maize has wider genetic variation in carotenoid content in the endosperm, a character that breeders can exploit through biofortification to develop maize cultivars with high provitamin A (proVA) content (Menkir et al., 2008). Over a decade ago, proVA maize was introduced in SSA through the efforts of HarvestPlus and partners (Bouis et al., 2011). Since then several proVA-biofortified maize hybrids and open pollinated varieties (OPVs) have been developed and released in or for several African countries. Both conventional and molecular breeding strategies can be employed in maize biofortification. However, a good understanding of the genetics and biochemical science of proVA synthesis is important for the designing and choosing of the correct breeding programme and strategy, respectively. Maize proVA biofortification in SSA faces its portion of challenges in the form of consumer scepticism, technical challenges and the negative stigma of the coloured maize. Therefore, this review seeks to discuss the science and technology of maize proVA biofortification and its impacts on agriculture-based livelihoods with SSA as a case study.

2.2.2 Vitamin A deficiency (VAD) status in sub-Saharan Africa

Vitamin A is an essential micronutrient that cannot be synthesised by the body and therefore must be provided through the diet. Yellow maize and other plants contain vitamin A precursors (provitamin A) in the form of carotenoids (Wurtzel et al., 2012). Vitamin A is responsible for the normal function of the visual and immunity systems among other key functions in the human body (WHO, 2009). Living on a diet that is chronically deficient of vitamin A is the underlying cause of VAD, a scenario common with most rural communities in SSA who are living on predominantly maize-based diets. Vitamin A deficiency can cause xerophthalmia (progressive blindness), increased infant morbidity and mortality, and depressed immunological responses. Vitamin A deficiency diagnosis can either be done through clinical assessment of eyes for signs of xerophthalmia and/or biochemical determination of serum or plasma retinol concentration. However, biochemical assessment of retinol concentration is the latest and most commonly used method. Vitamin A deficiency is diagnosed when the liver vitamin A content measured in terms of liver retinol is below 0.7 µmol/l (WHO, 2009).

Vitamin A deficiency is estimated to affect 190 million preschool children and 19 million pregnant and lactating women worldwide, mainly in Africa and Asia (WHO, 2009; Stevens et al., 2015). WHO (2009) declared VAD as one of the threats to human survival and well-being which needs urgent and consistent intervention.

2.2.3 Vitamin A deficiency interventions

Several strategies to curb VAD in vulnerable communities have been put forward. These include dietary diversification, vitamin A supplementation and food fortification (Bouis et al., 2011; Babu et al., 2013). Despite these interventions, VAD remains a threat to human survival in SSA, especially in rural areas. This could be due to the fact that diet diversification is beyond the financial reach of most poor rural farmers and is greatly affected by crop seasonality; therefore, cannot be readily and consistently available for most poor rural farmers. On the other hand, poor infrastructure in developing countries has limited widespread coverage of direct vitamin supplementation programmes with rural areas mostly affected (Nuss and Tanumihardjo, 2010). Mandatory exogenous vitamin A food fortification including maize flour that has been adopted by most countries has a limitation of side-lining rural farmers who do not buy processed fortified maize flour and other maize based products but rather process from their own grown maize. Furthermore, poor enforcement of the mandatory food fortification policy in some of the developing countries is resulting in some of the manufacturers not consistently adhering to the policy. A combination of these factors has led to higher VAD prevalence among rural populations in some of the SSA countries than their urban counterparts (Fig 2.1). The advent of endogenous maize fortification, which is also known as biofortification, can be a complimentary solution to the above-mentioned strategies in curbing VAD challenges in rural Africa. Biofortification, which is the genetic enhancement of vitamin A through crop breeding and biotechnology is more sustainable, cost effective and practical solution for VAD in chronically malnourished rural populations that have limited access to diverse diets and other micronutrient interventions (Nuss and Tanumihardjo, 2010; Bouis and Saltzman, 2017).

Developing agronomically competitive maize cultivars that are biofortified with high concentrations of vitamin A precursors has been regarded as a key approach towards alleviating VAD in maize consuming regions of SSA and Asia (Bouis and Saltzman, 2017). ProVA refers to the carotenoids that can be converted into physiologically activated vitamin A in the human body and these are α-carotene, β-carotene, and β-cryptoxanthin. HarvestPlus and its partners through the global challenge programme are credited for championing

biofortification of maize and other crops for enhanced vitamin A and other micronutrients content in SSA (Andersson et al., 2017).

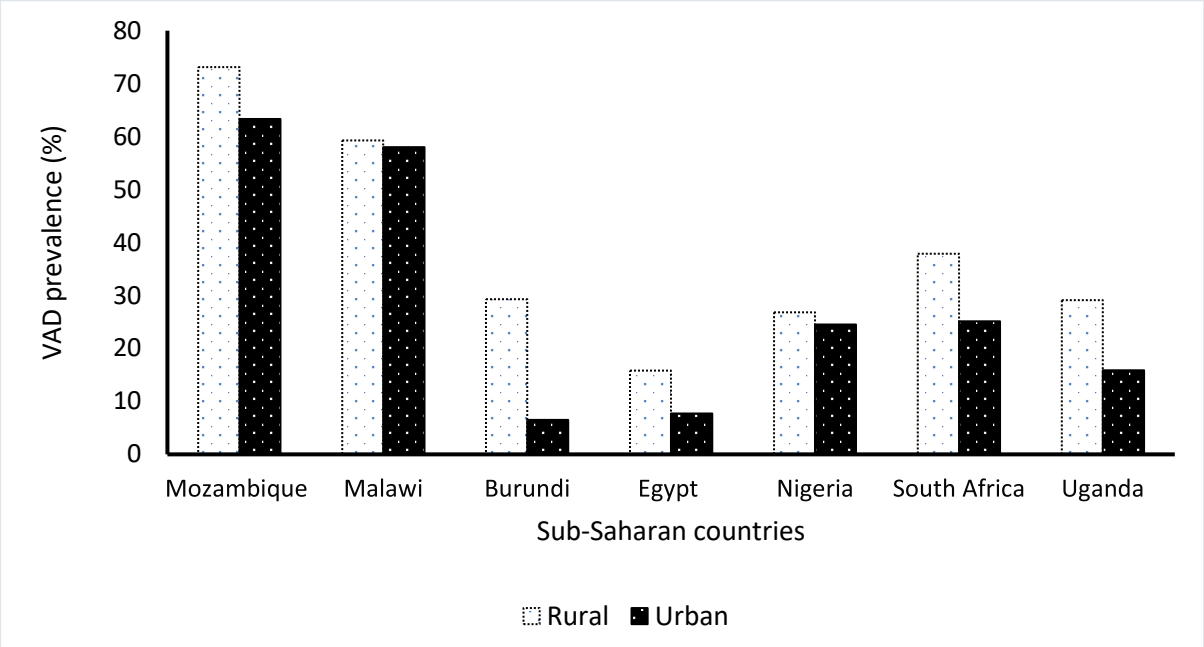

Figure 2.1: Disparities between rural and urban VAD prevalence in some of the maize consuming countries of the sub-Saharan Africa. Data source: http://www.who.int/nutrition/topics/vad/en//

2.2.4 The carotenoid biosynthetic pathway

Molecular and biochemical aspects of the carotenoid biosynthetic pathway have been studied comprehensively in many crops including maize (Harjes et al., 2008 Yan et al., 2010; Wurtzel et al., 2012). Carotenoids are categorised into proVA and non-proVA carotenoids. ProVA carotenoids, which are α-carotene, β-carotene and β-cryptoxanthine serve as dietary sources of vitamin A. On the other hand, non-provA carotenoids, which are lutein and zeaxanthin, have been reported to act as antioxidants in the human body (Chander et al., 2008). Lutein and zeaxanthin are the primary products of the biosynthetic pathway so are normally found in greater quantities in the maize endosperm than their proVA counterparts, which are the intermediates of the biosynthetic pathway (Nuss and Tanumihardjo, 2010). Among the three vitamin A precursors, β-carotene has higher proVA activity because of its unique double ring molecular structure (Harrison, 2015). Fig 2.3 shows an outline of the key steps of the biosynthetic pathway and the key genes that are responsible for the catalysis of relevant biochemical stages.

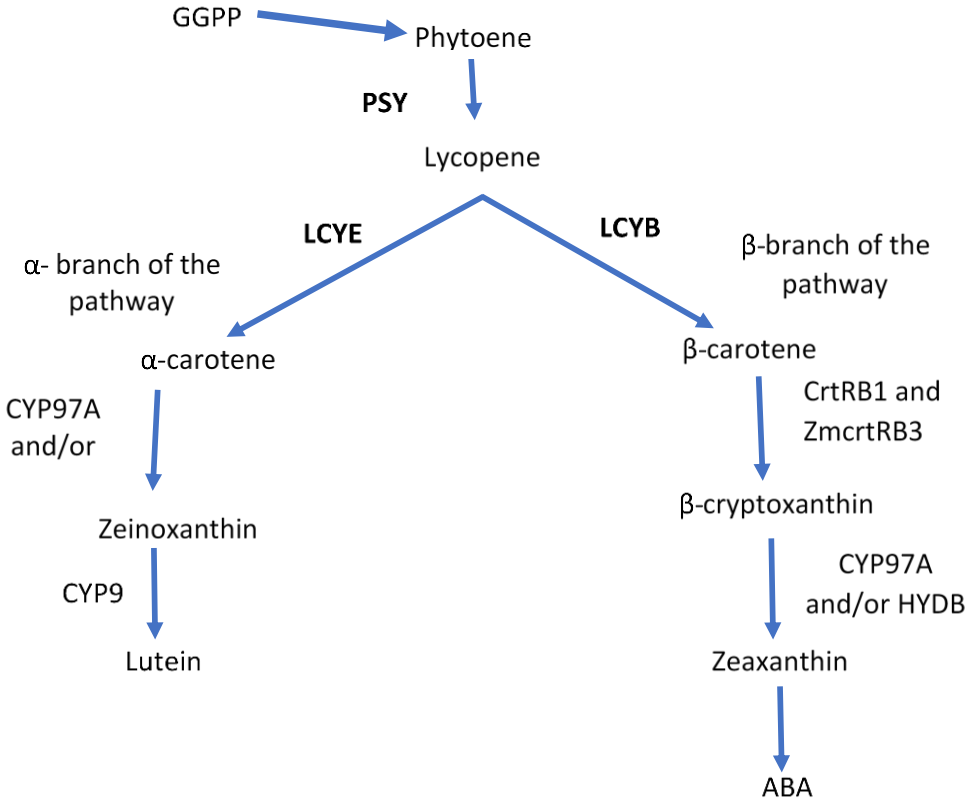

Figure 2.2: Carotenoid biosynthetic pathway and the major genes. GGPP: genanylgeranyl diphosphate, PSY: phytoene synthase, LCYB: β- cyclase, LCYE: E-cyclase, ABA: abscisic acid. Adopted from Babu et al. (2012).

2.2.5 Genetics of provitamin A content in maize

Understanding the heritability and gene action controlling the trait of interest is crucial in choosing a breeding strategy and designing a breeding programme. Provitamin A content is influenced by general combining ability (GCA) effects which reflect the predominance of additive gene action and has been reported to have moderate to high heritability (Babu et al., 2013; Suwarno et al., 2014). In maize proVA accumulation is affected by three key enzymes in the carotenoid biosynthetic pathway, namely phytoene synthase (PSY1), lycopene epsilon cyclase (LCYE) and β-carotene hydroxylase 1 (CRTRB1) (Pfeiffer and McClafferty, 2007). PSY1 gene encodes for phytoene synthase, an enzyme that is responsible for the shift from white to yellow grain colour by catalysing the conversion of genanylgeranyl (GGPP) to phytoene (Babu et al., 2013). Lycopene epsilon cyclase encodes for the enzyme lycopene

epsilon cyclase, which catalyses the conversion of lycopene into α-carotene or β-carotene (Harjes et al., 2008). Beta carotene hydroxylase 1 encodes for β-carotene hydroxylase enzyme that converts β-carotene into β-cryptoxanthin (Yan et al., 2010). It is through the manipulation of these genes using different breeding strategies that breeders enhance the proVA content of maize.

2.2.6 Biofortification objectives and pre-breeding activities

The primary objective of proVA biofortification is to develop cultivars with high proVA content, to provide approximately 50% of the estimated average requirements for Vitamin A. The initial maize proVA target is set at 15 µg g^{-1} (Bouis et al., 2011; Andersson et al., 2017). However, the cultivars should also be robust in other traits to increase adoption (Pillay et al., 2011). Suwarno et al. (2014) reported no significant correlation between grain yield and provA concentration, an indication that both traits can be improved simultaneously. It should be noted that, like any other breeding programme, the success of a biofortification programme relies on the availability of sufficient genetic variation in proVA concentration among the available germplasm (Pixley et al., 2013; Suwarno et al., 2014).

Thus, genetic diversity and population structure analysis for proVA concentration among the available germplasm should be undertaken as part of pre-breeding activities. Yellow maize has wide genetic variation and allelic diversity for carotenoid content, a characteristic that allows the application of both conventional and molecular breeding strategies. The availability of sufficient genetic variation allows breeders to exploit additive gene effects, transgressive segregation, and heterosis to improve proVA density in maize kernels. Conversely, when there is insufficient genetic variation among the available germplasm, transgenic approaches can be employed (Andersson et al., 2014). The following breeding strategies can be applied in maize biofortification.

2.2.7 Conventional breeding strategies

Backcross breeding has been a key strategy in developing proVA biofortified maize varieties during the early stages of biofortification in tropical and sub-tropical countries including SSA (Menkir et al., 2008; Azmach et al., 2013; Pixley et al., 2013). Temperate based germplasm has been found to be superior over the tropical and sub-tropical germplasm in proVA content especially in β-carotene content (Babu et al., 2013). Therefore, the base germplasm of proVA maize breeding in SSA was developed from backcrossing tropically adapted elite white maize with temperate yellow proVA donor lines (Pixley et al., 2013).

Recurrent selection is another breeding strategy that has been employed in maize biofortification. Under this approach, the breeding pipeline can be started by intermating landraces, popular or introduced varieties with superior proVA concentrations, followed by selecting the best progenies and repeat the process until high average stable proVA concentrations are achieved. Dhliwayo et al. (2014) improved the proVA content of open pollinated varieties (OPVs) from 25 to 67% through recurrent selection.

Hybridization has been an important strategy in breeding cross-pollinated crops like maize, mainly to exploit the associated heterosis and because of increasing adoption of hybrids in maize producing countries including SSA (Derera et al., 2007). In maize biofortification, hybridization involves the development of inbred lines with stable, robust, high-yielding and high proVA concentration, followed by crossing the selected inbred lines into single, three-way and double cross improved hybrids. The value of an inbred line in a hybrid combination depends on its ability to combine with other lines to produce high performing hybrids. Therefore, the chosen inbred parents should first undergo a rigorous screening and combining ability analysis for proVA concentration and other key agronomic traits (Menkir et al., 2015). To date many proVA hybrids have been released for SSA production.

2.2.8 Molecular breeding

The identification of key genes that govern the key steps of the carotenoid pathway and their allelic polymorphism enabled the incorporation of marker-assisted selection (MAS) technology into biofortification (Andersson et al., 2014). Fu et al. (2013) identified two polymorphisms in the gene PSY1, explaining 7 to 8% of the variation in total carotenoids. Favourable alleles of PSY1 increase proVA content by increasing the amount of substrate flowing into the carotenoid biosynthesis pathway (Sagare et al., 2015). Major breakthrough in the history of molecular biofortification came when three polymorphic sites in CRTRB1 gene that accounts for 40% of variation in β-carotene concentration in maize endosperm were identified (Yan et al., 2010). On the other branch of the carotenoid biosynthetic pathway (see Fig 2.2), Harjes et al. (2008) reported allelic polymorphism in the LCYE gene with the favourable allele associated with increase in total proVA content at the expense of lutein content. Based on the functional polymorphisms of these key genes of the carotenoid biosynthesis pathway, a number of maize molecular markers have been developed and validated for use in maize biofortification (Harjes et al., 2008; Yan et al., 2010; Babu et al., 2012; Fu et al., 2013). This resulted in accelerated genetic gain in breeding for increased provitamin A content in maize.

Table 2.1 gives a summary of maize genes encoding key enzymes in the carotenoid biosynthesis pathway and their respective favourable alleles.

Molecular markers based on functional polymorphisms within PSY1, LcyE and CRTRB1 provides a quick means of developing provitamin A enriched lines and cultivars. Marker assisted backcrossing can be handy in speeding up the introgression of favourable alleles of LCYE and CRTRB1 into tropical materials from temperate donors. Applying MAS CIMMYT and IITA breeders have developed several tropical maize lines and populations with proVA content that surpasses the current set target of 15 µg g^{-1} (Andersson et al., 2017; Menkir et al., 2017).

Transgenic technology is another approach that is applicable in proVA biofortification since proVA content is controlled by few genes. However, it has been deemed less necessary in maize proVA biofortification because maize has adequate natural genetic variation.

Table 2.1: Genes encoding key enzymes in the carotenoid biosynthesis pathway, and their allelic polymorphism.

Gene	Polymorphic site	Allelic diversity	Favourable allele	Reference
PSY1	PSY1-SNP7	A, C	A	(Babu et al., 2013; Fu et al., 2013)
	PSY1-InDel1	0,378	378	
LCYE	LCYE-5'TE	1,2,3,4	1,4	
	LCYE-SNP 216	G, T	G	(Harjes et al., 2008)
	LCYE-3'InDel	8,0	8	
CRTRB1	CRTRB1-5'TE	1,2,3	2	
	CRTRB1-InDe14	12,0	12	(Yan et al., 2010)
	CRTRB1-3'TE	1,2,3	1	

Adopted from Sagare et al. (2015) with modifications.

2.2.9 Provitamin A analysis

Provitamin A quantification is one of the daunting and crucial steps in maize biofortification. It is a challenging task because (1) maize has a complex mix of carotenoids (proVA and non proVA carotenoids), which takes a thorough laboratory analysis to extract and quantify each molecule; (2) carotenoids can be found in complex interaction with other molecules such as starch and proteins and (3) given their organic nature carotenoids are prone to degradation (Guild et al., 2017). These challenges can be reduced by carefully choosing the analysis method. Several methods have been considered and evaluated based on their accuracy, cost and speed to screen carotenoid content in maize kernels. These methods include visual colour scoring, near infrared reflectance and spectroscopy (NIRS) and liquid chromatography.

Despite its low cost, visual colour scoring was found less efficient in quantifying carotenoids in maize because of poor correlation between the key proVA carotenoids (β-carotene and β-cryptoxanthin) and the visual colour score (Harjes et al., 2008). Spectroscopic techniques such as NIRS are excellent in determining total carotenoid (proVA and non-ProVA) content but not good at partitioning the carotenoids as the absorption maxima is a similar wavelength region

for all carotenoids. Therefore, this method is not suitable for crops like maize that have a complex mixture of carotenoids (Guild et al., 2017).

Liquid chromatography analysis, which is either high performance liquid chromatography (HPLC) or ultra-performance liquid chromatography (UPLC) can partition and quantify the different carotenoids present. This is useful in crops like maize, which contain a mixture of carotenoids. High performance liquid chromatography (HPLC) has been the method of choice for precision analysis; but the high cost, low throughput and consequently longer time required for analysis are acting as deterrents for most resource constraint biofortification programmes in SSA. Due to its high throughput capacity, low cost for reagents, ultra-performance liquid chromatography (UPLC) is becoming a better choice for most breeders (Pixley et al., 2013).

2.2.10 Cultivars released

Since the inception of maize biofortification in Africa, over 50 proVA maize cultivars in the form of open pollinated varieties, synthetics, single-cross hybrids, and three-way hybrids have been released for production in many maize consuming SSA countries. These countries fall within the HarverstPlus' maize top Biofortification Priority Index (BPI) (http://www.harvestplus.org/knowledge-market/BPI). Fig 2.3 shows the general performance in terms of grain yield and proVA content of some of the cultivars that were released in two phases between 2012 and 2017. These cultivars were released in Zimbabwe, Ghana, Malawi, Mali, Nigeria, Tanzania, Zambia and DR Congo. ProVA content ranges from 5 to 15 µg g^{-1} with percentage target increment varying from 33% to 100% (HarvestPlus, 2014; Andersson et al., 2017). The phase three products are still in lines form and are expected to be released within the next years.

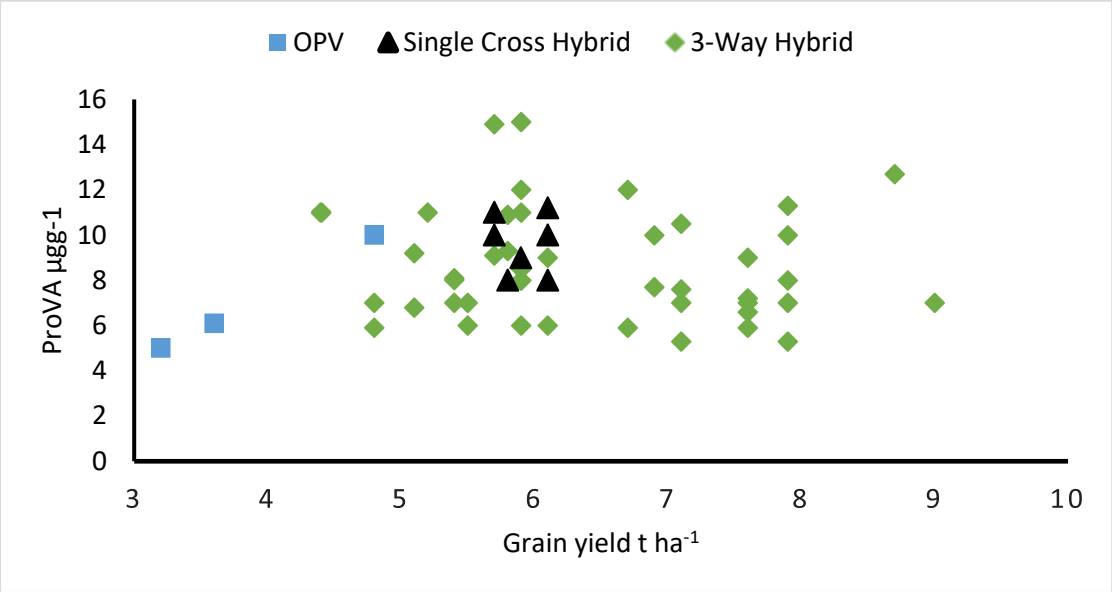

Figure 2.3 : ProVA content and grain yield performance of some of the released proVA maize cultivars in the form of OPVs, single cross and 3-way hybrids. Data sources: (HarvestPlus, 2014; Andersson et al., 2017 and cultivar release proposals from some of the National Research Institutes in SSA[2]).

The phase three inbred lines were developed using both conventional and molecular breeding methods with average proVA content as high as >15 µg g^{-1}. They have the CRTB genes introgressed using marker assisted backcrossing (Andersson et al., 2017). Apart from having high proVA content, the released cultivars and identified elite lines have high grain yield and strong farmer preferences. The International Maize and Wheat Improvement Centre (CIMMYT), International Institute of Tropical Agriculture (IITA) and selected National Research Institutes form the research and breeding component of the maize biofortification programme in Africa. Zambia and Nigeria are the primary countries where maize proVA biofortification is coordinated from while Zimbabwe, Mozambique, Malawi, Ghana, Benin, Ghana, Liberia, Sierra Leone and Mali among others constitute regional testing sites (HarvestPlus, 2014).

[2] Unpublished release proposal for provitamin A maize cultivars HP1301, HP1302 and HP1303 in 2017 by the Crop Breeding Institute, Department of Research and Specialist Services, Zimbabwe.

2.2.11 Challenges of provitamin A biofortification

Early maize proVA biofortification efforts in SSA were constrained by high preference for white maize over yellow maize by consumers and other maize value actors (De Groote and Kimenju, 2008). This resulted in poor adoption of yellow coloured biofortified maize, a challenge that slowed down the uptake of maize biofortification technology in SSA. Pillay et al. (2011) found that this skewed preference is due to lack of knowledge on the nutritional benefits of biofortified yellow maize. In Southern Africa, notably in Zimbabwe, Zambia and Mozambique yellow maize is shunned because it is perceived as a symbol of suffering and poverty. This is because yellow maize was imported into these countries during times of drought and famine (Muzhingi et al., 2008). To remedy the problem of skewed colour preferences, breeders changed the colour of biofortified maize to orange or deep yellow through conventional breeding, a measure, which greatly improved the acceptability of biofortified maize in SSA. Furthermore, to inform farmers and other maize value-chain actors about the nutritional benefits of biofortified maize, HarvestPlus and partners created parallel programmes to reach out to end users in the form of awareness campaigns. This resulted in improved acceptability of biofortified orange maize in SSA (HarvestPlus, 2014).

Quantification of carotenoids in the maize endosperm is another challenge facing maize biofortification for high provitamin A. High performance liquid chromatography (HPLC) which is the current method of choice is expensive, time consuming and low sample throughput, compromising its suitability for high sample volume breeding programmes in resource-constraint plant breeding programmes in sub-Saharan Africa and other developing countries. The cost of carotenoid analysis using HPLC is $50-$100 per sample, which is beyond the reach of most breeding programmes. Ultra-performance liquid chromatography (UPLC) provides a good alternative to HPLC due to lower cost and slightly higher throughput. However, the UPLC throughput still falls far below the quantities required by most of the breeding programmes.

The aspects of maize carotenoids degradation and retention during postharvest storage still require more elucidation and documentation. Although a number of researchers have raised the issue, there is no consensus on the average rate of degradation and level of proVA carotenoid retention (Burt et al., 2010; Messias et al., 2014; Mugode et al., 2014; De Moura et al., 2015). This poses a challenge to the quantification of the gains of biofortification especially in rural areas where maize is stored in different storage facilities for a longer period by subsistence farmers before consumption. Genotype, kernel physical properties, storage

temperature, light, oxygen and humidity are the main factors that affect postharvest storage rate of carotenoid degradation and level of retention (Taleon et al., 2017).

Elevated temperatures and humidity during postharvest periods accelerate carotenoid degradation (Ortiz et al., 2016). Disparities among genotypes in carotenoid stability are partially attributed to the differences in kernels physical properties. This means that kernel physical properties are other traits that breeders should consider when breeding for enhanced proVA content. Thus, kernels with small surface and low porosity can be selected to breed for increased carotenoid retention during postharvest storage (Ortiz et al., 2016). However, given the inadequate and diverging claims by several researchers concerning carotenoid retention during postharvest storage, there is need for further detailed research.

2.3 Breeding maize for drought tolerance

2.3.1 Importance of breeding for drought tolerance

Climate change exacerbated recurrent and episodic droughts are threatening agriculture and food security around the world. Maize productivity is highly vulnerable to drought. This threatens food security especially in sub-Saharan Africa (SSA) where maize is mostly grown by poor resourced subsistence farmers under rain fed conditions and is a staple food crop (Cairns et al., 2012). Drought was defined by Mitra (2001) as the inadequacy of water availability that includes precipitation and soil-moisture storage capacity in quantity and distribution during plant growth cycle, which restricts the full expression of the genetic potential of the plants.

Genetic improvement of maize for drought tolerance through breeding is a sustainable means of cushioning resource-poor farmers in SSA from drought (Blum, 2011). This can reduce maize grain-yield loss to drought by 20-25% (Edmeades, 2013). However, drought tolerance is a complex trait, which is controlled by many genes, each with minor effects to the ultimate performance of the cultivar (Mwadzingeni et al., 2016a). Most of the genes conditioning drought tolerance have been reported in terms of quantitative trait loci (QTL) and candidate genes (Messmer et al., 2009; Nikolić et al., 2012; Almeida et al., 2013). Furthermore, under drought stress, grain yield, which is the primary trait has low variation and heritability, which makes selection difficult (Bänziger et al., 2004). Drought tolerance is also highly affected by genotype by environment interaction.

Despite all these challenges, drought tolerance breeding is an achievable task as evidenced by many drought tolerant maize cultivars released and considerably high genetic gains

reported under drought stress in both tropical and temperate environments (Abdulmalik et al., 2017; Masuka et al., 2017a; Araus et al., 2018). Drought stress affects the wellbeing of plants at cellular, tissue and organ levels triggering several adaptive reactions. Maize being an isohydric crop, it readily shows visible and measurable responses to drought stress.

To counteract the effect of poor heritability and genetic variation of grain yield under drought stress, breeders select for morpho-physiological and biochemical traits, which are also referred to as secondary traits (Edmeades, 2013). Using different systematic phenotyping and selection methods breeders are able to select candidate lines that exhibit adaptive mechanisms in the form of drought escape, avoidance and tolerance (Blum, 2011). Many phenotypic and molecular based methods of screening maize genotypes for drought tolerance have been developed and applied in maize drought tolerance breeding (Tsonev et al., 2009; Zia et al., 2013; Reynolds and Langridge, 2016). Phenotypic methods range from the conventional low throughput manual based to sophisticated high-throughput remote sensing-based methods (Bänziger et al., 2004; Makanza et al., 2018). Molecular methods include use of candidate genes (CGs) and QTL analysis (Nikolić et al., 2012; Xu et al., 2014). Use of biochemical methods in screening of maize genotypes for drought tolerance has not been extensively explored, despite being widely applied in C_3 crops such as wheat and barley (Flexas et al., 2004; Mwadzingeni et al., 2016b).

Although, conventional breeding methods alone have achieved considerable genetic gains in maize drought tolerance improvement (Edmeades, 2013), the rate of genetic gain and cultivar turnover is inferior to what can be achieved with the aid of molecular based approaches (Beyene et al., 2016; Bankole et al., 2017). Despite great strides in the development of high-throughput phenotyping and genotyping technologies and methods, information about methods and technologies specifically applicable to drought tolerance breeding in maize is often reported in a disjointed manner. This review therefore, seeks to discuss some of the key morpho-physiological and biochemical traits that can be used in breeding for drought tolerance in maize and their level of genetic variation and heritability under drought stress. It will also highlight some of the modern breeding strategies applicable to drought tolerance improvement in maize and the genetic gains observed and reported in maize under drought stress conditions focusing mainly in tropical and subtropical SSA.

2.3.2 Effects of drought on crops

Drought reduces the water status of the soil, plant, atmosphere continuum (SPAC), disrupting the physiological, morphological and biochemical processes of crops at cell, tissue and the whole plant level. In maize, drought stress generally causes; (i) reduction in leaf area index due to reduced photosynthesis, (ii) slowed silk and tassel growth which in turn negatively affects pollen-silk synchronisation thereby reducing kernel number, (iii) kernel and ear abortion which causes poor kernel number and number of ears per pant, (iv) increased root to shoot ratio causing deeper roots as adaptive measure to enable effective water uptake, (v) lower stomatal conductance due to stomatal closure, and (iv) accelerated leaf senescence especially during the grain-filling stage (Lopes et al., 2011; Araus et al., 2012; Sun et al., 2016). In serious cases, drought stress can trigger remobilisation of stem reserves causing xylem embolism and cavitation, which in turn leads to premature and excessive stem and root lodging (Cochard, 2002). Drought can affect plant growth at any stage. It can occur at seedling, pre-reproductive or reproductive stages. At seedling stages, drought affects plant establishment, while at the reproductive stage drought can cause yield loss. Different genotypes react variably to drought stress.

2.3.3 Morpho-physiological and biochemical traits associated with drought stress

Given the poor heritability and lack of genetic yield gains under drought stress, morpho-physiological and biochemical traits can be selected during drought tolerance breeding (Edmeades, 2013). An ideal trait should be highly correlated to grain yield, have high genetic variation, easy and cheap to measure, and be stable during the data collection period. The following subsections give some of the key morpho-physiological traits. Table 2.2 shows some of the key morpho-physiological traits that can be used in drought tolerance maize breeding, their respective level of heritability and correlation to grain yield.

2.3.3.1 Anthesis-silking interval and ears per plant

Anthesis-silking interval (ASI) and number of ears per plant (EPP) are the most utilised morphological traits in drought-tolerance maize breeding. Anthesis-silking interval is the difference between number of days to silking and anthesis, whilst EPP is number of ears with at least one fully developed grain divided by the number of harvested plants in a plot (Bänziger et al., 2004). Anthesis-silking interval determines the pollen-silk synchronisation, which is key in maize hybrid production. Unlike grain yield, the heritability and variation of ASI and EPP does not decrease under drought stress but remains stable or even increases in some

occasions. Genotypes with reduced or negative ASI and many ears per plant should, thus be selected (Bänzinger et al., 2000). Selection for ASI and EPP have been used successfully for drought tolerance screening in maize breeding programmes in both tropical and temperate environments (Bolaños and Edmeades, 1996; Magorokosho et al., 2003; Campos et al., 2004;). Ribaut et al. (2009) ranked ASI and EPP as the best drought tolerance associated traits with high heritability and correlation with grain yield, requiring less time and cost for data collection. In another study, (Bolaños and Edmeades, 1996) reported high correlation coefficient values of -0.6 and 0.9 of grain yield with ASI and EPP, respectively, under drought conditions.

2.3.3.2 Stay green/leaf senescence

Leaf senescence refers to cell/tissue death triggered by the environmental factors such as drought. Loss of chlorophyll and progressive reduction in photosynthetic capacity are the key symptoms of leaf senescence (Tao et al., 2000). At whole plant level, senescence reduces the pollen receptiveness of silks and the viability of pollen, which can result in poor fertilisation (Bassetti and Westgate, 1993). Reduction in plant height under drought conditions can also be attributed to plant senescence (Shakeel et al., 2011). Plant height is a post-anthesis trait and in maize should be measured during grain filling period. Stay green generally means delayed senescence, which is a desirable trait in maize. Genotypes that exhibit stay green character are considered drought tolerant (Zheng et al., 2009). Stay green trait is genetically controlled and exhibits considerably positive correlation with grain yield under drought conditions in maize (Bänziger et al., 2004; Zheng et al., 2009). In another study, Bekavac et al. (1998) reported a high correlation between stay green character and leaf water content (r = 0.9). It has also been linked to stem and root lodging resistance (Belícuas et al., 2014). Maize plants that delay leaf senescence should be selected in drought tolerance breeding through visual scoring during the grain filling stage. However, caution should be taken when scoring for stay green trait as other factors such as soil fertility status, especially nitrogen content can have confounding effects on the stay green trait (Borrell et al., 2001; Subedi and Ma, 2005).

2.3.3.3 Stomatal conductance and leaf rolling

Plants also respond to drought stress by closing the stomata and rolling their leaves. In maize, leaf rolling is negatively correlated to grain yield under drought conditions (Bänzinger et al., 2000). Reduced stomatal conductance under drought stress reduces xylem embolism and

cavitation, which then increases maize survival (Cochard, 2002). Both stomatal conductance and leaf rolling are also strongly associated with leaf water potential. The rate of stomatal closure and level of leaf rolling during drought stress varies genetically among different maize genotypes; variation that is utilised by plant breeders in drought tolerance breeding.

The ability of a plant to adjust osmotically when drought stress sets in determines the level of leaf rolling in which plants with high osmotic adjustment exhibit less rolling and vice versa for those that exhibit high rolling. Thus, genotypes with reduced leaf rolling should be earmarked as drought tolerant candidates (Kadioglu and Terzi, 2007). Stomatal closure increases leaf temperature, which is associated with transpiration efficiency and lower carbon isotope discrimination (Khan et al., 2007). Drought tolerant genotypes exhibit lower stomatal conductance, which is associated with increased leaf temperature and high transpiration efficiency (Khan et al., 2007; Araus et al., 2012). On the other hand, drought susceptible genotypes are identified by higher stomatal conductance and lower leaf temperature, which in turn reduces transpiration. Heritability of stomatal conductance as a drought tolerant associated trait is not known. It is an area, which needs further studies in order to be considered as a key trait in drought tolerance selection.

2.3.3.4 Root characteristics

Roots are affected first when drought sets in because they are directly responsible for water uptake. Genotypes that have deep root systems are desirable because they can easily extract water which is often found in deeper soil layers during drought (Trachsel et al., 2011). Maize root system has a unique morphology and architecture, which is responsible for anchorage, water and nutrients uptake (Hochholdinger et al., 2005). The maize root system is made up of embryogenic and post embryogenic roots (Hochholdinger, 2009). The embryogenic root system consists of single primary and seminal roots that are formed during embryogenesis and are responsible for water and nutrient uptake during the seedling stage of a maize plant. Postembryonic root system is composed of shoot borne and lateral roots, which are responsible for water and nutrient transmission during the post seedling stage of a plant (Wang et al., 1995). Shoot borne roots are formed at both above and below ground nodes and they are called brace and crown roots, respectively (Trachsel et al., 2011). Genetic variation in the number of seminal roots has been reported to vary between 0-13 among different maize inbred lines (Hochholdinger, 2009). Thus, the number of seminal roots can be selected for when breeding for drought tolerance in maize seedlings. Similarly, the life span of embryogenic root system varies among maize of different genetic backgrounds. In some inbred lines it becomes

obsolete with the emergency of the postembryonic root systems while with other inbred lines it can remain functional throughout the life cycle of a plant supporting the post embryogenic roots (Hochholdinger, 2009). This suggest that the fate of embryogenic root system can be a selection criterion in drought tolerance breeding in maize.

In their study of rooting depth and water use efficiency, Hund et al. (2009) observed that deep root system coupled with high water use efficiency (WUE) can enhance drought tolerance in maize. Despite its importance in breeding for maize drought tolerance, there is scarcity of information about the heritability of root characteristics under drought conditions. It is an area, which requires attention. The major limitation of using the root structure as a trait for selection had been the difficulty in measuring non-destructively. Root capacitance meter can be used to measure root parameters without uprooting the plants but it is too expensive for most of the under resourced maize breeding programmes in developing countries (Messmer et al., 2011).

2.3.3.5 Proline content and abscisic acid

Apart from morpho-physiological changes, plants also respond to drought stress through certain biochemical changes, which include osmotic adjustment, increase in stress signalling hormones and key enzymes (Yang et al., 2010; Shakeel et al., 2011). Abscisic acid (ABA) and proline accumulation are some of the key biomolecules that are associated with drought response in plants. The ABA induces stomatal closure, growth reduction and is responsible for maintaining root elongation at low water potential (Ober and Sharp, 1994; Jovanović et al., 2000). It is also involved in the transcription and translation of several ABA-responsive genes, which are responsible for plant water-stress management (Obata and Fernie, 2012). Proline is an amino acid, which plays an osmoregulatory role in plants exposed to drought conditions. Drought triggered proline accumulation increases the cell solute concentration, which in turn increases tissue water potential, a process commonly referred to as osmotic adjustment. There is no consensus in literature on whether the accumulation of proline is triggered by ABA or is an independent process (Ober and Sharp, 1994; Yang et al., 2000). Both ABA and proline content have been shown to increase with increase in drought stress in most plants. Hong-Bo et al. (2006) reported correlations between proline content and soil water stress threshold in their wheat drought tolerance study.

In their drought tolerance evaluation study with rice, Vajrabhaya et al. (2001) observed a significant accumulation of proline in the leaves. In another study, Zegaoui et al. (2017) reported significant variation in proline accumulation among cowpea genotypes under drought

conditions. Given these findings, accumulation of proline under stress is linked with stress tolerance in many plant species. Its concentration has been shown to be generally higher in drought tolerant than in drought susceptible plants (Szabados and Savouré, 2010; Mwadzingeni et al., 2016b). Although several studies have been done on proline accumulation under drought stress, its suitability in drought tolerance breeding in maize is not known. Furthermore, proline correlation with maize grain yield and other drought tolerance associated traits is not yet established. Furthermore, there is need to investigate the heritability of proline accumulation as a drought tolerant trait.

The final criteria for selecting drought tolerant candidate genotypes should not be based on a single trait but rather a set of adaptive traits computed using tolerance indices (Bänzinger et al., 2000; Blum, 2011).

Table 2.2 : Morpho-physiological traits that are key in maize drought tolerance breeding, their level of heritability and their relationship with grain yield under drought stress.

Trait	Heritability	Correlation with grain yield	Selection	References
Anthesis-silking interval	Medium to high	High	Short anthesis silking interval	(Lu et al., 2011)
Number of ears per plant	High	High	Many ears per plant	(Bänzinger et al., 2000; Ribaut et al., 2009)
Plant height	Medium	Medium	Short plants	(Betrán et al., 2003a)
Stay green/ leaf senescence	Medium	Medium	Delayed leaf senescence	(Borrell et al., 2001)
Leaf rolling	Medium to high	Medium to low	Unrolled leaves	(Bänzinger et al., 2000)
Stomatal conductance	…….	……	Lower stomatal conductance	(Cochard, 2002)
Tassel size	Medium to high	Medium	Smaller tassel with fewer branches	(Ribaut et al., 2009)
Root structure	……..	High	Deep roots	(Hochholdinger, 2009; Hund et al., 2009)
$\Delta^{18}O$	…….	……	Higher $\Delta^{18}O$	(Cabrera Bosquet et al., 2009)

Broad sense heritability classes are: 0 – 30% (low), 30 – 60% (moderate), and ≥ 60% (high) according to Robinson et al. (1951).

2.3.4 Genetics of maize drought tolerance

Understanding the genetics of morpho-physiological traits is important in elucidating drought tolerance. Drought is a complex quantitative trait whose genetics has been reported in terms of quantitative trait loci (QTLs) and lately in terms of candidate genes (Tsonev et al., 2009; Xu et al., 2014). Following the advent of QTL mapping tools such as linkage and association mapping, several QTLs encoding for some of the key drought tolerance related morpho-physiological traits have been identified in major crops like maize, rice and wheat. For instance, in maize, QTLs for grain yield and yield components, ASI, root structure, stay green, leaf ABA among others have been reported (Landi et al., 2007; Hund et al., 2011; Messmer et al., 2011; Almeida et al., 2014).

Despite tremendous strides in QTL studies for drought tolerance improvement in maize, there is still a great challenge of identifying major and stable QTLs responsible for drought tolerance in maize. Majority of QTLs reported are minor QTLs accounting for less than 10% of the phenotypic variation (Sehgal and Yadav, 2009; Mir et al., 2012; Shikha et al., 2017). QTLs identified using given genotypes/populations and environment may not be detected using different genotypes and environments making it difficult for breeders to use the QTL in developing different populations (Hao et al., 2010). Modern technologies such as next generation sequencing (NGS) technologies have become instrumental in identifying candidate genes underlying drought tolerance QTLs in major crops including maize.

Candidate genes confer drought tolerance through specific processes like encoding for proteins that are involved in cell protection under drought stress and/or regulate other genes participating in drought response (Mir et al., 2012). Examples of such genes include pyrroline-5-carboxylate synthetase (*P5CS*), which is responsible for the enhanced accumulation of proline which in turn causes osmo-tolerance (Sun et al., 2016). NADP-malic (*NADP-Me*) is another enzyme, which is overexpressed to reduce stomatal conductance under drought stress thereby improving water use efficiency (WUE) (Cattivelli et al., 2008) and *DREBs* is a stress induced transcription factor that triggers the expression of downstream stress related genes that confers drought tolerance (Cattivelli et al., 2008; Sehgal and Yadav, 2009).

Knowledge of candidate genes encoding for drought tolerance is useful for understanding drought tolerance and can be utilised in developing drought tolerant maize cultivars through MAS (Mir et al., 2012). Identification of candidate genes and their subsequent validation enables the application of genetic engineering technique in drought tolerance improvement, an area which is still in its infancy, especially in developing countries due to lack of supporting

policies and technical know-how. Many drought-tolerance candidate genes have been identified (Ribaut et al., 2009; Sehgal and Yadav, 2009; Hao et al., 2010). Information about biotic and abiotic resistance related to QTLs, candidate genes and information on drought tolerance in maize is available on different websites including; http://www.plantstress.com, http://www.gramene.org and https://www.maizegdb.org/.

2.3.5 Genetic diversity analysis

Genetic diversity analysis, which can also be called genetic characterisation, is important in plant breeding as it helps breeders to classify germplasm according to their genetic relationships. Singh (1983) defined genetic diversity as the probability that randomly selected alleles are different. Genetic diversity is likely to be high if germplasm was collected from diverse sources and in that case, thorough characterisation is required. In hybrid maize development, high diversity allows plant breeders to develop new improved cultivars with robust performances due to heterosis, which can be efficiently exploited by crossing unrelated parents. Information obtained from genetic diversity analysis can be used for the conservation of plant genetic resources (Govindaraj et al., 2015).

Genetic diversity can be assessed using different methods that allow the estimation of genetic distances between different genotypes. The distance between different genotypes indicates the level of diversity among genotypes (Beyene et al., 2013). Genetic diversity studies can be carried out at both phenotypic and/or molecular levels. Diversity analysis at phenotypic level can be done using agro-morphological traits (Beyene et al., 2005), whilst molecular markers are used for molecular diversity analysis (Kassa et al., 2012).

Agro-morphological traits encompasses both agronomic and morphological traits, which can be qualitative or quantitative. In this study, only quantitative traits were used to differentiate among genotypes based on their agro-morphological performances. Diversity studies using agro-morphological traits are easy and do not require expensive technology. However, more land and labour force are required to implement the trials (Govindaraj et al., 2015). The major drawback of this method is that the traits are highly influenced by the environment, an attribute, which reduces accuracy. Therefore, to obtain a good understanding of the level of diversity among the available genotypes, agro-morphological diversity analysis should be complemented with molecular diversity analysis.

Several molecular markers have been successfully applied in plant genetic diversity analysis including restriction fragment length polymorphism (RFLP) (Barbosa et al., 2003), amplified

fragment length polymorphism (AFLP) (Barrett and Kidwell, 1998), simple sequence repeats (SSRs) (Adeyemo and Omidiji, 2013) and single nucleotide polymorphisms (SNPs) (Badu-Apraku et al., 2015). Each molecular marker system has its strengths and drawbacks. However, technological trends have shifted towards SNPs mainly because they are locus-specific, have high genomic abundance, potential for high throughput analysis, and lower genotyping error rates (Kassa et al., 2012).

The SNPs are DNA sequence variations that occur when a single nucleotide changes in the genome sequence as either transitions or transversions. To date, large numbers of SNPs markers and relevant genotyping platforms in maize have been developed compared to other crops. Thus, SNPs are becoming markers of preference in carrying out a variety of genotyping tasks in plant improvement including diversity analysis, QTL mapping, and whole genome sequencing (Prasanna et al., 2010).

2.3.6 Combining ability analysis and gene action

Combining ability effects can be classified into general combining ability (GCA) and specific combining ability (SCA). In maize GCA refers to the average performance of an inbred line in its hybrid combinations, whilst SCA is when hybrid performance deviates from the expected performance based on the average performance of its parental inbred lines (Hallauer and Miranda, 1988). Combining ability analysis helps breeders in determining the type of gene action controlling the expression of a trait.

Gene action can be defined as the behaviour of genes in a given genetic population. In maize hybrid development, information about gene action helps plant breeders to choose the suitable breeding strategy in order to maximise genetic gain (Hallauer and Miranda, 1988). Gene action is assessed in terms of genetic variance components or combining ability effects. Genetic components can be categorised into either additive or non-additive gene action with non-additive gene action further apportioned into dominance and epistasis effects. Additive gene action is when the behaviour of genes from both parents positively or negatively influence the expression of the trait whilst non-additive gene action explains the variation that cannot be accounted for by additive gene action (Dabholkar, 1992). Thus, additive gene action is associated with predominance of GCA effects whilst non-additive gene action is associated with the predominance of SCA effects.

In case of predominance of additive gene action, the decisions to integrate the materials with high GCA values into the breeding pipeline should be followed. However, when non-additive

gene action is predominant, superior materials should be advanced to hybrids stage for commercial purposes (Singh and Prasad, 2002). In case of both additive and non-additive effects having equal contribution towards the variance, then development of lines with superior performance should be undertaken (Singh and Prasad, 2002).

It is important to understand gene action for grain yield and other secondary traits under drought stress to develop effective strategies to use in breeding for drought tolerance without compromising yield. Betran et al. (2003b) and Derera et al. (2007) reported the predominance of additive gene action for grain yield of maize under drought conditions, whilst Murtadha et al. (2018) and Mhike et al. (2011) reported the predominance of non-additive gene action. Different cases where additive gene action was predominant and non-additive gene action was predominant for grain yield have been reported in previous drought tolerance studies.

2.3.7 Progress in breeding for drought tolerance

A substantial amount of financial resources are being channelled into temperate, tropical and sub-tropical maize drought tolerance improvement projects in both public and private sectors (Bänziger et al., 2004; Campos et al., 2004). In SSA, two major projects focusing on drought-tolerance maize improvement were recently undertaken under the banners of drought tolerance maize (DTMA) (http://dtma.cimmyt.org) and Water use Efficient Maize for Africa (WEMA) (https://wema.aatf-africa.org/). This resulted in several drought tolerant maize lines and cultivars being developed and released worldwide in the form of open pollinated varieties (OPVs) and hybrids (Anami et al., 2009; Edmeades, 2013).

Considerable success has been reported in terms of genetic gains under both drought stress and non-drought stress conditions through the improvement of one or more morpho-physiological traits using both conventional, molecular and transgenic breeding methods. The advantages of marker assisted selection (MAS) over conventional breeding in quantitative traits such as drought tolerance in terms of yield gain is well documented for both temperate and tropical maize. For instance, in temperate maize, a genetic advantage of 146 kg ha^{-1} yr^{-1} of maize cultivars developed through MAS over the conventionally developed has been reported under drought, occurring during the reproductive stage. Similarly, Abdulmalik et al. (2017) reported a genetic yield gain of 163 kg ha^{-1} yr^{-1} with tropical maize using MAS. Table 2.3 summarises some of the recently reported genetic gains in tropical maize drought stress breeding programmes. Morphological traits that are contributing to the genetic gains are also highlighted.

Table 2.3: Some of the latest yield genetic gains reported under drought stress conditions for tropical maize.

Rate of yield increase (kg ha^{-1} yr^{-1})	Cultivar	Targeted trait(s)	Environment	Reference
51.0	Hybrid	GY, ASI	Managed drought	(Beyene et al., 2016)
118.0	Hybrid	GY, ASI, PH	Managed drought	(Bankole et al., 2017)
163.0	Hybrid	GY, ASI, SEN	Managed drought	(Abdulmalik et al., 2017)
32.5	Hybrid	GY, ASI, PH	Managed drought	(Masuka et al., 2017a)
22.7	Hybrid	ASI, GY, PH	Random drought	(Masuka et al., 2017a)
29.2	OPV	GY, ASI, PH	Random drought	(Masuka et al., 2017b)

SEN – leaf senescence, GY – grain yield, OPV – open pollinated varieties, PH – plant height.

2.3.8 Conclusion and prospects

Biofortification of maize for enhanced vitamin A has proved to be an important innovation for addressing both food and nutrition insecurity in SSA. Given the genetics and heritability of proVA both conventional and molecular breeding can be applied in maize biofortification. The application of molecular markers accelerates the process of proVA biofortification. To increase the adoption of biofortified maize varieties in SSA, the released cultivars should be competitive in other traits such as grain yield, biotic and abiotic resistance. Enhancing drought tolerance in proVA maize cultivars developed for SSA could contribute to the acceptability of the biofortified maize in Southern Africa given the precedency of drought in this region. Given the predicted potential growth of the biofortification industry, there is need for the development of cheaper, efficient and high throughput proVA quantification technologies.

Drought tolerance improvement in maize, remains an important breeding objective, given the climate change effects and high population growth, which continues to place a high demand for food, in the region. Marker assisted breeding, next generation sequencing and genetic engineering are promising technologies that can increase maize genetic gains under drought

conditions. However, their optimum application to maize improvement is hindered by lack of validated major and stable QTLs/genes that control key traits associated with drought tolerance. Furthermore, there is need for policy support for genetic engineering technologies especially in developing countries. Since phenotyping is still key in plant breeding programmes, there is need for alternative low-cost high-throughput phenotyping technologies to support the poorly resourced breeding programmes in developing countries. Further studies should also focus on identifying new traits associated with drought tolerance in maize to increase the efficiency of phenotyping especially those related to the metabolic processes. Finally, given the complex nature of drought tolerance there is need of an integrated adaptive approach that encompasses morphology, physiology, genomics and biomolecular drought-stress response in maize.

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CHAPTER 3

Diversity Analysis of Provitamin A Maize Inbred Lines using Agro-morphological Traits and β-carotene Content

Abstract

Information on the agro-morphological trait values of the available genotypes is essential for any breeding programme as it helps breeders to make informed decisions when selecting parental combination during hybridisation. The study was conducted to assess the level and pattern of phenotypic diversity among 46 provitamin A maize germplasm sourced from CIMMYT and IITA using β-carotene content and agro-morphological trait values. Analysis of variance, cluster analysis, genetic parameters, genetic distances and phenotypic correlation analysis were used to describe the pattern and level of phenotypic diversity. Inbred lines varied significantly for most of the traits studied. Grain yield averaged 1.8 t ha^{-1} ranging from 0.70 t ha^{-1} to 2.70 t ha^{-1}. Grain yield, β-carotene and anthesis-silking interval exhibited high broad sense heritability (H^2) and genetic advance as a percentage of the mean (GAM). Cluster analysis grouped the genotypes into three distinct clusters. The highest phenotypic distance was 8.37 between genotypes TZM114 and CLHPO331. Grain yield, β-carotene content anthesis-silking interval and days to maturity were the most discriminating traits and recommended to be used in selecting materials to advance to the next stage. Inbred lines with high β-carotene content and grain yield were earmarked for use as parents in the hybridisation programme. Overall, the study indicated the existence of high trait diversity in CIMMYT and IITA inbred lines, which can be exploited for the genetic improvement of β-carotene content and grain yield of tropical and subtropical germplasm.

3.1 Introduction

Maize (*zea mays* L.) is the most important cereal crop in sub-Saharan Africa (SSA) especially in southern Africa where it is the staple crop for most people (Muthayya et al., 2013). White maize which is popularly consumed in southern and eastern Africa lacks vitamin A, which is causing high prevalence of vitamin A deficiency (VAD) in the region, especially in rural areas (Suwarno et al., 2014). This is because most people who rely on maize dominated diets in SSA are the marginalised poor rural people who mainly consume what they grow and, in most cases cannot afford diversified vitamin A rich food sources. On the other hand, maize productivity in SSA region is facing threats from biotic and abiotic constrains with drought being the major abiotic limiting factor (Cairns et al., 2013).

Developing provitamin A enhanced and drought tolerant yellow/orange maize cultivars can help to reduce VAD among maize consumers and at the same time reduce the impacts of drought to maize productivity (Edmeades, 2013; Bouis and Saltzman, 2017). Vitamin A content of maize and other crops can be improved through provitamin A biofortification which is the enhancement of provitamin A content in maize kernels using conventional plant breeding and/or biotechnology (Bouis et al., 2011). The International Maize and Wheat Improvement Centre (CIMMYT) and International Institute of Tropical Agriculture (IITA) are the two organisations that are currently spearheading maize biofortification in the world in partnership with HarvestPlus (www.harvestplus.org/). They are the major sources of biofortified maize germplasm used in most of the maize biofortification programmes worldwide.

Yellow maize kernels have high levels of variation in provitamin A carotenoids, which are β-carotene, α-carotene and β-cryptoxanthin (Pfeiffer and McClafferty, 2007). These are converted into vitamin A in the human body (Fierce et al., 2008). Among the three carotenoids, β-carotene has higher provitamin A activity which is twice greater than the combined activity of α-carotene and β-cryptoxanthin (Harrison, 2015). Hybrid, synthetic or open pollinated maize cultivars, exhibiting heterosis for provitamin A content and grain yield can be a sustainable solution to VAD and poor maize productivity in Africa (Menkir et al., 2014). Hybrid maize breeding needs to be well planned and implemented to enable maximum exploitation of heterosis. Heterosis can be best exploited if high performing and genetically divergent parents are crossed to give a heterozygous F_1 progeny (Hallauer and Miranda, 1988). Genetic distance (GD) estimates indicates the level of parental divergent with parental lines that have wider

genetic distances having higher chances to give a more vigorous hybrid performance (Yu et al., 2005).

Information about broad sense heritability (H^2), phenotypic coefficient of variation (PCV), genetic coefficient of variation (GCV), of the available germplasm is important as it assists plant breeders to predict and assess the magnitude of genetic improvement that can be achieved by selecting for the respective traits (Govindaraj et al., 2010). Heritability indicates the portion of variation that is due to genetic effects and varies with the level of genotypic differences within a population. Knowledge of trait heritability enables plant breeders to choose an efficient breeding approach. Selection of traits with high heritability can lead to high genetic gain. Genetic advance is the genetic progress achieved by a breeding programme over a stipulated period and shows the efficiency of a breeding programme (Bello et al., 2012). Thus, high genetic variability coupled with high heritability estimates provide suitable conditions for efficient selection. It is therefore, important to assess the level of variation among the available breeding materials through diversity analysis.

In maize, diversity analysis can be carried out using agro-morphological traits, biochemical and molecular markers (Govindaraj et al., 2015). The choice of a method depends on the objectives of the breeding programme, technology and resources available to the breeder. The major advantage of using agro-morphological traits is that the method does not require high technical competence and sophisticated instruments to apply, which makes it suitable for under resourced breeding programmes in SSA. The major disadvantage of using agro-morphological traits in diversity analysis is that they are prone to environmental influence and may require large tracks of land to implement.

In the present study, β-carotene content and agro-morphological traits were used to assess phenotypic diversity among 46 provitamin A maize inbred lines. The specific objectives of the study were: (i) to evaluate the agro-morphological performances and β-carotene content of provitamin A inbred lines sourced from CIMMYT and IITA, and (ii) to assess the levels and patterns of phenotypic diversity among provitamin A maize inbred lines. The information generated would assist in selection of parents and deciding parental combinations to use for hybridisation.

3.2 Materials and methods

3.2.1 Plant materials and the study sites

The study evaluated a total of 48 inbred lines for agro-morphological performances and only 46 of the lines were tested for β-carotene content. The inbred lines consisted of 30 experimental provitamin A and 3 provitamin A check inbred lines from CIMMYT-Zimbabwe, 13 extra yellow lines from IITA-Nigeria that were selected from a *Striga* resistance nursery and two non-provitamin A (white endosperm) drought tolerant inbred lines. The two non-provitamin A inbred lines were used to balance the experimental design (see section 3.2.2). The IITA inbred lines were given new code names after selfing them to multiply seed. Table 3.1 shows the list of the inbred lines, type and their respective sources. All the inbred lines type name "provitamin A". No pedigree information of the inbred lines could be provided were under germplasm protection policies by the respective source institution as they were still under development. The study was carried out at Ukulinga research farm (29^0 40' S, 30^0 24' E; 806 m above sea level) in Pietermaritzburg in South Africa from December to April during the 2016/17 growing season. The mean temperature and rainfall at Ukulinga research farm for the growing period is presented in Table 3.2. Generally, the 2016/17 season was optimum with regards to rainfall received despite some very few sporadic drought conditions experienced during the early vegetative growth stage.

Table 3.1: List of maize inbred lines used in the study.

Entry number	Genotype	Source	Type	Entry number	Genotype	Source	Type
1	CLHP00306	CIMMYT-Zimbabwe	ProVA-experimental	26	CLHP0156	CIMMYT-Zimbabwe	ProVA-experimental
2	CLHP0310	CIMMYT-Zimbabwe	ProVA-experimental	27	CLHP0005	CIMMYT-Zimbabwe	ProVA-experimental
3	CLHP0302	CIMMYT-Zimbabwe	ProVA-experimental	28	CLHP0003	CIMMYT-Zimbabwe	ProVA-experimental
4	CLHP0312	CIMMYT-Zimbabwe	ProVA-experimental	29	CLHP0049	CIMMYT-Zimbabwe	ProVA-experimental
5	CLHP0334	CIMMYT-Zimbabwe	ProVA-experimental	30	CLHP0020	CIMMYT-Zimbabwe	ProVA-experimental
6	CLHP00478	CIMMYT-Zimbabwe	ProVA-experimental	31	CML486	CIMMYT-Zimbabwe	ProVA-Check
7	CLHP00286	CIMMYT-Zimbabwe	ProVA-experimental	32	CML451	CIMMYT-Zimbabwe	ProVA-Check
8	CLHP0303	CIMMYT-Zimbabwe	ProVA-experimental	33	CML304	CIMMYT-Zimbabwe	ProVA-Check
9	CLHP00307	CIMMYT-Zimbabwe	ProVA-experimental	34	TZM114	IITA-Nigeria	ProVA-experimental
10	CLHP00322	CIMMYT-Zimbabwe	ProVA-experimental	35	TZM116	IITA-Nigeria	ProVA-experimental
11	CLHP00378	CIMMYT-Zimbabwe	ProVA-experimental	36	TZM25	IITA-Nigeria	ProVA-experimental
12	CLHP00432	CIMMYT-Zimbabwe	ProVA-experimental	37	TZM113	IITA-Nigeria	ProVA-experimental
13	CLHP0331	CIMMYT-Zimbabwe	ProVA-experimental	38	TZM1225	IITA-Nigeria	ProVA-experimental
14	CLHP0343	CIMMYT-Zimbabwe	ProVA-experimental	39	TZM1224	IITA-Nigeria	ProVA-experimental
15	CLHP0350	CIMMYT-Zimbabwe	ProVA-experimental	40	TZM112	IITA-Nigeria	ProVA-experimental
16	CLHP0326	CIMMYT-Zimbabwe	ProVA-experimental	41	TZM117	IITA-Nigeria	ProVA-experimental
17	CLHP0310	CIMMYT-Zimbabwe	ProVA-experimental	42	TZM1223	IITA-Nigeria	ProVA-experimental
18	CLHP0352	CIMMYT-Zimbabwe	ProVA-experimental	43	TZM106	IITA-Nigeria	ProVA-experimental
19	CLHP00294	CIMMYT-Zimbabwe	ProVA-experimental	44	TZM109	IITA-Nigeria	ProVA-experimental
20	CLHP0364	CIMMYT-Zimbabwe	ProVA-experimental	45	TZM1248	IITA-Nigeria	ProVA-experimental
21	CLHP0404	CIMMYT-Zimbabwe	ProVA-experimental	46	TZM1276	IITA-Nigeria	ProVA-experimental
22	CLHP0221	CIMMYT-Zimbabwe	ProVA-experimental	47	CML488	ARC-South Africa	Drought tolerant
23	CLHP0058	CIMMYT-Zimbabwe	ProVA-experimental	48	CML550	ARC-South Africa	Drought tolerant
24	CLHP0022	CIMMYT-Zimbabwe	ProVA-experimental				
25	CLHP0113	CIMMYT-Zimbabwe	ProVA-experimental				

Table 3.2: Monthly weather data during the field trial at Ukulinga research farm (2016/2017).

Month	T_{max} (°c)	T_{min}(°C)	RH_{max}(%)	RH_{min} (%)	ETO (mm)
December	27.14	16.16	98.43	52.71	102.36
January	24.46	15.70	98.46	49.16	120.22
February	27.21	16.33	97.16	55.18	104.55
March	29.18	15.36	99.12	46.12	81.15
April	24.78	14.51	98.31	50.12	78.14

T_{max} - average maximum temperature, T_{min} - average minimum temperature, RH_{max} - average maximum relative humidity, RH_{min} - average minimum relative humidity ETO - average total relative evapotranspiration

3.2.1 Experimental design and trial management

The experimental design used was an 8 x 6 alpha lattice with 2 replications. The plants were planted in two row plots of 5 m length with inter row spacing of 0.75 m and intra row spacing of 0.25 m. Two seeds were planted per hill and later thinned into one plant at 3 weeks after emergency. Compound fertilizer was applied at the rate of 65 kg N, 65 kg P and 65 kg K ha^{-1} at the time of planting. Top dressing fertilizer was applied at five weeks after emergence at a rate of 60 kg N ha^{-1}. Weeds were controlled using gramoxone at a rate of 5 L ha^{-1} and manual weeding to keep the field weeds free. Coragen and karate insecticides were used to control insects at a rate of 1 L $ha^{-1.}$

3.2.2 Data collection

Data on β-carotene content and twelve agro-morphological traits were collected. Carotenoid analysis was only done for β-carotene due to financial limitations. A sample of 20 g which constituted about 30 to 50 kennels of maize was randomly collected from each of the 46 provitamin A inbred lines and dispatched to Agricultural Research Council (ARC), Science analytical laboratory, Pretoria, South Africa (http://www.arc.agric.za) for β-carotene analysis. High performance liquid chromatography (HPLC) was used for analysis following a protocol for dried maize kernel as described by Menkir et al. (2008). The β-carotene analysis was done two times per sample giving two data points per each genotype. Table 3.3 shows the agro-morphological traits that were measured and the description on how the measurements were done.

Table 3.3: Agro-morphological traits measured and description of the measuring methods.

Acronym	Trait	Measuring procedure
GY	Grain yield (t ha^{-1})	Grain yield per plot, adjusted to 12.5% grain moisture and converted to tons per hectare using equation 3.1 below.
DA	Days to anthesis	Measured as number of days after planting when 50% of the plants shed pollen.
ASI	Anthesis-silking interval	Calculated as days to silking minus days to anthesis.
EPP	Ears per plant	Number of ears per plant. Counted as number of ears with at least one fully developed grain divided by the number of harvested plants.
PH	Plant height (m)	Measured as height between the base of a plant to the insertion of the first tassel branch of the same plant of 6 alternating plants in the plot.
SL	Stem lodging (%)	Measured as the percentage of plants per plot that have their stems broken below the ear.
RL	Root lodging (%)	Measured as percentage of the plants per plot which have their stems inclining by more than 45^{o}.
HKW	Weight of 100 kernels	Three samples of 100 kernels randomly selected from the total kernels and their weight measured and averaged.
SP	Shelling %	Field weight minus grain weight and the difference was expressed as a percentage.
DM	Days to maturity	Measured as number of days after planting to physiological maturity.
PS	Plant stand	Percentage of number of plants harvested in a plot with at least one ear to the total expected number of plants in a plot.
BCC	β-carotene content	Analysed using HPLC following a procedure by Menkir et al. (2008) (see section 3.2.3 for description).

$$\mathrm{GY} = \frac{\text{Field weight (kg)} * 10000(\mathrm{m}^2) * (100 - \mathrm{MOI}) * \text{Shelling\%}}{1000(\mathrm{kg}) * \text{Plot area } (\mathrm{m}^2) * (100 - 12.50)\%} \quad (3.1)$$

Where GY = calculated grain yield per ha; MOI= Grain moisture content measured at harvest; Shelling % = percentage of grain weight over field weight.

3.3 Data analysis

3.3.1 Mean performance, analysis of variance and genetic parameters

The overall performances of the inbred lines were assessed using descriptive statistics computed using Genstat® version 18 VSN, International (Payne et al, 2017). Analysis of variance (ANOVA) was performed for β-carotene and all the agro-morphological traits using the general linear model (GLM) procedure of Genstat. The model used for analysis of all the agro-morphological traits is shown in equation 3.2.

$$Y_{ijk} = \mu + r_i + b_j + G_k + e_{ijk} \quad (3.2)$$

Where, μ = overall trial mean, r_i = effect of i^{th} replications, b_j = effect of thej^{th} block within the i^{th} replication effects, G_k = effect of the inbred lines and e_{ijk} = random experimental error effects.

The model for used for β-carotene content analysis is presented in equation 3.3.

$$Y_{ij} = \mu + G_i + S(G)_{ij} \quad (3.3)$$

Where μ = overall trial mean, G_i = effect of ith inbred line, $S(G)_{ij}$ = error term.

Genetic parameters were estimated using the following equations 3.4 - 3.10 (Singh and Chaudhary, 1985):

$$\sigma_g^2 = \frac{MS_g - MS_e}{r\ x\ b} \quad (3.4)$$

$$\sigma_e^2 = MS_e \quad (3.5)$$

$$\sigma_P^2 = \sigma_g^2 + \sigma_e^2 \quad (3.6)$$

$$GCV(\%) = \frac{\sqrt{\sigma_g^2}}{\mu}\ x\ 100 \quad (3.7)$$

$$H^2 = \frac{\sigma_g^2}{\sigma_P^2}\, x\, 100 \qquad (3.8)$$

$$GA = i\left(\sqrt{\sigma_P^2}\right)(H^2) \qquad (3.9)$$

$$GAM(\%) = \left(\frac{GA}{\mu}\right)100 \qquad (3.10)$$

Where σ_e^2 = environmental variance; σ_g^2 = genotypic variance; σ_P^2 = phenotypic variance; $MS_e =$ error mean square; H^2 = broad sense heritability; GA = genetic advancement; $GAM(\%)$= Genetic advancement as a percentage of mean; i= the standard selection differentials at 5% selection intensity (the value of *i* at 5% = 2.063); r= number of replications; b= number of blocks; $PCV(\%)$= phenotypic coefficient of variance; $GCV(\%)$= genotypic coefficient of variance.

3.3.2 Cluster and correlation analysis

Prior to cluster analysis, the quantitative data was first standardised by converting the values to binary format. This was achieved by adding and subtracting the standard deviation values from their respective mean values to get the upper and lower category limits, respectively. This was then followed by assigning binary values to the traits value in accordance to the generated categories (Beyene et al., 2005). The cluster analysis was then performed using the unweighted pair-group arithmetic average method (UPGMA) using DARwin 6.0 software (Perrier and Jacquemoud-Collet, 2006). A dendrogram was generated from the genetic distance matrix. Pearson's correlation analysis was done using Genstat® version 18 VSN, International to determine phenotypic pairwise relationships between all the measured traits.

3.4 Results

3.4.1 Analysis of variance, performance and ranking of inbred lines

Table 3.4 presents a summary of combined ANOVA showing mean square values, coefficient of variation (CV%), least significant different (LSD) and the level of significance (F-probability) of the twelve measured traits. The inbred lines exhibited considerable significant differences for most of the traits. The mean squares for BCC, GY, EPP, PH and PS were highly significant

(p ≤ 0.001) whilst those for DM and SP were significant at p≤0.01. Anthesis-silking interval and DA were significantly different at p ≤ 0.05 while HKW, RL and SL were not significantly different. High CV% (>20%) was observed for ASI (38.37%) and EPP (26.39%).

Table 3.4: Summarised analysis of variance for provitamin A maize inbred lines.

Traits	Mean Squares	CV	LSD	F-pro
Beta carotene (µg g^{-1})(BCC)	1.85	11.8	0.42	***
Grain yield (t ha^{-1}) (GY)	0.36	11.0	0.39	***
Anthesis-silking Interval (days) (ASI)	2.51	38.37	2.26	*
Days to anthesis (DA)	11.10	5.74	4.66	*
Days to maturity (DM)	156.60	6.93	17.08	**
Ears per plant (EPP)	0.83	26.39	1.16	***
100 Kernel weight (HKW)	0.06	5.97	0.00	ns
Plant height (cm) (PH)	1273.23	10.68	38.61	***
Plant stand (%) (PS)	304.47	15.02	22.53	***
Root Lodging (%) (RL)	11.81	15.52	22.12	ns
Stem lodging (%) (SL)	2.52	16.95	12.54	ns
Shelling percentage (%) (SP)	0.01	10.33	0.10	**

*, **, ***, ns indicate level of significance of the data at P≤ 0.05, P ≤0.01, P ≤ 0.001 and not significant, respectively.

Table 3.5 shows top 10 and bottom 5 of the 46 provitamin A inbred lines ranked in descending order of BCC. Mean BCC was 2.04 µg g^{-1} ranging from 0.60 µg g^{-1} to 4.22 µg g^{-1}. Inbred line CLHP00306 had the highest BCC of 4.22 µg g^{-1}whilst CLHP0326 had the least BCC of and 0.6 µg g^{-1}. Among the three provitamin A checks, CML451 had the highest BCC of 2.89 µg g^{-1}. Table 3.6 shows performance of the inbred lines ranked according to GY. The observed mean GY was 1.8 t ha^{-1} with inbred line CLHP0020 having the highest yield of 2.7 t ha^{-1} and CLHP0302 as the least yielding with 0.7 t ha^{-1}. Only two checks (CML304 and CML451) were in the top ten of high yielding inbred lines with both having 2.2 t ha^{-1}. Four inbred lines that featured in the top ten for both BCC and GY were CLHP00306, CML451, CLHP0350 and CLH0364.

Table 3.5: Means of BCC and agro-morphological traits of top ten and bottom five inbred lines (ranked according to β-carotene content).

Entry	Name	BCC	GY	ASI	EPP	HKW	DA	DM	PS	PH	SL	RL	SP
Top ten genotypes													
1	CLHP00306	4.22	1.6	5.14	1.13	0.031	60.49	107.36	87.52	149.41	7.76	22.73	0.40
11	CLHP00378	4.21	2.1	4.06	1.79	0.031	62.05	120.02	87.57	150.09	10.77	24.13	0.52
6	CLHP00478	3.66	1.0	3.12	1.36	0.031	66.26	109.73	61.03	153.29	9.08	26.26	0.43
20	CLHP0364	3.52	2.1	3.83	2.38	0.031	61.60	129.28	84.66	146.04	7.00	17.56	0.54
5	CLHP0334	3.43	1.6	3.56	1.29	0.031	68.05	121.52	82.57	142.09	11.77	17.13	0.49
30	CLHP0020	3.05	2.7	3.94	3.26	0.029	63.14	132.89	82.36	143.00	11.08	23.68	0.57
32	*CML451	2.89	2.2	2.62	0.86	0.031	66.26	106.23	70.03	156.29	10.08	20.76	0.42
15	CLHP0350	2.88	2.1	3.01	2.55	0.027	66.02	122.80	84.52	150.33	7.39	25.28	0.51
12	CLHP00432	2.88	1.2	4.77	2.81	0.032	63.89	131.58	88.20	160.84	10.19	22.93	0.52
40	TZM112	2.80	2.0	-0.83	1.86	0.028	67.64	115.42	78.42	250.39	9.96	23.06	0.45
Bottom five genotypes													
13	CLHP0331	0.78	2.0	3.12	2.36	0.035	62.76	133.23	86.03	158.79	10.08	23.26	0.60
22	CLHP0221	0.71	0.8	1.99	2.50	0.033	68.17	130.11	84.91	203.25	7.97	25.03	0.44
26	CLHP0156	0.70	1.5	4.93	1.61	0.031	63.15	121.81	85.39	168.66	8.84	22.42	0.44
14	CLHP0343	0.63	1.4	2.55	2.08	0.031	67.38	118.82	75.37	157.46	10.77	24.66	0.49
16	CLHP0326	0.60	1.5	4.83	1.88	0.029	63.10	123.78	71.66	168.54	10.50	21.06	0.47
	Mean	2.04	1.8	3.00	2.14	0.030	65.8	121.73	81.98	177.60	9.53	22.66	0.50
	CV%	11.8	11.0	38.37	26.39	5.970	5.4	6.9	11.9	10.7	17.0	15.5	10.33
	LSD	0.42	0.4	2.26	1.16	0.004	7.2	17.1	19.8	38.6	3.3	7.1	10.35

BCC – β-carotene content (µg/g), GY – grain yield (t ha^{-1}), ASI – anthesis-silking Interval (days), EPP – ears per plant, HKW 100 kernel weight (kg), DA – days to anthesis, DM – days to maturity, PS – plant stand (%), PH – plant height (cm), SL – stem lodging percentage (%), RL – root lodging percentage (%), SP - shelling percentage (%), LSD – least significant differences, CV% – coefficient of variation. *stared inbred lines are provitamin A check.

Table 3.6: Means of agro-morphological traits of top ten and bottom five inbred lines (ranked according to grain yield).

	Entry name	GY	BCC	ASI	EPP	HKW	DA	DM	PS	PH	SL	RL	SHN
Top ten genotypes													
30	CLHP0020	2.7	3.05	2.94	3.26	0.029	63.1	132.9	88.3	143.0	11.1	23.7	56.78
18	CLHP0352	2.4	1.61	3.55	2.58	0.032	64.9	125.8	84.9	163.5	7.8	25.7	55.55
9	CLHP00307	2.2	2.69	3.33	1.88	0.032	62.6	124.8	87.0	142.5	8.0	20.6	53.35
37	TZM113	2.2	2.65	2.95	1.54	0.030	64.8	104.2	86.5	203.5	9.8	24.8	49.55
33	*CML304	2.2	2.23	3.43	3.11	0.030	65.2	130.3	91.4	180.2	8.3	22.9	55.30
23	CLHP0058	2.2	2.56	1.44	2.99	0.030	67.2	131.6	84.1	146.0	9.0	24.3	54.48
32	*CML451	2.2	2.89	2.62	2.86	0.032	66.3	106.2	70.0	156.3	10.1	20.8	46.62
20	CLHP0364	2.1	3.52	3.83	2.58	0.033	61.6	129.3	84.7	146.0	7.0	17.6	53.85
15	CLHP0350	2.1	2.88	3.01	2.55	0.031	66.0	122.8	84.5	150.3	7.4	25.3	51.26
41	TZM117	2.1	0.87	2.30	3.07	0.035	74.2	135.1	101.6	254.6	10.4	23.2	53.32
Bottom five genotypes													
6	CLHP00478	1.0	3.66	3.12	1.36	0.031	66.3	109.7	61.0	153.3	9.1	26.3	42.53
2	CLHP0310	0.9	1.16	3.87	1.50	0.031	62.0	116.2	82.4	155.3	8.7	22.5	43.01
22	CLHP0221	0.8	0.71	4.99	2.30	0.031	68.2	130.1	71.4	203.3	8.0	25.0	43.52
17	CLHP0310	0.8	1.59	4.14	1.13	0.027	65.0	111.4	76.2	127.9	8.3	22.2	42.03
3	CLHP0302	0.7	2.28	4.14	1.13	0.027	60.5	117.4	84.9	133.4	8.8	24.7	41.62
	Mean	1.8	2.04	3.31	2.18	0.031	65.2	121.8	82.6	163.9	8.8	23.3	49.52
	CV%	11.0	11.8	38.37	26.39	5.970	5.4	6.9	11.9	10.7	17.0	15.5	10.33
	LSD	0.4	0.3	2.26	1.16	0.004	7.2	17.1	19.8	38.6	3.3	7.1	10.35

GY – grain yield in t ha^{-1}, ASI – anthesis silking interval (days), EPP – ears per plant, HKW – 100 kernel weight, DA – days to anthesis (days), DM – days to maturity, PS – plant stand, PH – plant height, SL – stem lodging percentage (%), RL – root lodging percentage (%), SP – shelling percentage (%), LSD – least significant differences. *stared inbred lines are provitamin A checks.

Inbred line CLHP00478 was ranked highly in BCC rankings but performed poorly in terms of GY whilst inbred line CLHP0221 had both low β-carotene content and GY as it was ranked in the bottom five for both traits. Appendix 3.1 shows all the mean values for all the traits assessed and all the 46 inbred lines. Mean values of EPP, PH, PS, DM and SP were 2.18, 163.9 cm, 82.6.3%, 121.8 days and 49.52%, respectively. Anthesis-silking interval and DA had mean values of 2.31 days and 65.2 days, respectively (Table 3.7).

Table 3.7: Descriptive statistics for 12 morpho-agronomic traits of 50 inbred lines and β-carotene of 46 provitamin A inbred lines.

Traits	Mean	Max	Min	Median	Range	SD
BCC ($\mu g g^{-1}$)	2.04	4.24	0.60	1.91	3.63	0.96
GY (t ha^{-1})	1.7	2.70	0.70	1.85	1.93	0.45
ASI (days)	2.31	5.40	-1.92	3.12	7.06	1.38
DA (days)	65.2	74.24	60.49	66.26	13.75	3.08
DM (days)	122.1	135.12	99.38	123.90	35.73	9.22
EPP	2.17	3.50	0.86	2.14	2.64	0.66
HKW (g)	0.03	0.04	0.03	0.03	0.01	0.002
PH (cm)	178.97	264.59	127.91	169.50	136.68	36.61
PS%	74.31	101.59	61.03	83.12	40.56	7.49
RL%	22.52	28.06	17.13	22.70	6.58	2.67
SL%	9.56	12.40	6.50	9.58	5.90	1.32
SP%	50	60	38	50	21	6

BCC – β-carotene, GY – grain yield, ASI – anthesis-silking interval, DA – days to anthesis, DM – days to maturity, EPP – ears per plant, HKW – 100 kernel weight, PH – plant height, PS – plant stand, RL – root lodging, SL – stem lodging, SP shelling percentage, SD – standard deviation.

3.4.2 Genetic parameters

Estimates of genetic parameters (H^2, PCV, GCV and GAM) are presented in Table 3.8. According to the heritability categories indicated by Robinson et al. (1949), β-carotene, GY and PH exhibited high heritability values of 91.26%, 83.11% and 76.24%, respectively. Anthesis-silking interval had moderate heritability while the rest of traits had low heritability. Beta carotene, ASI and GY had the highest values of GCV of 47.02%, 32.07% and 24.27%, respectively. The traits SL and RL exhibited too high PCV values than their respective GCV

values. BCC had the highest GAM value of 92.53% whilst DA had the least GAM value of 0.01%.

Table 3.8: Estimates of' phenotypic and genotypic coefficients of variability, heritability and genetic advance as proportion of the mean.

Trait	H^2	%PCV	%GCV	GAM
β-carotene (µgg^{-1})	91.26	49.22	47.02	92.53
Grain yield (t ha^{-1})	83.11	26.63	24.27	45.58
Anthesis silking Interval (days)	49.82	45.43	32.07	46.63
Days to anthesis	0.49	0.87	0.06	0.01
Days to maturity	44.01	6.97	4.63	6.32
Ears per plant	45.05	35.49	23.82	32.94
100 Kernel weights	11.26	6.54	2.19	1.52
Plant height (cm)	76.24	20.55	17.94	32.27
Plant stand (%)	47.74	20.12	13.91	19.79
Root Lodging (%)	7.49	48.07	13.15	7.42
Stem lodging (%)	10.61	67.48	21.98	14.75
Shelling percentage (%)	35.12	12.72	7.54	9.21

H^2 (%) broad sense heritability, PCV (%) phenotypic coefficient variation, GCV (%) genotypic coefficient variation, GAM genetic advance as a percentage of mean,

3.4.3 Cluster analysis

Cluster analysis using β-carotene and eleven agro-morphological traits classified the 46 provitamin A inbred lines into three distinct major clusters labelled A, B and C (Figure 3.1). Clusters A, B and C constituted of 30.4%, 34.8% and 34.8% respectively of the total inbred lines. Cluster A was made up of CIMMYT inbred lines only, while clusters B and C had a mixture of CIMMYT and IITA inbred lines. The average phenotypic distance was 4.00 with maximum and minimum distances of 8.37 and 1.41, respectively (Table 3.9). The maximum genetic distance was between inbred line TZM114 and CLHPO331 whilst the minimum genetic distance was between inbred lines TZM1276 and TZM1223. Appendix 3.2 shows mean performances of inbred lines within their respective clusters ranked according to BCC. Clustering pattern and the mean performance indicate that clustering was mainly based on BCC, GY, ASI and DM. Cluster A had the highest mean BCC of 2.3 µg g^{-1} followed by Cluster B with 2.00 µg g^{-1} then cluster C having the lowest of 1.88 µg g^{-1}. In terms of GY, Cluster B

had the highest mean of 1.83 t ha^{-1} followed by cluster C with 1.77 t ha^{-1} and cluster A had lowest GY of 1.65 t ha^{-1}. In terms of ASI, the ascending order ranking was clusters A, B and C with 3.83, 3.23 and 2.04 days respectively. Cluster C had the earliest maturing genotypes with mean DM of 114 days followed by cluster A having 127 days and cluster B had the late maturing genotypes with mean DM of 130 days (Appendix 3.2).

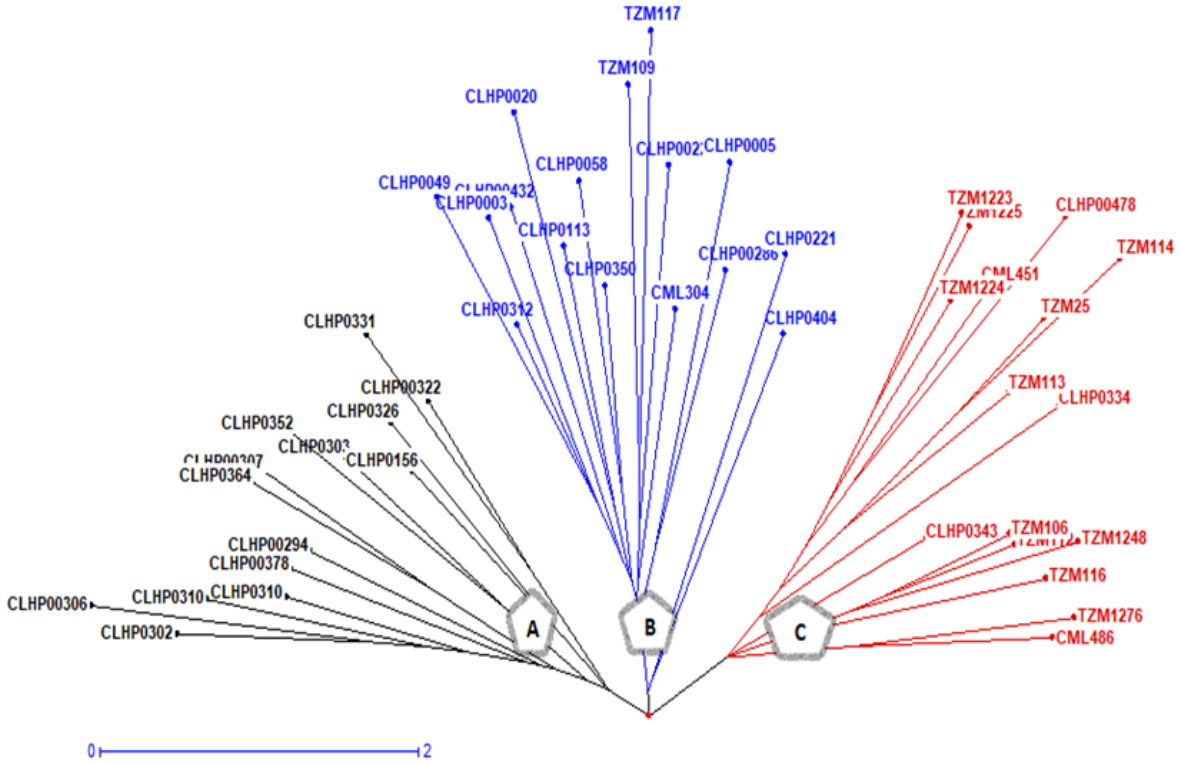

Figure 3:1: Dendrogram showing different clusters of study maize inbred lines characterised using 12 agro-morphological traits.

Table 3.9:Top and bottom ten pairs of inbred lines ranked in descending order according to genetic distances between them.

Top ten pairs			Bottom ten pairs		
Inbred Line 1	Inbred line 2	Distance	Inbred Line 1	Inbred Line 2	Distance
TZM114	CLHP0331	8.37	CLHP0049	CLHP0058	3.46
TZM113	CLHP0331	7.96	TZMI109	CLHP0020	3.16
TZM117	TZM114	7.94	TZM1248	TZM106	3.00
TZM114	CLHP00294	7.81	TZM116	CLHP0113	2.82
TZM112	CLHP0331	7.55	TZMI109	CLHP0404	2.64
TZM1224	CLHP0331	7.54	CLHP0049	CLHP0350	2.44
TZM117	TZM113	7.28	CLHP0020	CLHP0404	2.24
TZM1225	CLHP0331	7.21	CML304	CLHP0003	2.23
TZM114	CLHP0364	7.07	CML304	CLHP0005	1.73
TZM112	TZM114	7.00	TZM1276	TZM1223	1.41

3.4.4 Correlation analysis

Phenotypic pair-wise correlation analysis indicated that some of the measured traits had statistically significant relationships (Table 3.10). No significant correlation was observed between β-carotene and all agro-morphological traits. Grain yield had moderate to high and positive significant ($p \leq 0.001$) correlations with EPP (0.92**), DM (0.54**) and PS ($r = 0.73$**) and a negative significant correlation with ASI ($r = -0.73$**). Another notable positive significant correlation was between EPP and DM ($r = 0.59$**). ASI was also significantly and negatively correlated to EPP (- 0.77**).

Table 3.10: Phenotypic correlation coefficients among β-carotene content and twelve morpho-agronomic traits.

	HKW	ASI	BCC	DA	DM	EPP	PH	PS	RL	SL	SP	GY
HKW												
ASI	0.12											
BCC	0.02	0.19										
DA	- 0.21	- 0.16	- 0.29									
DM	- 0.03	- 0.42*	- 0.17	0.07								
EPP	- 0.07	- 0.77**	- 0.13	0.11	0.59**							
PH	0.15	0.16	- 0.26	0.26	- 0.14	- 0.07						
PS	- 0.08	- 0.95**	- 0.05	0.15	0.92**	0.89**	- 0.12					
RL	0.06	0.25	0.02	0.17	- 0.29	- 0.08	0.01	- 0.19				
SL	- 0.09	0.21	- 0.07	0.21	- 0.15	- 0.07	0.10	- 0.24	0.22			
SP	- 0.05	- 0.76**	- 0.09	0.16	0.72**	0.63**	- 0.20	0.74**	- 0.18	- 0.21		
GY	- 0.04	- 0.73**	0.29	0.01	0.54**	0.92**	- 0.17	0.73**	- 0.25	- 0.22	0.70**	1

HKW - 100 kernel weight, ASI - anthesis-silking interval (days) , BCC - β-carotene content (µg g^{-1}), DA - days to anthesis, DM - days to maturity, EPP - ears per plant, PH - plant height, PS - plant stand (%), RL - root lodging, SL - stem lodging percentage, SP- shelling percentage, *,** indicate level of significant of the correlation at P ≤ 0.05 and P≤0.001, respectively

3.5 Discussion

The present study revealed that there is considerable diversity among the provitamin A inbred lines sourced from CIMMYT and IITA in terms of agro-morphological performances and β-carotene profiles. High significance differences observed for β-carotene content, grain yield, number of ears per plant, plant height and plant stand indicate that the genotypes significantly vary with respect to these traits. The results are comparable with those reported by some of the previous researchers. For instance, the mean β-carotene content observed in this study (2.04 µg g^{-1}) is higher than 0.44 µg g^{-1} reported by Chander et al. (2008) for the Chinese elite provitamin A inbred lines but less than 13.6 µg g^{-1} reported by Harjes et al. (2008). The mean grain yield of 1.8 t ha^{-1} observed in this study was higher than that reported by Halilu et al. (2016) of 0.82 t ha^{-1} among the tropical provitamin A inbred lines.

The observed wide ranges for most of the traits further confirms the presence of high genetic variation, for instance, β-carotene content's range of 3.63 µg g^{-1} is greater than that reported by Menkir et al. (2014) of 1.3 µg g^{-1}. This shows that there is greater opportunity to select parental lines with diverse β-carotene profiles for the genetic improvement of β-carotene content for tropical and sub-tropical production. Similarly, the observed wider range of grain yield suggests that there is a greater scope of selecting high yielding parents for the development of high yielding provitamin A hybrids, synthetic cultivars and/or OPVs. The observed wide range in days to maturity provides an opportunity for developing cultivars with different maturity dates suitable for production in different climatic conditions in South Africa and other tropical or subtropical environments. The observed significant variation and wide range of anthesis silking interval indicates a high probability of finding many parental lines with good pollen-silking synchronisation, which makes it a requirement for a successful maize cultivar development programme.

The observed differences between the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) values for β-carotene content, grain yield, number of ears per plant and plant height was very small suggesting that these traits were less affected by the environment. This was also confirmed by low CV% values for these four traits. Halilu et al. (2016) also reported a smaller difference between PCV and GCV values for β-carotene content and other carotenoids in their genetic study with provitamin A experimental hybrids. This diversity provides opportunities for improvement of provitamin A maize with respect to these traits (Matin et al., 2017).

High to moderately high broad sense heritability (H^2) estimates, which were observed for β-carotene content, grain yield, plant height, anthesis-silking interval, days to maturity, number of ears per plant and plant stand indicates that there is great potential for selection of these traits. The observed high heritability can be explained by the fact that the materials under study are homozygous inbred lines, which means that their general performance is predominantly controlled by additive gene action. This is of great importance as it can increase selection efficiency since additive variance is transferable to the progenies (Govindaraj et al., 2010).

The high H^2 estimate for β-carotene content (91.3%) observed in this study is almost equal to 92.5% reported by Chander et al. (2008) in a related study with Chinese provitamin A inbred lines, whilst that of grain yield is comparatively similar to the one reported by Halilu et al. (2016) in their study with tropical provitamin A materials. The estimate of genetic advance as a percentage of mean (GAM) for β-carotene content exhibited herein was higher than that reported by Halilu et al. (2016). However, since the study was carried out for one season and on one site, the observed genetic values may change if implemented across multi-environments. The study was not repeated across seasons and sites since it was complimented with molecular diversity analysis given in chapter 4. Further evaluation across several environments is therefore, recommended to ascertain the stability of these materials. On the other hand, the observed very low heritability value for days to anthesis could be attributed to the highly fluctuating night temperatures during the trial period, which add on the environmental effect.

The clustering pattern was not in perfect agreement with the sources of the germplasm as they were mixture of CIMMYT and IITA inbred lines in clusters B and C whilst cluster A was made up of CIMMYT inbred lines only. This suggest the presence of gene flows between the two centres possibly through germplasm sharing (Morjan and Rieseberg, 2004).

Information about correlations between different agro-morphological traits can be utilised in making indirect simultaneous selections, which can therefore reduce the breeding work load. A positive significant correlation coefficient (r) shows the direct relationship between traits. That is, an increase in one trait can result in an increase of the other trait. On the other hand, a negative correlation coefficient value indicates inverse association between the two traits. Which means an increase in one trait is associated with a decrease in the other trait. The observed lack of significant correlation between β-carotene content and grain yield agrees

with findings by previous researchers (Menkir and Maziya-Dixon, 2004; Menkir et al., 2014). This suggest that both β-carotene content and grain yield can be improved concurrently through direct selection without compromising each other. This also qualifies the use of backcross and recurrent selection breeding approaches in breeding for combined higher yield and β-carotene (Dhliwayo et al., 2014).

The observed negative significant correlations between anthesis silking interval and grain yield and ears per plant implies that selection for short anthesis-silking interval could significantly increase these traits. The observed high coefficient of variation (CV) (>20%) for anthesis-silking interval and ears per plant can be attributed to the method used to compute these traits since they are derived traits in which the negative values lower the mean ASI value with little or no effect to the heritability value (Gasura et al., 2013). Significant moderately strong positive correlation observed between grain yield and days to maturity infers that late physiological maturity was associated with higher grain yield. This is also confirmed by having higher grain yield in cluster B which is characterised with many days to maturity (Appendix 3.2).

3.6 Conclusion

This study revealed that provitamin A inbred lines from CIMMYT and IITA exhibit high variation for β-carotene content and the studied agro-morphological traits. β-carotene content, grain yield, anthesis-silking interval and plant height had the highest H^2 and GAM estimates. Therefore, these traits should be used when selecting superior genotypes. Inbred lines CLHP00306, CML451, CLHP0350 and CLH0364 were ranked highly in both β-carotene content and grain yield rankings and therefore, are earmarked as the best inbred lines. Lack of significant correlation between grain yield and β-carotene content means these two important traits of the current study can be improved concurrently through various breeding approaches. Since both grain yield and β-carotene content are equally important in provitamin A biofortification, it is recommendable to use a selection index that give equal importance to both traits. To ascertain the findings of this study, there is need to complement this study with diversity analysis using molecular markers. Furthermore, it is important to carry out a similar study across different sites to cater for the effect of the genotype by environment interaction.

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3.8 Appendices

Appendix 3.1: Mean performance of the inbred lines with respect to β-carotene and twelve agro-morphological traits.

Entry number	GEN	BCC	GY	ASI	EPP	HKW	DA	DS	DM	PS	PH	SL	RL	SHNL
1	CLHP00306	4.22	1.6	5.14	1.13	0.031	60.5	65.6	107.4	87.52	149.4	7.76	22.73	39.62
2	CLHP0310	1.16	0.9	3.87	2.50	0.032	62.0	65.8	116.2	88.32	155.3	8.75	22.46	49.55
3	CLHP0302	2.28	0.7	4.14	2.13	0.035	60.5	64.6	117.4	86.52	133.4	8.76	24.73	41.62
4	CLHP0312	2.03	2.0	3.56	2.29	0.034	67.5	71.1	124.0	78.57	177.6	10.27	25.63	51.99
5	CLHP0334	3.43	1.6	3.56	1.29	0.031	68.0	71.6	121.5	82.57	142.1	11.77	17.13	48.99
6	CLHP00478	3.66	1.0	3.12	1.36	0.031	66.3	69.4	109.7	61.03	153.3	9.08	26.26	42.53
7	CLHP00286	2.61	2.0	4.12	2.36	0.031	66.8	70.9	128.7	89.03	146.3	7.08	19.26	56.53
8	CLHP0303	2.26	1.8	2.86	1.82	0.031	63.3	66.1	118.4	73.73	148.3	8.53	26.83	52.82
9	CLHP00307	2.69	2.2	3.33	1.88	0.032	62.6	65.9	124.8	76.16	142.5	8.00	20.56	53.35
10	CLHP00322	1.91	2.0	2.99	2.00	0.033	60.7	63.7	125.6	81.41	173.3	11.47	23.53	55.02
11	CLHP00378	4.21	2.1	4.06	1.79	0.031	62.0	66.1	120.0	87.57	150.1	10.77	24.13	51.99
12	CLHP00432	2.88	1.2	4.77	2.81	0.032	63.9	68.7	131.6	88.20	160.8	10.19	22.93	52.31
13	CLHP0331	0.78	2.0	3.12	2.36	0.035	62.8	65.9	133.2	86.03	158.8	10.08	23.26	59.53
14	CLHP0343	0.63	1.4	2.55	2.08	0.031	67.4	69.9	118.8	75.37	157.5	10.77	24.66	49.05
15	CLHP0350	2.88	2.1	3.01	2.55	0.027	66.0	69.0	122.8	84.52	150.3	7.39	25.28	51.26
16	CLHP0326	0.60	1.5	4.83	1.88	0.029	63.1	67.9	123.8	71.66	168.5	10.50	21.06	46.85
17	CLHP0310	1.59	0.8	3.14	1.13	0.031	65.0	68.1	111.4	87.02	127.9	8.26	22.23	46.62
18	CLHP0352	1.61	2.4	3.55	1.58	0.032	64.9	68.4	125.8	71.37	163.5	7.77	25.66	55.55
19	CLHP00294	1.80	1.4	3.83	1.88	0.030	62.6	66.4	126.8	86.16	161.5	6.50	28.06	46.85
20	CLHP0364	3.52	2.1	3.83	2.38	0.031	61.6	65.4	129.3	84.66	146.0	7.00	17.56	53.85
21	CLHP0404	1.95	1.4	2.50	2.68	0.034	67.4	69.9	127.9	69.49	183.7	9.68	19.67	52.25
22	CLHP0221	0.71	0.8	1.99	2.50	0.033	68.2	70.2	130.1	84.91	203.3	7.97	25.03	43.52

Appendix 3.1 continued ……

Entry number	GEN	BCC	GY	ASI	EPP	HKW	DA	DS	DM	PS	PH	SL	RL	SHNL
23	CLHP0058	2.56	2.2	4.44	2.99	0.030	67.2	71.6	131.6	84.12	146.0	8.97	24.33	54.48
24	CLHP0022	2.72	2.0	1.27	2.81	0.030	65.9	67.2	132.6	90.70	170.3	9.19	21.93	50.31
25	CLHP0113	1.66	2.1	2.49	2.50	0.030	67.2	69.7	129.1	83.91	129.8	10.97	24.03	56.52
26	CLHP0156	0.70	1.5	4.93	1.61	0.031	63.2	68.1	121.8	85.39	168.7	8.84	22.42	44.30
27	CLHP0005	0.93	1.2	3.37	3.50	0.031	66.0	69.3	130.2	93.82	179.3	9.25	18.96	56.55
28	CLHP0003	1.27	1.7	3.36	2.82	0.031	68.3	71.6	131.9	81.23	185.8	8.53	23.33	55.82
29	CLHP0049	1.63	1.7	4.86	2.82	0.031	67.3	72.1	128.9	80.73	162.3	11.03	27.83	57.32
30	CLHP0020	3.05	2.7	3.94	3.26	0.029	63.1	67.1	132.9	82.36	143.0	11.08	23.68	56.78
31	CML486	1.20	1.9	3.49	1.50	0.028	69.2	72.7	117.6	77.91	183.3	9.47	24.53	58.52
32	CML451	2.89	2.2	2.62	0.86	0.031	66.3	68.9	106.2	70.03	156.3	10.08	20.76	42.03
33	CML304	2.23	2.2	3.43	3.11	0.030	65.2	68.6	130.3	91.39	180.2	8.34	22.92	55.30
34	TZM114	0.98	1.9	1.67	1.36	0.028	65.1	66.8	103.9	89.42	220.4	9.46	18.06	40.42
35	TZM116	1.41	1.5	1.45	2.94	0.028	66.8	68.3	125.2	80.95	212.5	9.30	20.31	51.01
36	TZM25	1.42	2.1	3.67	1.36	0.029	64.1	67.8	107.4	86.42	216.4	9.46	18.06	42.42
37	TZM113	2.65	2.2	2.95	1.44	0.030	64.8	67.8	104.2	84.95	203.5	9.80	24.81	43.01
38	TZM1225	2.74	2.0	2.58	1.65	0.028	67.9	70.5	99.4	69.12	227.7	10.24	20.92	39.42
39	TZM1224	1.68	2.1	1.30	1.57	0.029	71.7	73.0	115.6	81.59	194.6	10.40	21.67	38.32
40	TZM112	2.80	2.0	-0.83	1.86	0.028	67.6	66.8	115.4	78.42	250.4	9.96	23.06	45.42
41	TZM117	0.87	2.1	2.30	3.07	0.027	74.2	76.5	135.1	101.59	254.6	10.40	23.17	53.32
42	TZM1223	2.63	1.8	2.08	1.65	0.028	70.4	72.5	109.9	72.62	181.7	10.74	24.92	49.42
43	TZM106	1.40	2.1	0.80	2.07	0.027	67.2	68.0	122.1	75.09	219.6	10.40	22.67	49.32
44	TZM109	2.53	1.4	2.30	3.07	0.030	67.7	70.0	133.1	89.59	244.6	12.40	17.67	48.32
45	TZM1248	1.65	1.8	-1.92	2.15	0.028	69.9	68.0	119.4	82.62	222.7	11.24	21.42	49.42
46	TZM1276	0.87	1.6	3.58	2.15	0.028	69.4	73.0	120.4	75.12	262.7	10.74	22.42	50.42

Entry number	GEN	BCC	GY	ASI	EPP	HKW	DA	DS	DM	PS	PH	SL	RL	SHNL
Mean	Mean	2.04	1.8	3.31	2.18	0.031	65.2	68.5	121.8	82.60	163.9	8.80	23.30	49.52
	CV%	11.8	26.0	38.37	26.39	5.970	5.4	5.2	6.9	11.90	10.7	17.00	15.50	10.33
	LSD	0.42	0.4	2.26	1.16	0.004	7.2	7.3	17.1	19.80	38.6	3.30	7.10	10.35

Appendix 3.2: Agro-morphological mean performance inbred lines ranked according to BCC within their respect clusters (clusters A, B and C).

ENTRY	GEN	BCC	GY	ASI	EPP	HKW	DA	DS	DM	PS	PH	SL	RL	SHNL
	Cluster A													
1	CLHP00306	4.22	1.58	5.14	1.13	0.03	60.49	65.64	107.36	87.52	149.41	7.76	22.73	39.62
11	CLHP00378	4.21	2.09	4.06	1.79	0.03	62.05	66.11	120.02	87.57	150.09	10.77	24.13	51.99
20	CLHP0364	3.52	2.12	3.83	2.38	0.03	61.60	65.44	129.28	84.66	146.04	7.00	17.56	53.85
9	CLHP00307	2.69	2.19	3.33	1.88	0.03	62.60	65.94	124.78	76.16	142.54	8.00	20.56	53.35
3	CLHP0302	2.28	0.74	4.14	2.13	0.04	60.49	64.64	117.36	86.52	133.41	8.76	24.73	41.62
8	CLHP0303	2.26	1.83	2.86	1.82	0.03	63.27	66.13	118.40	73.73	148.32	8.53	26.83	52.82
10	CLHP00322	1.91	2.03	2.99	2.00	0.03	60.67	63.65	125.61	81.41	173.25	11.47	23.53	55.02
19	CLHP00294	1.80	1.41	3.83	1.88	0.03	62.60	66.44	126.78	86.16	161.54	6.50	28.06	46.85
18	CLHP0352	1.61	2.36	3.55	1.58	0.03	64.88	68.43	125.82	71.37	163.46	7.77	25.66	55.55
17	CLHP0310	1.59	0.79	3.14	1.13	0.03	64.99	68.14	111.36	87.02	127.91	8.26	22.23	46.62
2	CLHP0310	1.16	0.93	3.87	2.50	0.03	61.97	65.84	116.19	88.32	155.29	8.75	22.46	49.55
13	CLHP0331	0.78	2.05	3.12	2.36	0.03	62.76	65.89	133.23	86.03	158.79	10.08	23.26	59.53
26	CLHP0156	0.70	1.50	4.93	1.61	0.03	63.15	68.09	121.81	85.39	168.66	8.84	22.42	44.30
16	CLHP0326	0.60	1.49	4.83	1.88	0.03	63.10	67.94	123.78	71.66	168.54	10.50	21.06	46.85
	Mean	2.10	1.65	3.83	1.86	0.03	62.47	66.31	121.56	82.40	153.37	8.78	23.23	49.82

Appendix 3.2 continued ….

ENTRY	GEN	BCC	GY	ASI	EPP	HKW	DA	DS	DM	PS	PH	SL	RL	SHNL
	SD	1.20	0.54	0.74	0.42	0.00	1.42	1.40	7.00	6.32	13.39	1.45	2.66	5.73
	Cluster B													
30	CLHP0020	3.05	2.67	3.94	3.26	0.03	63.14	67.07	132.89	82.36	143.00	11.08	23.68	56.78
15	CLHP0350	2.88	2.10	3.01	2.55	0.03	66.02	69.03	122.80	84.52	150.33	7.39	25.28	51.26
12	CLHP00432	2.88	1.15	4.77	2.81	0.03	63.89	68.66	131.58	88.20	160.84	10.19	22.93	52.31
24	CLHP0022	2.72	2.01	1.27	2.81	0.03	65.89	67.16	132.58	90.70	170.34	9.19	21.93	50.31
7	CLHP00286	2.61	2.04	4.12	2.36	0.03	66.76	70.89	128.73	89.03	146.29	7.08	19.26	56.53
23	CLHP0058	2.56	2.16	4.44	2.99	0.03	67.16	71.61	131.59	84.12	146.00	8.97	24.33	54.48
44	TZM109	2.53	1.43	2.30	3.07	0.03	67.74	70.04	133.12	89.59	244.59	12.40	17.67	48.32
33	CML304	2.23	2.17	3.43	3.11	0.03	65.15	68.59	130.31	91.39	180.16	8.34	22.92	55.30
4	CLHP0312	2.03	1.96	3.56	2.29	0.03	67.55	71.11	124.02	78.57	177.59	10.27	25.63	51.99
21	CLHP0404	1.95	1.38	2.50	2.68	0.03	67.37	69.86	127.90	69.49	183.72	9.68	19.67	52.25
25	CLHP0113	1.66	2.09	2.49	2.50	0.03	67.17	69.65	129.11	83.91	129.75	10.97	24.03	56.52
29	CLHP0049	1.63	1.67	4.86	2.82	0.03	67.27	72.13	128.90	80.73	162.32	11.03	27.83	57.32
28	CLHP0003	1.27	1.70	3.36	2.82	0.03	68.27	71.63	131.90	81.23	185.82	8.53	23.33	55.82
27	CLHP0005	0.93	1.20	3.37	3.50	0.03	65.97	69.34	130.19	93.82	179.29	9.25	18.96	56.55
41	TZM117	0.87	2.10	2.30	3.07	0.03	74.24	76.54	135.12	101.59	254.59	10.40	23.17	53.32
22	CLHP0221	0.71	0.83	1.99	2.50	0.03	68.17	70.15	130.11	84.91	203.25	7.97	25.03	43.52
	Mean	2.03	1.83	3.23	2.82	0.03	66.98	70.22	130.05	85.89	176.12	9.55	22.85	53.29
	SD	0.78	0.48	1.04	0.34	0.00	2.42	2.25	3.22	7.23	34.57	1.48	2.75	3.73
Cluster C														
6	CLHP00478	3.66	0.97	3.12	1.36	0.03	66.26	69.39	109.73	61.03	153.29	9.08	26.26	42.53
5	CLHP0334	3.43	1.59	3.56	1.29	0.03	68.05	71.61	121.52	82.57	142.09	11.77	17.13	48.99

Appendix 3.2 continued ….

ENTRY	GEN	BCC	GY	ASI	EPP	HKW	DA	DS	DM	PS	PH	SL	RL	SHNL
32	CML451	2.89	2.15	2.62	0.86	0.03	66.26	68.89	106.23	70.03	156.29	10.08	20.76	42.03
40	TZM112	2.80	2.00	-0.83	1.86	0.03	67.64	66.81	115.42	78.42	250.39	9.96	23.06	45.42
38	TZM1225	2.74	1.99	2.58	1.65	0.03	67.94	70.52	99.38	69.12	227.67	10.24	20.92	39.42
37	TZM113	2.65	2.18	2.95	1.44	0.03	64.84	67.78	104.18	84.95	203.47	9.80	24.81	43.01
42	TZM1223	2.63	1.77	2.08	1.65	0.03	70.44	72.52	109.88	72.62	181.67	10.74	24.92	49.42
39	TZM1224	1.68	2.08	1.30	1.57	0.03	71.74	73.04	115.62	81.59	194.59	10.40	21.67	38.32
45	TZM1248	1.65	1.84	-1.92	2.15	0.03	69.94	68.02	119.38	82.62	222.67	11.24	21.42	49.42
36	TZM25	1.42	2.08	3.67	1.36	0.03	64.14	67.81	107.42	86.42	216.39	9.46	18.06	42.42
35	TZM116	1.41	1.46	1.45	2.94	0.03	66.84	68.28	125.18	80.95	212.47	9.30	20.31	51.01
43	TZM106	1.40	2.07	0.80	2.07	0.03	67.24	68.04	122.12	75.09	219.59	10.40	22.67	49.32
31	CML486	1.20	1.87	3.49	1.50	0.03	69.17	72.65	117.61	77.91	183.25	9.47	24.53	58.52
34	TZM114	0.98	1.90	1.67	1.36	0.03	65.14	66.81	103.92	89.42	220.39	9.46	18.06	40.42
46	TZM1276	0.87	1.56	3.58	2.15	0.03	69.44	73.02	120.38	75.12	262.67	10.74	22.42	50.42
14	CLHP0343	0.63	1.42	2.55	2.08	0.03	67.38	69.93	118.82	75.37	157.46	10.77	24.66	49.05
	Mean	2.00	1.77	2.04	1.70	0.03	67.65	69.69	113.55	77.70	200.27	10.18	21.98	46.23
	SD	0.96	0.33	1.61	0.49	0.00	2.11	2.24	7.77	7.28	35.48	0.75	2.72	5.39

CHAPTER 4

Diversity Analysis of Provitamin A Maize Inbred Lines using Single Nucleotide Polymorphism Markers

Abstract

Assessment of genetic diversity among provitamin A maize germplasm is important as it can assist plant breeders to design efficient provitamin A breeding biofortification programmes. In this study, diversity analysis was carried out for 46 provitamin A inbred lines using 3046 single nucleotide polymorphism (SNP) markers. Cluster analysis detected two distinct clusters, which were largely in accordance with the sources of genotypes. The average genetic distance observed was 0.59 ranging from 0.07 to 0.68. An average of 1.615 effective alleles (Ne) per locus and mean polymorphic information content (PIC) of 0.359 ranging from 0.347 to 0.369 were detected. The average gene diversity among genotypes was 0.363 ranging from 0.351 to 0.371. For population genetic parameters, based on AMOVA most of the variation (78%) was attributed to among individual genotypes and the remaining 22% was due to among population and within individual variation. Significant population genetic diversity parameters, high gene flow (2.12), comparatively high average genetic distances and moderate genetic differentiation (0.105) between the two populations suggest that there are considerable genetic differences between provitamin A maize inbred lines that are developed by CIMMYT and IITA. The findings of this study will facilitate selections of parents and their combination in hybrid development. Inbred lines in different clusters are genetically divergent, therefore hybridisation programmes would consider crossing parents drawn from different clusters to achieve high heterosis.

4.1 Introduction

Vitamin A deficiency (VAD) is one of the serious nutritional disorders that has been on the rise in most developing countries, notably in Africa and Asia (WHO, 2009). High cases of visual impairment, increased morbidity and mortality of preschool-age children and pregnant women in Africa and Asia are some of the indicative consequences of VAD (UNDP, 2012; Stevens et al., 2015). Provitamin A maize biofortification is a sustainable solution to curb VAD among maize consumers. It complements other strategies of reducing VAD that are reviewed by Sommer and Davidson (2002). HarvestPlus and partners have been spearheading biofortification of major food crops including maize in Africa and Asia for the past 15 years (Pfeiffer and McClafferty, 2007). According to the Biofortification Priority Index (BPI) (www.harvestplus.org/knowledge-market/BPI), provitamin A maize biofortification is ideal for Africa especially southern Africa due to the wide production and consumption of maize in this region. Biofortification is the enhancement of micronutrients content in staple crops using plant breeding and/or biotechnology.

In plant breeding, the availability of adequate genetic variation is a pre-requisite for the success of any breeding programme. Yellow endosperm maize exhibit natural variation in kernel vitamin A precursors (provitamin A), which are carotenoids. These are β-carotene, α-carotene and β-cryptoxanthin (Pixley et al., 2013). Beta-carotene has more efficient provitamin A activity as compared to the other two carotenoids, which makes it the most important carotenoid (Harrison, 2015). Carotenoid variations in tropical maize was obtained from exotic temperate donor lines via backcrossing of locally adapted lines with temperate donors with high carotene content (Menkir et al., 2015) and from local yellow endosperm landraces (Hwang et al., 2016). Tapping from these sources provitamin A maize cultivars have been developed mainly by CIMMYT and IITA, which are the major suppliers of provitamin A maize germplasm in the world. The developed cultivars range from open pollinated varieties (OPVs) to three-way hybrids (Andersson et al., 2017).

Irrespective of cultivar type, development of any superior maize cultivar requires breeders to cross genetically distanced superior parental lines. Molecular diversity analysis allows breeders to identify genetically distanced lines through genetic distance estimations and cluster analysis. Phenotypic based genetic diversity analysis like the one conducted in chapter 3 allows breeders to identify the field performance of their materials. Therefore, there is need

to use both results when selecting parents. Genetic diversity analysis information also helps in the conservation, evaluation and utilisation of genetic resources (Govindaraj et al., 2015).

Several molecular markers have been successfully applied in maize genetic studies such as restriction fragment length polymorphism (RFLP) (Tuberosa et al., 1998), amplified fragment length polymorphism (AFLP) (Barbosa et al., 2003), simple sequence repeats (SSRs) (Adeyemo and Omidiji, 2013) and single nucleotide polymorphisms (SNP) (Badu-Apraku et al., 2015). Each molecular marker system has its own advantages and limitations. However, technological trends have shifted towards SNP markers because they have high locus-specificity, high genomic abundance, potential for high throughput analysis, and lower genotyping error rates (Semagn et al., 2012b).

SNPs are DNA sequence variations that occur when a single nucleotide (A, T, G or C) in the genome sequence is changed as either transitions for example G/A or C/T or transversions for example C/A, A/T or C/G (Rafalski, 2002). To date, many SNPs markers and relevant genotyping platforms have been developed in maize compared to other crops (Lu et al., 2009; Semagn et al., 2013). Thus, SNPs are now the markers of preference in carrying out a variety of genotyping tasks in maize improvement including diversity analysis, quantitative trait loci (QTL) mapping, and whole genome sequencing (Prasanna et al., 2010). Genetic distances computation, population structure, cluster and polymorphism analysis are some of the parameters that have been utilised by plant breeders in molecular genetic diversity analysis (Semagn et al., 2012a; Dao et al., 2014).

In the current study, SNPs were used to (i) assess the level and pattern of existing genetic diversity among provitamin A maize inbred lines sourced from CIMMYT and IITA and (ii) to generate the molecular bases of selecting genetically divergent parental inbred lines for further hybridisation programmes.

4.2 Materials and methods

4.2.1 Plant materials, sample collection and SNP genotyping

The study evaluated a total of 46 inbred lines for molecular genetic diversity. Description of the materials used in terms of genetic background, β-carotene content and agro-morphological performances is given in Chapter 3. The genotyping service was outsourced to LGC Genomics Ltd in London, United Kingdom (https://www.lgcgroup.com). The 46 maize inbred lines that were analysed for phenotypic diversity analysis in chapter 3 were randomly

planted in a greenhouse at the University of KwaZulu-Natal (UKZN) campus. Leaf sampling was done using supplied LGC sampling kit. Four leaf sample discs (punches) were collected from randomly selected four plants in each plot representing each genotype at three weeks after germination. The sampled leaves were placed into an LGC's 96-well plate with each well representing an individual genotype. The plates with samples were placed in plastic bag, held tightly together with silica gel sachet to reduce desiccation before they were shipped to LGC Genomics laboratory for DNA extraction and subsequent genotyping.

DNA extraction was done according to the LGC protocol (www.lgcgroup.com). Genotyping was done using the 3K array system following an Infinium HD Assay Ultra protocol described by Steemers and Gunderson (2007). The 3K array system comprises of 3046 markers that were randomly selected from the 50K array described by Ganal et al. (2011).

4.3 Data analysis

Monomorphic and SNPs with minor allele frequency of less than 2% were filtered out and from the 3046 SNPs, only 86.1% (2623) remained. The Bayesian genotypic clustering approach of STRUCTURE 2.3.4 (Pritchard et al., 2000) was used to determine the population structure. An admixture model with independent allele frequencies, without prior population information, was used to simulate the population. Each individual genotype was grouped into a given cluster using 'membership coefficient' for each cluster interpreted as a probability of membership. To assign individual genotype to a given population and for optimal alignment of genotypes, 10 replicates structure analysis were conducted. The genotype membership was determined by the computer program CLUMPP (Jakobsson and Rosenberg, 2007). The online genetic software STRUCTURE HARVESTER (Earl and von Holdt, 2012) visualized the structure analysis results.

Genotypic data were subjected to analyses of molecular variance (AMOVA) with various measures of genetic diversity within and among inferred subpopulations using GenAlex software version 6.5 (Goudet, 2001; Peakall and Smouse, 2012). Genetic diversity parameters such as total number of alleles per locus (Na), number of effective alleles per locus (Ne), observed heterozygosity (Ho), Shannon's Information Index (I), gene diversity or expected heterozygosity (He) and polymorphic information content (PIC) were determined using the protocol of Nei and Li (1979).

The genotypic data were used to obtain a dissimilarity matrix using the Jaccard index. The matrix was used to run a cluster analysis. Cluster analysis was done based on neighbor-joining algorithm using the un-weighted pair group method using arithmetic average (UPGMA) in DARwin 6.0 software (Perrier and Jacquemoud-Collet, 2006). Bootstrap analysis was performed for node construction using 10,000 bootstrap values.

4.4 Results

4.4.1 Population structure, cluster analysis and genetic distances

The population structure of the inbred lines was assessed using distance-based and model-based analyses. Using the Evanno criterion (Earl and vonHoldt, 2012), two distinct groups were identified at K = 2, which was found to be the highest level of structure with maximum value of delta K (Table 4.1). Similarly, cluster analysis based on genetic distance revealed the presence of two distinct clusters (A and B) with genotype CLHP0049 separated from the rest of genotypes (Figure 4.1). Clusters A and B constituted 46% and 52% of the genotypes, respectively. The observed clustering pattern was largely consistent with the sources of the genotypes (CIMMYT and IITA). However, all the provitamin A inbred lines that were used as checks (chapter 3) obtained from CIMMYT (CML454, CML304 and CML486) were clustered together with the IITA lines.

Table 4.1: The Evanno output showing the number of clusters that can be deduced from the given populations.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-119639.630000	19.265458	—	—	—
2	10	-109683.720000	28.085892	9955.910000	3232.210000	115.083046
3	10	-102960.020000	572.954400	6723.700000	4879.580000	8.516524
4	10	-101115.900000	4124.937457	1844.120000	26041.690000	6.313233
5	10	-125313.470000	93442.252559	-24197.570000	58218.250000	0.623040
6	10	-91292.790000	1913.296053	34020.680000	39560.440000	20.676591
7	10	-96832.550000	10507.258554	-5539.760000	12365.180000	1.176823
8	10	-114737.490000	66636.048635	-17904.940000	39446.400000	0.591968
9	10	-93196.030000	9197.937365	21541.460000	170942.060000	18.584825
10	10	-242596.630000	222025.812980	-149400.600000	—	—

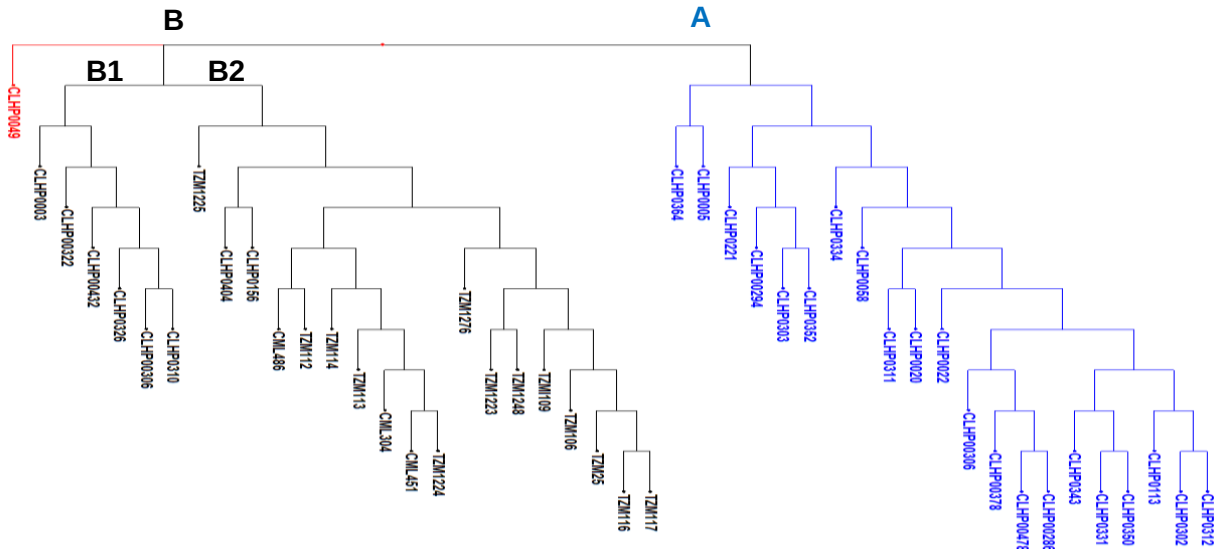

Figure 4:1: Dendrogram of 46 experimental provitamin A inbred lines obtained using 2623 SNP markers.

Genetic distances based on pairwise comparison of all the 46 genotypes ranged from 0.14 to 0.68 with an average of 0.59. The maximum genetic distance was observed between genotypes TZM116 and CLHP0343, which were from IITA and CIMMYT respectively, whilst CLHP0310 and CLHP00306 had a minimum genetic distance between themselves and were

both from CIMMYT. Table 4.2 shows top and bottom ten cross combinations with highest and lowest genetic distances.

Table 4.2: Top and bottom ten pairs of inbred lines with biggest and smallest genetic distances between them

Top ten pairs			Bottom ten pairs		
Line 1	line 2	Genetic distance	line 1	line 2	Genetic Distance
TZM116	CLHP0343	0.68	CLHP0310	CLHP00306	0.14
TZM1224	CLHP0331	0.67	CLHP0312	CLHP0302	0.20
TZM1223	CLHP0058	0.67	CLHP0350	CLHP0331	0.35
TZM112	CLHP0113	0.67	TZM116	CML486	0.36
TZM1248	CLHP00294	0.67	CLHP0326	CLHP0310	0.36
TZM25	CLHP00378	0.67	CLHP00286	CLHP00478	0.40
TZM106	CLHP0022	0.67	CLHP0113	CLHP0312	0.42
TZM117	CLHP00286	0.67	CLHP0350	CLHP0343	0.43
CLHP0343	CLHP0310	0.66	CLHP0020	CLHP0311	0.43

4.4.2 Gene diversity and polymorphism among individual genotypes

Table 4.3 presents the genetic diversity parameters measured from 46 maize inbred lines using 2623 SNPs out of a total of 3046 SNPs after removing SNPs with minor allele frequency of <0.02. The mean PIC value was 0.359 ranging from 0.347 to 0.369. The number of polymorphic SNPs per chromosome varied from 150 on chromosome ten to 383 on chromosome one, with an overall mean of 277 per chromosome. More than half of the variant SNPs (58%) were located on the first five chromosomes with chromosome one having the highest number of SNPs (383). The mean number of effective alleles (N_e) was 1.615 with the highest being 1.638 on chromosome 8. Observed heterozygosity ranged from 0.040 to 0.06 per chromosome. High mean fixation rate (F_{IS}) of 86.1% was observed. Gene diversity (H_e) ranged from 0.351 to 0.371 with a mean of 0.363.

Table 4.3: Genetic diversity within and among 46 maize genotypes based on 3046 SNPs markers.

Chromosome	No SNPs used	Polymorphic SNPs	% P	Ne	Ho	He	F_{IS}	PIC
1	462	383	82.9	1.623	0.040	0.365	0.881	0.361
2	321	277	86.3	1.630	0.048	0.371	0.866	0.367
3	349	303	86.8	1.599	0.050	0.357	0.852	0.353
4	338	295	87.3	1.607	0.043	0.360	0.878	0.356
5	281	233	82.9	1.616	0.058	0.364	0.834	0.360
6	227	202	89.0	1.637	0.047	0.372	0.866	0.367
7	245	208	84.9	1.593	0.046	0.352	0.862	0.348
8	262	236	90.1	1.638	0.060	0.374	0.829	0.369
9	241	214	88.8	1.587	0.046	0.351	0.864	0.347
10	179	150	83.8	1.621	0.049	0.365	0.853	0.361
Unknown	141	122	86.5	1.616	0.044	0.366	0.868	0.362
Overall mean	277	238	86.1	1.615	0.048	0.363	0.861	0.359
SE				0.006	0.001	0.002	0.002	0.002

%P - percentage polymorphic markers, N_e - number of effective alleles per locus, H_o - observed heterozygosity, H_e - expected heterozygosity (gene diversity), F_{IS} - inbreeding coefficient, PIC - polymorphic information content, SE - Standard error.

4.4.3 Genetic diversity among genotypes and populations

Computation of genetic diversity parameters was also done between the two populations based on the sources of the inbred lines (Table 4.4). Inbred lines that originated from CIMMYT revealed the highest variation for most of the genetic parameters. The mean observed (Na) and effective (Ne) number of alleles was higher (1.994 and 1.612) for CIMMYT and lower (1.892 and 1.452) for IITA inbred lines. The expected heterozygosity of CIMMYT lines was higher (0.033) than that of IITA lines (0.079). CIMMYT lines had expected mean gene diversity of 0.36 whilst IITA lines had a gene diversity of 0.284. Shannon information Index (I) value of the CIMMYT lines was 0.532 against that of IITA of 0.420. The mean fixation index was significantly ($p \leq 0.001$) higher for CIMMYT lines (0.902) against that of IITA lines of 0.631. CIMMYT lines had 99.39% polymorphic loci while the IITA had 89.25 polymorphic loci.

Table 4.4: Genetic diversity within and among the 46 maize genotypes classified by structure analysis.

Population	N	Na	Ne	I	Ho	He	F_{IS}	%P	P_A
CIMMYT	31	1.994	1.612	0.532	0.033	0.363	0.902	99.39	282
IITA	13	1.892	1.452	0.420	0.079	0.284	0.631	89.25	16
Overall mean	-	1.943	1.532	0.476	0.056	0.323	0.774	94.32	-
SE	-	0.003	0.004	0.003	0.001	0.002	0.004	5.07	-

N - number of genotypes tested per population, N_e - average number of effective alleles per locus per population, I - Shannon information index, H_o - observed heterozygosity per population, H_e - expected heterozygosity per population, F_{IS} - inbreeding coefficient, %P - percentage of polymorphic loci; P_A - number of private alleles, SE- Standard error.

Table 4.5 shows the analysis of molecular variance (AMOVA). Total molecular variation was partitioned into among population, among individuals and within individuals. All the three sources of variation were highly significantly different ($p \leq 0.001$). Larger genetic variability (78%) was attributed to variation among individuals and the remaining 22% variation was explained by variation among populations and within individual variation.

Table 4.5: Analysis of molecular variance (AMOVA) among 46 maize genotypes classified based on structure analysis using 3046 SNPs marker data.

Source	Df	SS	MS	Est. Var.	Per. Var.	F-Statistics
Among populations	1	3570.4	3570.4	65.2	12%	F_{ST} ($P \leq 0.001$)
Among individual	45	41011	932.07	435.5	78%	F_{IS} ($P \leq 0.001$)
Within individual	46	2809	61.065	61.1	10%	F_{IT} ($P \leq 0.001$)
Total	91	47390	-	561.8	100%	-

DF - degree of freedom, SS - sum of squares, MS - mean sum of squares, Est. var. - estimated variance, Per. Var. - Percentage variation, Pair-wise estimates of genetic differentiation (F_{ST}).

Pair-wise estimates of genetic differentiation (F_{ST}), gene flow (N_m), genetic distance (GD) and genetic identity between the two populations were 0.11, 2.12, 0.14 and 0.87, respectively.

4.5 Discussion

Genetic diversity information enables the breeders to take stock of the available genetic variation, conserve and efficiently utilise their materials in various breeding programmes. Therefore, it should be carried out from time to time to avoid sudden and unexpected genetic

drift (Govindaraj et al., 2015). In this study, SNP markers were used to assess the level and pattern of genetic variation among CIMMYT and IITA provitamin A inbred lines.

Population structure analysis revealed the presence of two main genetically distinct clusters, which are to a larger extent in agreement with the sources of germplasm. This largely suggests that the two breeding programmes use different parental materials. However, there is still signs of germplasm sharing between the two institutions as indicated by the fusion of CIMMYT and IITA genotypes in clusters B1 and B2 (Figure 3.1). This is also indicated by high gene flow value between the two populations of 2.12 (Morjan and Rieseberg, 2004).

The average pairwise genetic distance observed in this study (0.68) was much higher than that reported by Semagn et al. (2012b) of 0.35 using maize inbred lines from southern and eastern Africa. In another diversity analysis study involving 18 inbred lines using AFLP and SSR markers, Barbosa et al. (2003) reported a mean genetic distance of 0.58 and 0.64, respectively which are comparatively lower than the one observed herein.

The mean PIC value of 0.359 observed in this study is comparably lower than the one reported by Adeyemo and Omidiji (2013) of 0.43 using 122 tropical yellow endosperm maize inbred lines. The PIC value observed in this study is one of the highest among the previously reported maize diversity analysis studies (Lu et al., 2009; Dao et al., 2014; Nyombayire et al., 2016). This shows that the SNP markers used in this study are comparatively informative and discriminative (Smith et al., 2000). The mean genetic diversity realised in this study (0.363) is lower than that reported by Legesse et al. (2007) and Adeyemo and Omidiji (2013) who used SSR markers in yellow endosperm maize. However, it is comparatively higher than that reported by Dao et al. (2014) of 0.256. The observed comparably high genetic diversity implies there is greater opportunity to employ breeding approaches that require higher variation such as recurrent selection in developing OPVs and synthetic provitamin A cultivars (Dhliwayo et al., 2014).

In cross-pollinated crops such as maize, genetic purity is an important quality control criterion in breeding and seed system. This directly affects both the quality of hybrid seed and the development of new inbred lines (Semagn et al., 2012a). In this study, about 56% of the SNPs were fixed whilst the remaining 44% were considered either not fixed or likely to have been contaminated by pollen or seed of another source during maintenance (Ertiro et al., 2017). The lower genetic purity exhibited by IITA inbred lines as compared to the CIMMYT lines could be attributed to differences in sample sizes of the lines included in the present study from

these institutions which were 13 and 33, respectively. However, this could also be due to pollen contamination and seed admixture during inbred line development and maintenance (Ertiro et al., 2017).

The high among individual genotype variance could be due to efficient genetic selection systems by both institutions. The structure and cluster analysis results further confirm that the inbred lines derived from the two institutions are diverse. This was also confirmed by a significant gene diversity and Shannon information index values. The low within genotype variation can be explained by the fact that the lines are inbred lines, which are largely homozygous. The two populations were significantly different ($p \leq 0.001$) as confirmed by moderate genetic differentiation value of 0.11 and a genetic distance of 0.14 between them (Wright, 1978). However, the observed relatively low variation (12%) among population could be explained by the high gene flow of 2.1 detected in this study. High gene flow is not desirable for the preservation of genetic diversity as it can promote genetic drift (Robinson et al., 1949). This can be further explained by the continuous germplasm exchange among maize breeders at CIMMYT and IITA and/or outcrossing nature of maize, which with time can lead to genetic homogeneity of the two populations. In agreement with our results, Semagn et al. (2012a) also reported low among population variances when they characterised maize inbred lines from eastern and southern Africa using SNP markers.

4.6 Conclusion

The current study revealed that there is significant molecular genetic diversity, which is largely located among individual genotypes. The pattern of genetic diversity is largely in agreement with two sources of the inbred lines. The SNP markers used in this study were efficient in detecting genetic diversity among the 46 genotypes, therefore, are recommended for use in other diversity studies given their comparably high PIC values. Lastly, inbred lines in different clusters are genetically divergent, therefore, parental lines for hybridisation should be selected from different clusters to achieve high genetic gain. It is recommended to complement the molecular diversity findings of this study with agro-morphological based diversity analysis result in chapter 3 to get the field performance of the materials.

4.7 References

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CHAPTER 5

Screening of Provitamin A Maize Inbred Lines for Drought Tolerance using β-carotene Content, Morpho-physiological and Biochemical traits

Abstract

Provitamin A maize is important to combat vitamin A deficiency, which is prevalent in sub-Saharan Africa (SSA), where maize is the common staple food. However, adoption of provitamin A improved maize cultivars is very low in the region due to various factors, which include unavailability of drought tolerant cultivars and high cost of hybrid maize seed. Drought is a major abiotic constraint to maize (*zea mays* L.) productivity in the region. Breeding maize for drought tolerance offers a sustainable solution to the problems of drought. Screening for drought tolerance is an important step in developing drought tolerant cultivars. This study was conducted to screen provitamin A maize inbred lines for drought tolerance using an integration of morpho-physiological and biochemical traits. A total of 50 inbred lines were screened for drought tolerance in the greenhouse and field under optimum and drought conditions. Grain yield (GY), β-carotene content (BCC), anthesis-silking interval (ASI), number of ears per plant (EPP), plant height (PH), stomatal conductance (G_s), leaf senescence (SEN), chlorophyll content (CC), leaf rolling (LR), and proline content (PC) were measured. Analysis of variance, Pearson's correlation coefficient, principal component analysis and selection index (SI) were computed. The study demonstrated that the applied morpho-physiological and biochemical traits were effective in discriminating among genotypes and selecting drought tolerant provitamin A candidate inbred lines. Most of the genotypes that performed well under both optimum and drought conditions in terms of GY were ranked highly in the SI ranking. There were significant correlations ($p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$) between GY and most of the traits measured under both optimum and drought stress environments. In addition to EPP, and ASI, G_s also had higher contribution to the total variation under both optimum and drought stress conditions. Proline content significantly increased to higher levels under drought conditions in drought tolerant genotypes indicating that it can be used in maize drought stress screening. The study selected twenty highly ranked inbred lines according to SI as parents to use in the hybridisation programme.

5.1 Introduction

Provitamin A maize is a yellow/orange endosperm maize, which was recently identified as a complementary solution to vitamin A deficiency (VAD), especially for maize consuming countries in the developing world (Bouis et al., 2011). It contains carotenoids, which are precursors of vitamin A, namely β-carotene, α-carotene and β-cryptoxanthin (Fierce et al., 2008). Among the three carotenoids, β-carotene has higher provitamin A activity, which is twice than that of α-carotene and β-cryptoxanthin. Therefore, it is considered the most efficient and important carotenoid. Developing provitamin A maize cultivars can thus help to fight VAD.

However, smallholder maize production in sub-Saharan Africa (SSA) is highly vulnerable to the impacts of drought due to poor coping capacity by farmers. In the past few decades, southern Africa experienced overwhelming evidence of climate change in the form of recurrent and episodic droughts. For instance, in 1992 most of the southern African countries experienced worst drought resulting in 60% maize yield loss in the whole region (Magorokosho et al., 2003). In 2013, 770 million people in the Southern African Development Community (SADC) were reported to be at risk of food insecurity due to mid-season dry spell (DAFF, 2013). In 2016, eight of South Africa's nine provinces were declared food insecure due to drought (FAO, 2016).

Drought stress affects maize at almost all growth stages, but flowering and grain filling stages are the most susceptible, with yield losses of over 90% reported when drought coincides with these growth stages (Lu et al., 2011). Genetic improvement of maize for drought tolerance through breeding is a sustainable solution to reduce the impacts of drought. However, breeding for drought tolerance is a complex task because the trait is controlled by many genes and is highly affected by genotype by environment interaction (GxE). Plants respond to drought stress through morphological, physiological (morpho-physiological) and biochemical changes.

Many strategies have been applied in drought tolerance breeding ranging from conventional to molecular based methods (Blum, 1988; Ali et al., 2017). Irrespective of the breeding strategy, screening materials for drought tolerance remains a critical stage in drought tolerance breeding (Araus et al., 2012; Tuberosa, 2012). Screening maize for drought tolerance involves the selection of high yielding genotypes under both water stress and optimum conditions (Bänzinger et al., 2000; Derera et al., 2007).

The challenge of direct selection for grain yield under water stress is of low genetic variation, which makes selection difficult (Ge et al., 2011; Almeida et al., 2014). To circumvent this, plant breeders select for secondary traits, which include morpho-physiological and biochemical traits as proxies of grain yield. Morpho-physiological traits include leaf senescence (SEN), anthesis-silking interval (ASI), leaf rolling (LR), plant height (PH), and stomatal conductance (G_s) among others (Betrán et al., 2003). Stomatal conductance analysis as a physiological response to drought stress has not been widely applied in screening maize for drought tolerance and its level of correlation with maize grain yield under drought stress and optimum conditions is not well established. Biochemical changes, on the other hand, are induced by drought stress in plants and include increase in stress signalling hormones and key enzymes such as proline and abscisic acid (ABA) among others (Yang et al., 2010; Shakeel et al., 2011). Proline is an amino acid, which plays an osmoregulatory role in plants under drought conditions (Hong-Bo et al., 2006). Despite reports of genetic variation of proline content in plants under drought stress and wide application of proline analysis in other crops such as wheat (Vendruscolo et al., 2007) and cowpea (Zegaoui et al., 2017), among others, it has not been applied in maize drought tolerant screening studies.

Given the importance of provitamin A biofortification in maize and the prevailing devastating impacts of drought to maize productivity in the developing countries, it is important to investigate the effectiveness of integrated application of morpho-physiological and biochemical traits in screening provitamin A maize inbred lines for drought tolerance. The objective of this study was to screen and select drought tolerant provitamin A inbred lines based on their grain yield, β-carotene content, morpho-physiological and biochemical performances under drought stress and optimum conditions.

5.2 Materials and methods

5.2.1 Plant materials and study sites

The study screened 50 inbred lines for drought stress tolerance in both greenhouse and field conditions. Inbred lines included 43 yellow endosperm (provitamin A) experimental inbred lines, three provitamin A checks and four white endosperm (non-provitamin A) drought tolerant maize inbred lines as checks. The names, agro-morphological and molecular diversity of the 46 provitamin A inbred lines are given in Chapters 3 of this thesis. The four drought tolerant checks were obtained from the Agricultural Research Council (ARC) of South Africa and the

entry numbers and code names are (47) CML488, (48) CML550, (49) K64R and (50) CML569. The study was carried out across three environments (Env), which were two greenhouse and one field trials in the KwaZulu-Natal Province of South Africa. Greenhouse trials were carried out at the University of KwaZulu-Natal (UKZN), Pietermaritzburg campus from December 2016 to April 2017 (Env 1) and April to August 2017 (Env 2), whilst the field trial was carried out at Ukulinga Research Farm in Pietermaritzburg ($29^0$40'S, $30^0$24'E; 806 m above sea level) from March to August 2017 (Env 3). The average greenhouse day and night temperatures were 32^0C and 21^0C respectively for Env1 whilst for Env 2 was 28^oC and 19^0C respectively. Relative humidity ranged between 42% and 80%. Tables 5.1-5.3 show the weather data for environments 1, 2, and 3, respectively during the periods of the respective experiments.

Table 5.1: Monthly weather data at environment 1.

Month	Rs (MJ/m^2)	T_{max}	T_{min}	RH_{aver} (%)	ETO (mm)
December	19.98	27.64	16.71	75.97	126.16
January	18.46	29.59	17.18	77.50	105.96
February	16.04	26.43	16.34	79.09	98.31
March	13.98	24.83	15.55	77.80	79.98
April	12.65	22.44	12.12	68.04	72.17

Rs - average total radiation, T_{max} - average maximum temperature, T_{min} - average minimum temperature, RH_{aver} - average relative humidity, ETO - average total relative evapotranspiration.

Table 5.2: Monthly weather data at environment 2.

Month	Rs (MJ/m2)	T_{max}	T_{min}	RH_{aver}	ETO (mm)
April	12.65	22.44	12.12	68.04	102.36
May	21.36	30.59	16.71	78.50	94.98
June	12.65	21.83	17.18	66.00	63.22
July	16.04	24.31	16.34	64.22	74.86
August	20.19	23.81	15.55	70.56	89.46

Rs - average total radiation, T_{max} - average maximum temperature, T_{min} - average minimum temperature, RH_{aver} - average relative humidity, ETO - average total relative evapotranspiration.

Table 5.3: Monthly weather data at environment 3.

Month	ETO (mm)	T_{max} (°C)	T_{min} (°C)	RH_{max} (%)	RH_{min} (%)	Rainfall (mm)
April	103.91	28.55	16.42	97.64	54.71	53.0
May	120.12	23.36	17.42	97.33	56.39	21.33
June	81.36	21.36	13.76	86.95	36.88	0.0
July	78.34	19.30	12.65	80.23	53.83	0.0
August	88.31	24.15	14.11	66.31	42.78	0.0

ETO - average total relative evapotranspiration, T_{max} - average maximum temperature, T_{min} - average minimum temperature, RH_{max} - average maximum relative humidity, RH_{min} - average minimum relative humidity.

5.2.2 Experimental design and crop establishment

Two water regimes (water stress, WS and optimum conditions, WW) were applied across all the three environments. A 5 x 10 alpha lattice design with two replications for each water regime was used to lay out the trial. In the field, the plot size was two rows of 5 m with 0.75 m between the rows and intra row spacing of 0.30 m. Plots were planted with two seeds per station and thinned to one plant 3 weeks after planting. In the greenhouse, a plot was made of eight 5 L plastic pots with two plants in each pot, which was thinned to one plant per pot after 3 weeks. Pine bark growing media mixed with loam soil at a ratio of 3:1, respectively, was used in the greenhouse. In the field, the soil was predominantly clay loam soil. The WS treatment for all the experiments was implemented in accordance with CIMMYT protocols of withholding irrigation at three weeks before expected anthesis date (Bänzinger et al., 2000). The water stress condition was maintained until 5 weeks after 50% of the genotypes had flowered then a single irrigation was applied at grain filling stage. In the field the optimum treatment (control treatment, WW) involved a 7-day interval sprinkler irrigation throughout the growing period. In the greenhouse, WW involved drip irrigation for 3 min, four times per day. Weed management and other agronomic practices were done as described in Chapter 3.

5.2.3 Data collection

Eleven morpho-physiological traits were measured under both water regimes for both greenhouse and field trials. Measurement of most of the traits in this study followed the CIMMYT protocol (Bänzinger et al., 2000). Table 5.4 shows the traits measured and a brief description of how the measurements were taken. Detailed description of some the traits is also given. Chlorophyll content (CC) and stomatal conductance were measured at midday periods (1200–1400 hrs) as described in Table 5.4.

5.2.4 Proline analysis

Proline analysis was performed at UKZN, Crop Science laboratory following a protocol by Bates et al. (1973). Fresh leaf samples were collected from the second top fully expanded leaves for the WS and WW treatments of both field and greenhouse experiments at 3 weeks after imposing the WS treatment. The leaf samples were freeze-dried at very low temperature (-74^{o}C) using liquid nitrogen before grinding them into fine powder. A 0.5 g of ground leaf sample was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate was filtered. Two ml of filtrate was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid for 1 hour in a water bath at 100^{o}C.

After cooling 4 ml of toluene were added and then mixed vigorously using a test tube rotor. The top mixture containing proline within toluene was decanted from the aqueous phase then taken for UV visible spectrophotometer analysis for the absorbance of proline at a wavelength of 520 nm (Figure 5.1), using a model UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The proline concentration was determined from a standard curve and calculated using the formula shown below in equation 5.1.

PC (µg per gram of dry leaf tissue) = [(µg proline/ml) X ml toluene)/115.5 µg/µmole]/ [(g sample)/5]. (5.1)

Where PC = proline content and 115.5 is the molecular weight of proline.

Table 5.4: Morpho-physiological traits measured in this study.

Acronym	Trait	Measuring procedure
GY	Grain yield (t ha^{-1})	Weighed grain yield per plot, adjusted to 12.5% grain moisture and converted to tonnes per hectare using equation 5.2.
DA	Days to anthesis	Measured as number of days after planting when 50% of the plants shed pollen.
ASI	Anthesis-silking interval	Calculated as silking date minus anthesis date.
EPP	Ears per plant	Computed as number of ears with at least one fully developed grain divided by the number of harvested plants.
PH	Plant height (m)	Measured as height between the base of a plant to the insertion of the first tassel branch of the same plant of 6 alternating plants in the plot.
LR	Leaf rolling (%)	Scored using a 1 to 10 scale where 1 = unrolled leaf and 10 = leaf rolled like an onion. Measured twice before anthesis but after imposing drought treatment.
SEN	SEN (%)	Leaf senescence (stay green) was scored using a scale from 1 to 10 (1 = 10%; 2 = 20%; 3 = 30%; 4 = 40%; 5 = 50%; 6 = 60%; 7 = 70%; 8 = 80%; 9 = 90%; and 10 = 100 % dead leaf area) at 3, 5 and 7 weeks after 50% of the plant reached anthesis.
CC	Chlorophyll content	Measured from the adaxial surface of the second top fully expanded leaf of five plants per plot at 3, 5 and 7 weeks after 50% of the plants reached anthesis using SPAD-502-*Plus* chlorophyll meter (Konica Minolta, Osaka, Japan).
Gs	Stomatal conductance	Measured from the abaxial surface of the second top fully expanded leaf using a SC–1 leaf porometer (Decagon Devices®, Pullman, WA, USA) at 3, 5 and 7 weeks after 50% of the plants reached anthesis.
PC	Proline content	Following a laboratory method described by Bates et al. (1973).
BCC	B-carotene	Using HPLC following a protocol by Menkir et al. (2008) described in chapter 3.

$$\mathrm{GY} = \frac{\text{Field weight (kg)} * 10000(\mathrm{m}^2) * (100 - \mathrm{MOI}) * \text{Shelling\%}}{1000(\mathrm{kg}) * \text{Plot area } (\mathrm{m}^2) * (100 - 12.50)\%} \qquad (5.2)$$

Where GY = calculated grain yield per hectare, MOI = Grain moisture content measured at harvest, Shelling % = percentage of grain weight over field weight.

Figure 5:1: Proline analysis showing colour differences of reactants of leaf samples collected from a WS (left) and WW (right) genotype after adding toluene.

5.3 Data analysis

Combined analysis of variance for all morpho-physiological and biochemical traits evaluated was done in GenStat software version 18 (Payne et al., 2017). A linear mixed model presented in equation 5.3 was followed.

$$Yijklm = \mu + Ei + R(E)ij + BR(E)ijk + Wl + EWil + RW(E)ijl + BRW(E)ijkl + Gm + GEim + GWlm + GEWilm + eijklm \quad (5.3)$$

Where Y_{ijklm} = the performance of the *m* th inbred line screened in the i^{th} environment in the l^{th} water regime in j^{th} replication within the k^{th} block, μ = the overall mean; E_i = the effect of the i^{th} *environment;* $R(E)_{ij}$ = the effect of the j^{th} replication within the i^{th} environment*;* $BR(E)_{ijk}$ = the effect of the interaction of the interaction of the block and replication within an environment; W_l = the effect of the l^{th} water regime; EW_{il} = the effect of the interaction of the environment and water regime*;* $RW(E)_{ijl}$ = the effect of the interaction of the environment by water regime by replication; $BRW(E)_{ijkl}$ = the effect of the environment, water regime, replication and the

block; G_m = the effect of the genotype; GE_{im} = effect of genotype by environment interaction; GEW_{ilm} = effect of genotype by environment by water regime interaction; e_{ijklm} = experimental error. $BR(E)_{ijk}$ are the experimental error terms for the calculation of F-values for E_i, and $R(E)_{ij}$ and $BRW(E)_{ijkl}$ was the experimental error term for W_l, EW_{il} and $BRW(E)_{ijkl}$. The residual (e_{ijklm}) was used for the computation of the F-values for G_m, GE_{im} and GEW_{ilm}.

Correlation analysis was done using GenStat. A combined principal component analysis (PCA) and PCA biplot was computed using R software version 3.5.1 (R Development Core Team, 2013). Pearson's correlation coefficients (*r*) were computed to reveal relationships between measured parameters. Principal component analysis was carried out based on the correlation matrix to determine the influential traits for selection, while the PCA biplots were plotted to graphically show the relationships among the genotypes with respect to measured traits. A selection index (SI) was computed using all the measured traits the following formulas described by Bänzinger et al. (2000).

Phenotypic values (Pi) were standardized using equation 5.4:

$$Pi = (X_{ij} - M_i)/S_i \quad (5.4)$$

Where M_i and S_i are the mean and standard deviation of traits i in a population and X_{ij} is the value of the trait i measured on genotype j under drought stress conditions. The SI equation is presented below (Equation 5.5):

$$SI = b_1P_1 + b_2P_2 + \ldots b_nP_n \quad (5.5)$$

Where P_i is the observed standardized phenotypic value of the trait i and b_i is the weight given to that trait. Weights were assigned based on the economic value of the trait to the breeding programme, the heritability of the trait estimated in Chapter 3 of this study, correlation with grain yield and other traits as observed in this study.

Grain yield (GY) and BCC were given equal maximum weights because of their economic value. Grain yield was the only trait with both values from optimum and drought conditions included in the equation whilst only values obtained under drought conditions were considered for the rest of the traits. Other traits were given weights based on their respective level of correlation with GY (Table 5.5).

5.4 Results

5.4.1 Variation and mean performances under water stress and optimum conditions

Single environment analysis of variance (ANOVA) showed significant ($p \leq 0.001$, $p \leq 0.05$) effects of genotype and water regime and their interaction for most of the studied traits including GY, a combined ANOVA was carried out. Results of the combined ANOVA are shown in Table 5.6 showing the F-values and level of significance effect of genotype (Gen), water regime (WR), environment (Env), blocking factors and all their interactions for all the ten traits. Mean square values were presented for the residuals. Significant ($p \leq 0.001$) differences were observed among the main effects genotypes (Gen), water regimes (WR) and their interaction (WR.Gen) for all the ten traits. Genotype by environment interaction (Env.Gen) had a significant ($p \leq 0.001$) effect on most traits except on PC. Environment (Env) main effect had significant ($p \leq 0.001$) effect on GY, CCI and EPP only. Genotype by environment by water regime (Env.WR.Gen) interaction had significant ($p \leq 0.001$, $p \leq 0.01$) effects on all the traits except PC.

Table 5.5: Weights assigned to different traits in this study for the SI computation.

Trait	Weight	Sign	H^2(%)	Correlation coefficient with GY	
				Drought	Optimum
Grain yield (t ha^{-1})	5	+	83.11	1	1
B-carotene content (µg g^{-1})	5	+	91.26	---	0.29^{ns}
Ears per plant (count number)	3	+	45.05	0.90**	0.82**
Anthesis-silking interval (days)	2	-	49.82	- 0.58**	- 0.50**
Leaf senescence (SEN, %)	2	-	---	- 0.64**	- 0.47
Chlorophyll content index (µmol m^{-2})	2	+	---	0.60**	- 0.60**
Stomatal conductance (µmol m^{-2} s^{-1})	2	-	---	- 0.41**	0.67**
Leaf rolling (%)	1	-	---	- 0.57**	0.13^{ns}
Proline content (µg g^{-1})	1	+	---	0.58**	0.30^{ns}
Plant height (cm)	1	+	---	0.46**	0.50***
Days to anthesis	1	-	---	- 0.44**	0.13*

(+) - increasing, (-) - decreasing, H^2 - heritability, ns - not significant, *** - significant at $p \leq 0.001$.

Table 5.6: Combined analysis of variance showing F-values of the ten morpho-physiological and biochemical traits of 50 genotypes after

Sources of variation	DF	GY	PC	ASI	CCI	DA	EPP	SEN	Gs	PH	LR
Env	2	71.06***	0.49^{ns}	0.39^{ns}	27.66***	0.35^{ns}	14.76***	0.08^{ns}	2.20^{ns}	0.55^{ns}	1.72^{ns}
Env.Rep	3	0.54^{ns}	0.20^{ns}	0.06^{ns}	0.22^{ns}	0.01^{ns}	0.32^{ns}	0.44^{ns}	0.65^{ns}	0.17^{ns}	0.01^{ns}
Env.Rep.Bloc	24	40.17***	3.65***	19.05***	4.66***	10.71***	2.55***	19.39***	3.43***	4.75***	1.00^{ns}
WR	1	371.98***	818.57***	182.28***	2582.25***	23.95***	71.16***	656.66***	2560.67***	74.76***	521.36***
Env.WR	2	13.32***	0.04^{ns}	4.14*	8.84***	0.46^{ns}	0.17^{ns}	0.20^{ns}	2.45^{ns}	0.26^{ns}	2.43^{ns}
Env.WR.Rep	3	0.46^{ns}	0.47^{ns}	0.07^{ns}	0.10^{ns}	0.03^{ns}	0.22^{ns}	0.40^{ns}	0.86^{ns}	0.20^{ns}	0.01^{ns}
Env.WR.Rep.Bloc	24	17.25***	4.27***	16.85***	4.81^{ns}	18.21***	2.86***	16.94***	2.70***	8.00***	0.92^{ns}
Gen	49	234.03***	8.88***	44.11***	16.22***	18.99***	8.68***	65.41***	6.36***	22.48***	205.87***
Env.Gen	98	45.29***	1.28^{ns}	11.38***	3.40***	9.56***	2.78***	4.47***	4.15***	2.74***	30.81***
WR.Gen	49	32.16***	9.33***	36.31***	13.20***	15.83***	3.28***	61.16***	7.47***	17.42***	186.84***
Env.WR.Gen	98	10.60***	1.17^{ns}	7.56***	3.24***	10.11****	2.06***	3.52**	4.05***	2.08***	25.17***
Residual	246	0.0084	587.1	2.049	5.77	12.20	0.20	23.41	2256.	282.0	450.80

*,**,*** and ^{ns} - significant at P ≤ 0.05, p≤0.01, p≤ 0.001 and not significant respectively, Env - environment, Env.Rep - environment by replication interaction, Env.Rep.Bloc - environment by replication by bloc interaction, WR - water regime, Env.WR - environment by water regime interaction, Env.WR.Rep - Environment by water regime by replication interaction, Env.WR.Rep.Bloc - environment by water regime by replication by block interaction, Gen - genotype, Env.Gen - environment by genotype interaction, WR.Gen - water regime by genotype interaction, Env.WR.Gen – environment by water regime by genotype interaction, DF - degrees of freedom, GY - grain yield (t ha^{-1}), PC - proline content (µg g^{-1}), ASI - anthesis silking interval (days), CCI - chlorophyll content index (µmol m^{-2}), DA - days to anthesis, EPP - ears per plant, SEN - leaf senescence (%) , Gs - stomatal conductance (µmol m^{-2} s^{-1}), PH - plant height (cm), LR - leaf rolling (%). NB: shown residual values are mean square values for the respective traits.

The mean performance values, coefficients of variation (CVs), least significant difference at 5% significant levels and standard error of mean (SE) for the top 10 and bottom 5 genotypes ranked according to the selection index (SI) are presented in Table 5.7.

Appendix 5.1 shows the pooled mean values for all the traits ranked according to their SI values. Significant differences were observed among the means of all the morpho-physiological and biochemical traits used in this study. The highest SI value was 55.0 exhibited by entry 23 (CLHP0058) whilst entry 2 (CLHP0310) had the least SI value of -14.9. The best performing provitamin A check was entry 32 (CML451), which was ranked 19th with a SI value of 34.8. Thus, 39% of experimental provitamin A lines were ranked above the highest performing check.

Drought stress significantly reduced grain yield (GY) and altered morpho-physiological and biochemical performances of the genotypes as indicated by different trait values observed under drought stress and optimum conditions. The mean GY under optimum and stress conditions were 1.9 t ha^{-1} and 0.5 t ha^{-1}, respectively, resulting in a 73.7% mean yield loss. The lowest percentage yield loss due to drought stress was 4.3% exhibited by entry 6 (CLHP00478) whilst the maximum percentage yield loss was 98.6% exhibited by entry 41 (TZM25) (Appendix 5.1). Under water stress genotypes entry 9, 17 and 23 were the highest yielding with similar yields of 1.1 t ha^{-1} and they were ranked 2nd, 12th and 1st respectively using the SI ranking (Table 5.7 and Appendix 5.1). Entry 5 was the lowest yielding genotype under water stress conditions (Appendix 5.1). Entry 4 was the highest yielding under optimum conditions with 2.86 t ha^{-1} and was ranked 5th in the SI ranking. Other inbred lines that maintained higher yields under both stress and optimum conditions were entries 20, 37, 24, 19, 15 and 10 among several others (Appendix 5.1). The highest yielding provitamin A check under water stress conditions was entry number 31 (CML486) with 0.8 t ha^{-1} and it was ranked 20th in the SI ranking (Appendix 5.1).

Leaf free proline content (PC), which is the only biochemical trait under study, increased from a mean of 32.8 µg g^{-1} under optimum conditions to a mean of 149.8 µg g^{-1} under water stress conditions with genotype entry 37 (TZM113), which was ranked 10th, having the highest PC of 329.5 µg g^{-1} under water stress conditions. Drought stress resulted in early anthesis and mean days to anthesis (DA) being reduced from 76 days under optimum conditions to 69 days under water stress conditions with entries 21 (CLHP0404) and (46) TZM1276 being the earliest that reached anthesis stage in 51 days under water stress conditions. Mean anthesis-silking

interval (ASI) increased from a mean of 2 days under optimum conditions to a mean of 9 days under drought stress condition. Number of ears per plant (EPP) was reduced by drought stress from 2.3 under optimum to 1.8 under drought stress conditions. Mean leaf rolling (LR) increased from 10.6% under optimum conditions to 38.8% under water stress with entries 27 (CLHP0005) and 44 (TZM109) having the highest and lowest leaf rolling values of 90% and 10%, respectively, under drought stress conditions. Only 16% (31.8 cm) plant height reduction due to drought stress was observed with a mean of 174.7 cm observed under water stress compared to 206.5 cm under optimum conditions.

Stomatal conductance (G_s) which is one of the physiological traits was severely reduced from a mean of 362.2 mmol m^{-2} s^{-1} under optimum conditions to a mean of 49.7 mmol m^{-2} s^{-1} under drought stress conditions. Under drought stress conditions, entry 41 (TZM117), which was ranked 39th had the highest G_s with 117.9 mmol m^{-2} s^{-1} whilst entry 44 (TZM109), which was ranked 17th had the lowest G_s of 16.8 mmol m^{-2} s^{-1} (Appendix 5.1). On the contrary, under optimum conditions, entry 40 (TZM112) had the highest G_s with 581.6 mmol m^{-2} s^{-1} and it was ranked 3rd whilst entry 22 (CLHP0221), which was ranked 45th, had the lowest G_s of 107.9 mmol m^{-2} s^{-1}. Leaf senescence (SEN) increased due to drought stress from a mean of 11.8% under optimum conditions to 53.9% under water stress conditions.

Table 5.7: Mean performance of the top 15 and bottom 5 provitamin A maize inbred lines ranked according to their SI value.

Entry	GY			BCC	PC		ASI		DA		EPP		PH		SEN		LR		Gs		CCI	
Top ten																						
	WS	WW	SI	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW
23	1.1	2.5	55.0	2.6	207.2	49.0	5.5	2.0	78.5	87.3	2.8	3.0	193.8	217.5	24.6	12.5	17.5	9.4	31.7	389.7	21.7	33.6
9	1.1	2.3	51.1	2.7	190.3	39.8	11.8	0.3	77.0	86.3	2.8	2.3	175.8	212.0	23.2	10.1	27.5	9.0	57.1	581.9	25.9	32.9
40	1.0	2.1	50.5	2.8	183.5	50.4	-1.0	2.3	61.3	66.8	3.0	3.0	209.3	204.5	37.5	10.0	12.5	9.6	28.4	581.6	17.4	39.3
20	0.6	2.3	50.5	3.5	89.8	34.8	7.0	2.5	73.5	77.8	2.3	2.5	202.5	262.8	19.9	10.0	27.5	8.4	21.1	440.5	29.8	45.3
4	0.8	2.9	48.6	2.0	170.3	34.1	6.0	3.0	58.8	60.8	2.0	2.5	280.3	166.8	39.6	10.0	17.5	12.7	46.4	456.4	27.0	47.1
24	1.0	2.2	44.8	2.7	170.9	53.0	4.0	3.0	80.5	80.8	2.3	2.5	194.8	213.3	33.1	10.0	17.5	10.8	42.0	420.5	15.2	41.0
15	0.8	2.1	44.0	2.9	200.6	41.3	5.3	3.0	79.8	76.3	2.3	2.5	193.3	180.0	30.7	10.0	12.5	9.8	33.9	435.2	17.6	41.9
11	0.7	2.4	43.8	4.2	108.4	34.2	14.8	3.0	77.5	87.3	2.0	2.8	170.8	226.8	44.6	10.0	27.5	6.7	50.2	454.8	17.9	42.2
30	0.7	2.7	43.6	3.0	129.4	42.2	7.3	1.8	83.8	87.3	1.8	3.0	180.0	259.3	40.2	10.0	27.5	11.0	27.3	415.9	17.0	42.4
37	0.9	1.9	43.1	2.6	329.5	31.7	12.5	3.3	75.5	81.3	2.0	2.5	182.3	170.8	32.4	10.1	27.5	10.3	30.2	237.1	22.3	38.8
Bottom five																						
35	0.0	1.3	-1.1	1.4	97.7	28.5	14.0	1.8	76.5	80.3	1.0	1.5	159.5	180.8	75.2	12.5	77.5	13.5	72.8	110.6	15.0	33.1
26	0.1	1.3	-1.9	0.7	110.3	28.0	13.3	3.3	73.5	80.8	1.3	2.0	131.8	180.3	80.0	10.0	52.5	11.9	74.7	238.7	15.8	29.1
21	0.1	1.4	-2.3	2.0	125.8	21.8	21.0	2.5	50.8	58.8	1.0	2.0	180.0	181.0	87.5	12.4	72.5	9.8	71.6	245.4	10.5	33.2
22	0.1	0.8	-8.7	0.7	93.9	24.7	13.5	2.8	57.0	80.3	1.0	1.0	150.0	158.3	87.9	17.5	37.5	12.2	42.1	107.9	10.5	36.0
2	0.0	1.1	-14.9	1.2	113.3	23.8	13.0	3.3	77.8	81.8	1.0	1.8	143.3	181.3	80.5	15.1	77.5	8.2	64.7	307.9	12.0	36.3
Mean	0.5	1.9		2.04	150	32.8	9.2	1.7	69	76.1	1.8	2.3	175	206.5	53.9	22.2	39	10.0	49.7	362.2	18	38.6
SE	0.1	0.3		0.1	42.5	0.42	1.9	1.1	1.6	0.9	0.3	0.4	3.09	15	11.7	2.97	4.9	0.3	1.05	31.8	1.3	1.07
LSD (5%)	0.1	0.2		0.5	59.6	0.6	2.7	1.6	2.3	1.3	0.5	0.6	4.2	21.1	16.3	4.16	6.8	0.2	1.2	44.2	0.7	1.5
CV (%)	7.9	9.1		0.5	28.5	1.3	21	66	2.4	1.2	18	17	1.7	7.3	22.2	25.6	13	22.1	1.8	8.7	2.7	2.8

GY - grain yield, BCC - β-carotene content, PC - proline content, ASI - anthesis silking interval, DA - days to anthesis, EPP - number of ears per plant, PH - plant height, SEN - leaf senescence, LR - leaf rolling, Gs - stomatal conductance, CCI - chlorophyll content index, CV - coefficient of variation, LSD - least significant difference, SE - standard error. WS - Water stress conditions and WW - well watered condition.

5.4.2 Correlation analysis of morpho-physiological and biochemical traits

Table 5.8 shows Pearson's correlation coefficients (r) among grain yield, morpho-physiological and biochemical traits under study. In this study correlation with coefficients from ± 0.9 to ± 1.00 were very high correlations, ± 0.7 to ± 0.9 were high correlations, ± 0.5 to ± 0.7 were moderate correlations, ± 0.3 to ± 0.5 were low correlations and ± 0.00 to ± 0.3 were negligible correlation (Mukaka, 2012). Grain yield had highly significant ($p \leq 0.001$) positive correlations with EPP, CCI and PH under both optimum and water stress conditions (Table 5.8). Anthesis silking interval was the only trait that had a negative, highly significant ($p \leq 0.001$) correlation with GY under both optimum and waters stress conditions with correlations coefficients values of - 0.502 and - 0.694 respectively. Grain yield had highly significant ($p \leq 0.001$) correlations with LR (r = - 0.569**), SEN (r = - 0.643**) and PC (r = - 0.584**) under water stress. It also had highly significant correlations with stomatal conductance (G_s) of r = - 0.407** and r = 0.669** under stressed and optimum conditions, respectively.

Number of ears per plant (EPP) had highly significant strong correlations with GY (0.895** and 0.824** for drought stress and optimum conditions, respectively). It had moderate correlation correlations with ASI and CCI for both optimum and water stressed conditions. Stomatal conductance (G_s) had moderate correlations with EPP (- 0.647**), LR (- 0.694**) and SEN (- 0.643*) under drought conditions. Under water stress conditions, proline content had highly significant ($p \leq 0.001$) correlations with GY (r = 0.584**), CCI (r = - 0.597**), EPP (r = 0.569**), Gs (r = - 0.549**) and SEN (r = 0.660**).

Table 5.8: Pearson's correlation coefficients describing association of measured traits under WS (lower diagonal) and WW (upper diagonal) conditions.

Stressed conditions	Optimum conditions									
	ASI	CC	DA	EPP	GY	*Gs*	PH	LR	SEN	PC
ASI	1	- 0.032	0.080	- 0.537**	- 0.502**	- 0.188	0.459*	- 0.205	0.022	0.074
CCI	- 0.332*	1	0.007	0.522**	0.601**	0.450*	0.361	0.040	- 0.517	0.295*
DA	0.177	-0.061	1	0.026	0.133*	- 0.139	0.235*	- 0.38	- 0.024	0.148
EPP	- 0.578**	0.599**	- 0.538*	1	0.824**	0.559**	0.582**	0.193	0.470**	0.399*
GY	- 0.694 **	0.596**	- 0.444**	0.895**	1	0.669**	0.498**	0.132*	- 0.453*	0.301
Gs	0.458	0.573**	0.196	- 0.647**	-0.407**	1	0.539**	0.348	0.001	0.427
PH	- 0.394	0.567*	- 0.288	0.748**	0.405**	0.456**	1	0.051	- 0.339	0.442
LR	0.498**	- 0.590**	0.329	- 0.785**	- 0.569**	- 0.694**	- 0.482*	1	0.236*	0.417
SEN	0.214	- 0.831**	0.406	- 0.749**	-0.643**	- 0.643*	- 0.517*	0.725**	1	0.462
PC	0.447*	- 0.597**	0.017	0.569**	0.584**	- 0.549**	0.348	0.043	0.660**	1

ASI - anthesis silking interval, CCI - chlorophyll content index, DA - days to anthesis, EPP - ears per plant, GY - grain yield, G_s - stomatal conductance, PH - plant height, LR - leaf rolling, PC - proline content, SEN - leaf senescence, *,** indicate level of significant of the correlation at $p < 0.05$ and $p < 0.01$ respectively.

5.4.3 Principal component analysis

The proportion of total variation explained by principal components and their correlations with the morpho-physiological traits are shown by the correlation biplot (Figure 5.2). The first two principal components (Dim1 and Dim2) with eigen values equal or greater than one were selected to explain the cumulative variation of 73.9% for combined water stress and optimum conditions. The 55.8% variation explained by Dim1 was mainly due to the contrast effects of GY, CCI, SEN, LR, ASI, Gs, PH and EPP as shown by their higher correlation to Dim1. Dim2 was largely correlated to DS, DA and PC.

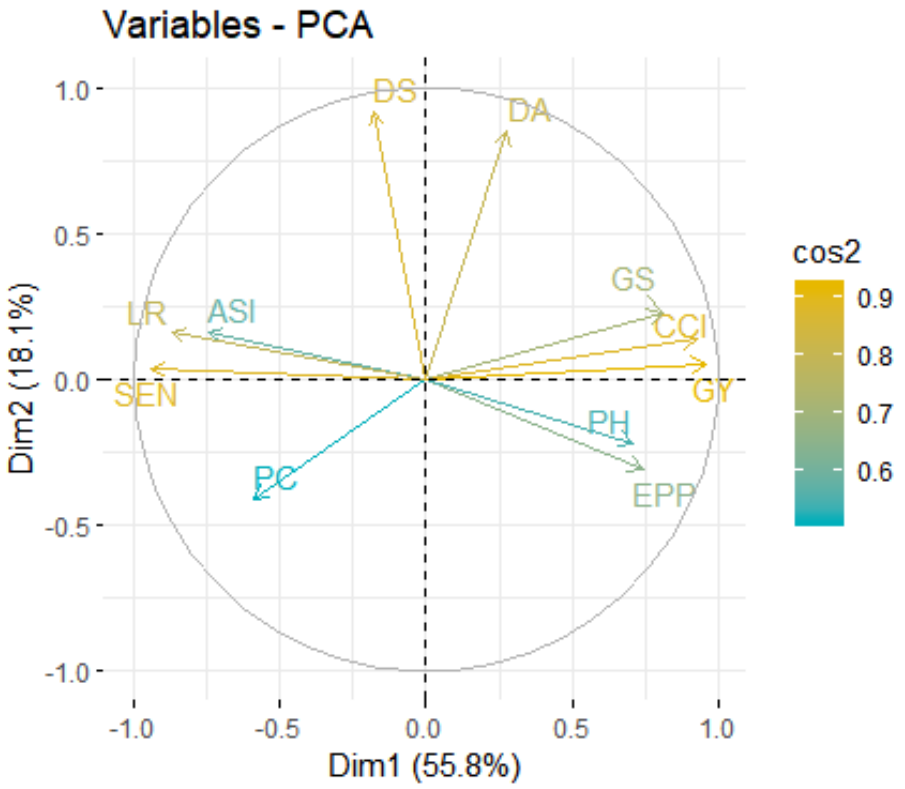

Figure 5.2: Principal component correlation biplot showing the correlation between the first two components and traits. EPP - ears per plant, ASI - anthesis silking interval, CCI - chlorophyll content index, DA - days to anthesis, GY - grain yield in tons per ha, G_s - stomatal conductance, PH - plant height, LR - leaf rolling percentage, PC - proline content

5.4.4 Principal component biplot analysis

The relationship between different genotypes and traits was illustrated visually using a combined principal components biplot (Figure 5.3). All the entries screened under water stressed conditions (those prefixed with SE) were located on the negative side of the x-axis of the biplot whilst majority of entries under optimum conditions (those prefixed with W) were on the positive side of the biplot. Genotypes clustered close to a trait or a group of trait were largely discriminated by the respective trait(s). Grain yield, CCI, GS, PH, EPP and DA were more discriminating under optimum conditions whilst LR, ASI, SEN, PC and DS were more discriminating under water stress conditions.

For example, entry SE37 (TZM113) exceptionally excelled in proline content (PC). Genotypes WE13 (CLHP0331) and WE14 (CLHP0343) were further on the Gs vector. Genotypes that were located far on the senescence vector were entry SE5 (CLHP0334), SE7 (CLHP00286) and SE8 (CLHP0303).

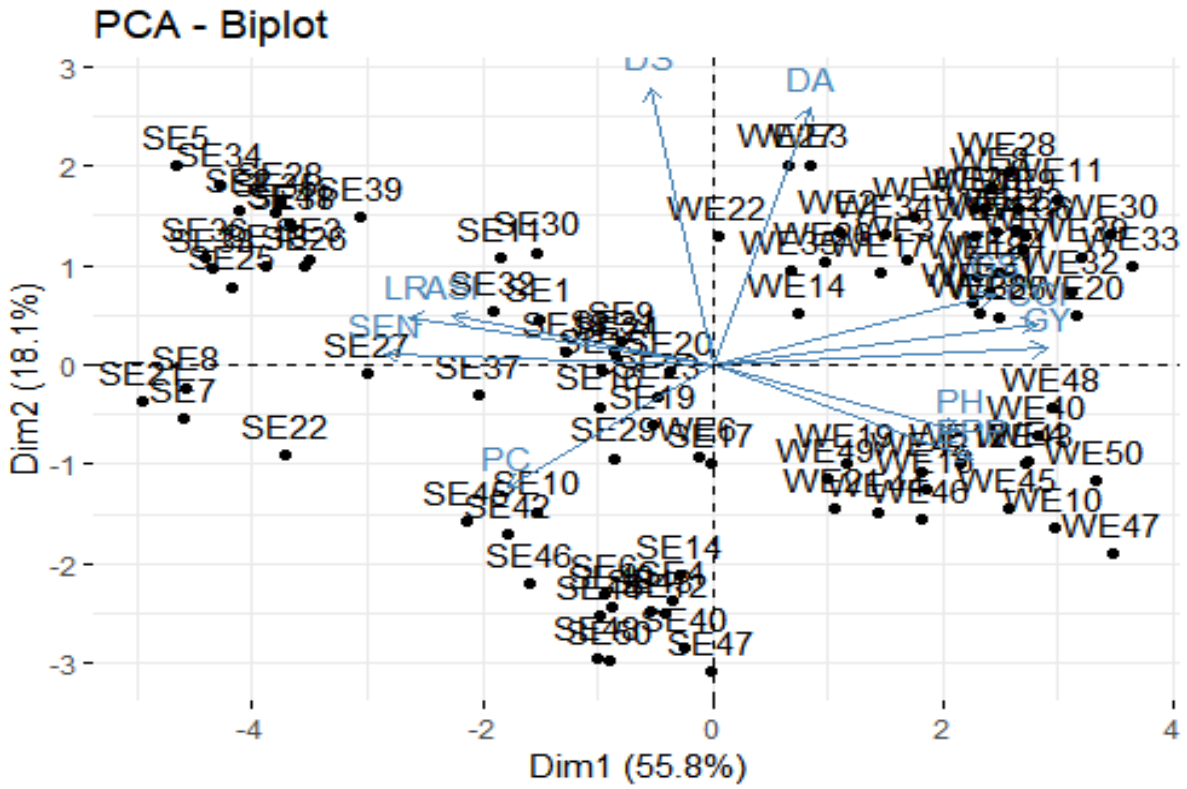

Figure 5:3: Principal component biplot showing entry clustering under WS and WW conditions. ASI - anthesis silking interval, CCI - chlorophyll content index, DA - days to anthesis, DS – days to silking. EPP - ears per plant, GY - grain yield, Gs - stomatal conductance, PH - plant height, LR - leaf rolling percentage, PC - proline content, SE - entries under water stress conditions, WE - entries grown under optimum conditions.

5.5 Discussion

Analysis of variance revealed that genotypes responded differently with respect to all the measured morpho-physiological and biochemical traits. This further confirms the findings of chapters 3 and 4 that the genotypes understudy are significantly diverse. The observed significance of water regime and its interaction with the genotype effect indicate that water regime was effective in discriminating among the genotypes. Therefore, genotypes that performs well under water stressed conditions should be considered as drought tolerant whilst genotypes that maintain higher performances across both water stressed and optimum conditions should be considered as stable genotypes. However, the significance of genotype by environment interaction effect on most of the traits shows the need of carrying genotype by environment interaction analysis to ascertain the stability of the genotypes. The observation that proline content was not significantly affected by the environment and its interaction with genotype effect but significantly affected by water regime and its interaction by genotype effect suggests that the use of proline content analysis for drought tolerance screening can be done in an environment provided the water regime procedure is followed.

The observation that 39.1% of the experimental provitamin A inbred lines under study performed better than the best provitamin A check (entry 32; CML451) in terms of the SI ranking, indicate a great opportunity of developing drought tolerant provitamin A maize cultivars. The use of a SI that uses multiple traits ensures that both yield and drought tolerance are improved simultaneously (Bänzinger et al., 2000) resulting in maximum advance for selection. Most genotypes that were highly ranked in the SI ranking maintained higher GY under both drought stress and optimum conditions, agreeing with Foulkes et al. (2007) that drought tolerant genotypes should yield highly under both conditions.

In the current study, the observed grain yield (GY) reduction due to drought stress can be largely associated with an integrated effect of changes in morpho-physiological processes namely number of ears per plant (EPP), anthesis-silking interval (ASI), plant height (PH), stomatal conductance (*Gs*), chlorophyll content (CC), enhanced leaf senescence (SEN) and leaf rolling (LR). Number of ears per plant and ASI are by far the most applied traits in maize drought tolerance studies (Bolaños and Edmeades, 1996; Bänzinger et al., 2000; Magorokosho et al., 2003). Under drought stress, EPP was the largest contributor to the total genetic variation as shown by the highest PCA loading scores. The observed high positive correlation between GY and EPP can be attributed to the fact that, at reproductive stage,

drought stress induces kernel abortion and poor grain filling which then resulted in reduced number of ears with fully developed kernels. T ahus, selecting for EPP can boost yield under both conditions. Contrary to the study by Monneveux et al. (2008), which did not find any significant association between GY and ASI, in this study a highly significant negative correlation was observed between these two traits. Thus, this study confirms and validates the findings by Cairns et al. (2012) that ASI is still an important trait to be used for selection in maize drought stress breeding. This is because one of the physiological effects of drought stress is to cause poor partitioning of assimilates to the developing ears, silks and tassels, which in turn causes stunted ear growth, increased kernel abortion and poor flower synchronisation (Araus et al., 2012). Hence, the observed longer ASI interval under drought stress than under optimum conditions. The yield loss observed in this study due to drought stress was lower than the 81% reported by Cairns et al. (2012) who attributed greater variation to kernel number whilst the observed levels of correlations between GY and ASI, and EPP were comparatively similar to the findings by Betrán et al. (2003).

The observed positive correlation between GY and PH under both stress and optimum conditions infers that selection for taller plants can help to achieve higher yields. This disagrees with findings by Bolaños and Edmeades (1996) who reported an increase in drought tolerance with decrease in PH. Findings of this study can, therefore, be explained from the standpoint that PH is one of the “sinks”, a product of dry matter accumulation and is a key indicator of growth rate in maize (Lee and Tollenaar, 2007). Thus, the plant channels photosynthetic assimilates to PH effecting growth, which is higher before flowering especially in determinate maize cultivars. However, in this study, a reduction in PH due to drought stress was very small as compared to the observation by Betrán et al. (2003) who reported a reduction of 257 cm. The observed smaller reduction in PH could be attributed to the high-early growth rate that resulted in plants reaching their full height potential before the setting in of the effects of the imposed drought stress three weeks before 50% anthesis. This explanation is further suggested by the observed weak correlations between GY and PH under both conditions. Alternatively, the observed small reduction in PH due to drought stress could be due to the effective selection for taller PH that the materials under study went through during their developmental processes.

Stomatal conductance (G_s) also largely contributed to the total observed variation, especially under optimum conditions and the observed high correlation with GY and other traits such as EPP, SEN, PH and LR. The observed huge decrease in G_s due to drought stress supports the

suggestion by Grzesiak et al. (2006), that G_s is the major physiological trait that discriminates between drought tolerant and susceptible maize and wheat genotypes. Thus, drought tolerant genotypes are more efficient in conserving tissue water status via decreased G_s, which in turn reduces transpiration rate and water loss in contrast to the susceptible genotypes.

The observed moderately weak but significant negative correlation between GY and G_s under drought stress could be attributed to the longer period of drought stress exposure, which ended up reducing photosynthesis, probably by limiting gaseous exchange in addition to water limitation, since both factors are essential for photosynthesis (Cabrera-Bosquet et al., 2009). This notion is also suggested by the observed high correlations between G_s and other photosynthesis related traits such SEN and CC. Thus, under drought conditions, reduced G_s, CC and enhanced SEN, cooperatively resulted in reduced photosynthetic capacity by limiting gaseous exchange and the ability to intercept photosynthetic active radiation (PAR). This and other above discussed factors, contributed to the observed GY reduction under drought conditions. In this view, the findings of our study can be related to the findings by Jiang et al. (2006) who reported a strong correlation between G_s and leaf CO_2 concentration, photosynthesis light interception, and net photosynthesis in barley under saline stress.

The observed moderate positive correlation (0.569**) between G_s and PH under optimum conditions further indicates the importance of G_s in differentiating genotypes' responses to drought stress. Since PH represents the above ground dry matter in maize, these findings agree with the reported correlation between G_s and above ground dry matter in barley (Jiang et al., 2006). However, to substantiate this notion in maize, it is recommended that further investigations to quantify the effect of terminal drought stress on gaseous exchange and photosynthesis parameters in maize be conducted. Finally, the observed significant positive correlation between LR and G_s, CC, and SEN under drought stress condition suggests that LR participates in controlling water loss and use by reducing leaf area available for photosynthesis (Vadez, 2014).

Proline content (PC), which was the only biochemical trait investigated in this study, has been previously applied in different abiotic stress studies of different types crops, which are, salinity in wheat (Jain et al., 2013), drought stress in wheat (*Triticum aestivum* L.) (Mwadzingeni et al., 2016), drought stress in cowpea (*Vigna unguiculata* L.) (Zegaoui et al., 2017), and German chamomile (*Matricaria chamomilla* L.) (Salehi et al., 2016). However, according to our literature search, there are no reported cases of where proline content analysis was used in

screening maize genotypes for drought tolerance. This could be attributed to intensive and time-consuming laboratory analysis, which makes PC analysis less applicable to large-scale field-based drought screening programmes. In other crops, in which proline content analysis was applied, significant differences among genotypes and increase in free PC has been reported after exposure to drought (Chorfi and Taïbi, 2011; Qayyum et al., 2013). Moayedi et al. (2011) reported an increase in free PC of 47% and 114% after exposing wheat genotypes to reproductive and grain filling drought stresses, respectively. In another study, Sánchez et al. (1998) reported a range from 4 to 40% of free PC increase after exposing cowpea genotypes to drought stress.

Sizes of angles between dimension vectors and the direction of vectors of a principal component biplot indicate the level of correlation among the traits in discriminating genotypes. Thus, smaller angles ($< 90^0$) between dimension vectors in the same direction mean high correlation of the involved traits in discriminating genotypes whilst larger angles ($> 90^0$) imply weak correlation between the traits in distinguishing genotypes. Genotypes performing well with respect to a particular trait are located closer to the vector representing that trait, and further in the direction of the vector, ideally on the vertices of the convex hull. Genotypes located at or close to the center where vector lines meet indicate that the respective genotypes perform averagely in all traits. The observation that entry 37 had high proline content under water stress and is highly ranked in SI ranking confirms that high proline content is correlated to high yield. The fact that genotypes 13 and 41 had higher stomatal conductance (Gs) under drought conditions but they were lowly ranked in the SI ranking means that genotypes that exhibited high stomatal conductance under drought stress are drought susceptible.

The observed moderate correlations between GY and free PC under stress coupled with considerably high principal component (PC-2) loadings suggest that genotypes that yielded high free PC can be selected as drought tolerant. The observed significant correlation between free PC and other morpho-physiological traits, further suggest that increase in free PC is indeed a proxy of drought tolerance in maize. This is further supported by the observation that majority of genotypes that had high free PC were ranked highly in the SI ranking, for instance entry 37 had the highest PC and it was ranked 10^{th} in the SI ranking (Appendix 5.1). The results, therefore, support the claim that under drought conditions proline is released to effect plant cell osmotic adjustments which helps to conserve cell turgor (Hong-Bo et al., 2006; Changhai et al., 2010; Marcińska et al., 2013).

5.6 Conclusion

This study showed that the morpho-physiological and biochemical traits applied in the screening of provitamin A inbred lines were effective in discriminating among genotypes for drought tolerance. The observed high genotypic variation with respect to grain yield, morpho-physiological and biochemical traits under study, confirmed the findings of the phenotypic and molecular diversity studies in Chapters 3 and 4, respectively, that there is considerably high genetic diversity among the provitamin A materials used. The study also revealed that G_s and free PC are traits that are capable of discriminating genotypes according to their response to drought stress. However, the application of free PC analysis in maize drought screening needs further investigation across more than one field sites and exploring fast and easy ways of detecting free PC in plant samples.

The highly ranked genotypes in the SI ranking (Table 5.8) are drought tolerant provitamin A maize inbred lines from which parents for the hybridization programme were selected. Additionally, the highly ranked genotypes can be used as drought tolerance donors in breeding programmes in South Africa and other countries with similar agro-ecological environments.

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5.8 Appendices

Appendix 5.1: Means of morpho-physiological and biochemical traits, β-carotene content and stress index (SI) of 46 provitamin A maize inbred lines when screened under WS and WW conditions.

Entry	GY			BCC	PC		ASI		DA		EPP		PH		SEN		LR		Gs		CCI	
	WS	WW	SI	BCC	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW
23	1.1	2.5	55.0	2.6	207.2	49.0	5.5	2.0	78.5	87.3	2.8	3.0	193.8	217.5	24.6	12.5	17.5	9.419	31.7	389.7	21.7	33.6
9	1.1	2.3	51.1	2.7	190.3	39.8	11.8	0.3	77.0	86.3	2.8	2.3	175.8	212.0	23.2	10.1	27.5	9.011	57.1	581.9	25.9	32.9
40	1.0	2.1	50.5	2.8	183.5	50.4	-1.0	2.3	61.3	66.8	3.0	3.0	209.3	204.5	37.5	10.0	12.5	9.625	28.4	581.6	17.4	39.3
20	0.6	2.3	50.5	3.5	89.8	34.8	7.0	2.5	73.5	77.8	2.3	2.5	202.5	262.8	19.9	10.0	27.5	8.371	21.1	440.5	29.8	45.3
4	0.8	2.9	48.6	2.0	170.3	34.1	6.0	3.0	58.8	60.8	2.0	2.5	280.3	166.8	39.6	10.0	17.5	12.65	46.4	456.4	27.0	47.1
24	1.0	2.2	44.8	2.7	170.9	53.0	4.0	3.0	80.5	80.8	2.3	2.5	194.8	213.3	33.1	10.0	17.5	10.8	42.0	420.5	15.2	41.0
15	0.8	2.1	44.0	2.9	200.6	41.3	5.3	3.0	79.8	76.3	2.3	2.5	193.3	180.0	30.7	10.0	12.5	9.815	33.9	435.2	17.6	41.9
11	0.7	2.4	43.8	4.2	108.4	34.2	14.8	3.0	77.5	87.3	2.0	2.8	170.8	226.8	44.6	10.0	27.5	6.749	50.2	454.8	17.9	42.2
30	0.7	2.7	43.6	3.0	129.4	42.2	7.3	1.8	83.8	87.3	1.8	3.0	180.0	259.3	40.2	10.0	27.5	11.05	27.3	415.9	17.0	42.4
37	0.9	1.9	43.1	2.6	329.5	31.7	12.5	3.3	75.5	81.3	2.0	2.5	182.3	170.8	32.4	10.1	27.5	10.29	30.2	237.1	22.3	38.8
43	0.8	2.2	41.0	1.4	166.1	34.1	0.3	2.5	63.0	64.5	3.0	3.0	210.5	201.5	47.5	12.5	17.5	13.18	20.1	382.2	18.0	46.2
17	1.1	1.4	40.1	1.6	190.3	20.0	2.8	2.0	74.5	78.8	3.0	1.8	207.5	186.5	25.0	10.1	17.5	12.99	49.0	300.7	23.5	37.4
6	0.9	0.8	40.0	3.7	200.7	19.1	4.5	1.0	58.3	60.0	2.0	1.0	190.0	128.5	27.5	20.0	27.5	12.9	26.0	262.5	28.1	29.8
12	0.9	1.5	38.6	2.9	171.5	25.8	4.3	-0.3	59.0	62.8	2.0	2.0	268.8	198.8	32.1	12.6	17.5	12.11	46.7	444.3	20.3	45.1
18	0.8	2.3	36.1	1.6	184.7	39.4	6.3	2.8	79.3	79.8	2.3	2.5	178.5	203.0	49.8	15.0	12.5	9.458	55.0	461.3	18.4	34.4
10	0.8	2.0	35.9	1.9	149.9	22.4	7.8	-0.3	61.5	61.3	2.0	2.8	181.5	248.5	32.5	10.1	37.5	7.341	20.9	428.1	19.5	40.7
44	0.8	1.6	35.2	2.5	163.2	20.2	6.0	1.8	57.0	60.3	2.0	2.0	241.3	212.3	50.0	10.0	10.0	7.362	16.8	138.4	18.8	38.5
19	0.9	1.5	34.9	1.8	177.0	26.7	4.3	3.5	77.0	60.8	2.8	2.0	227.5	170.3	40.0	15.0	17.5	9.825	23.7	381.0	18.4	32.5
32	0.6	2.4	34.8	2.9	159.4	37.2	7.5	0.3	78.3	83.3	1.3	3.0	164.5	250.0	42.5	9.9	10.0	7.076	54.9	385.6	14.0	38.6
31	0.8	2.0	34.5	2.2	185.0	32.3	3.5	2.8	80.3	79.0	2.0	2.5	181.5	219.5	32.9	10.0	12.5	10.04	45.1	387.7	23.2	39.7

Appendix 5.1 continue….

Entry	GY			BCC	PC		ASI		DA		EPP		PH		SEN		LR		Gs		CCI	
	WS	WW	SI	BCC	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW
29	0.6	2.0	33.9	1.6	125.7	29.8	-6.5	1.8	73.5	87.3	2.0	2.5	168.3	209.3	35.4	10.0	47.5	7.728	57.1	362.2	22.1	35.8
42	0.7	1.6	30.5	2.6	144.4	37.5	6.3	-1.0	60.8	64.0	2.0	2.0	164.8	202.0	52.7	12.5	17.5	11.05	21.2	431.1	14.4	33.4
45	0.7	1.8	27.2	1.6	192.5	32.5	7.5	2.8	60.5	59.8	1.5	3.0	157.3	200.5	42.4	10.0	27.5	8.784	44.0	496.4	17.2	41.7
46	0.7	1.7	26.8	0.9	206.1	35.9	7.0	0.8	58.8	60.3	2.3	2.5	166.0	188.0	42.3	12.5	17.5	12.07	37.6	332.2	18.4	36.4
16	0.8	1.6	26.3	0.6	192.1	24.1	3.8	0.0	76.0	61.3	2.0	2.3	190.8	171.5	34.5	10.0	12.5	10.73	34.4	404.7	20.2	37.3
14	0.9	1.3	25.4	0.6	132.3	27.1	5.8	3.0	59.8	76.3	2.3	1.5	233.5	206.0	32.4	17.5	10.0	6.69	56.4	124.1	22.3	33.5
1	0.9	1.9	25.2	4.2	200.0	26.3	13.5	2.8	76.5	83.8	2.0	2.3	170.0	261.5	32.5	10.0	17.5	8.905	24.9	315.4	24.9	45.2
39	0.1	2.6	17.0	1.7	80.1	47.5	12.3	1.8	77.5	86.3	1.0	2.8	156.8	271.0	62.3	10.0	52.5	9.442	69.6	236.2	16.3	47.3
33	0.1	2.7	14.6	2.2	101.3	27.8	22.3	2.3	66.3	82.8	1.0	3.0	142.0	259.0	85.0	9.9	62.5	9.118	75.7	481.5	13.7	44.5
25	0.1	2.3	9.1	1.7	104.4	55.1	14.8	2.5	70.5	77.8	1.0	2.5	161.8	218.8	87.5	10.0	72.5	12.36	44.8	372.1	9.4	41.0
5	0.0	1.6	8.6	3.4	97.6	25.3	20.3	3.8	75.0	83.0	1.0	2.0	113.8	200.8	80.0	10.1	82.5	6.314	75.7	366.0	12.5	38.0
38	0.0	1.7	8.4	2.7	88.7	27.7	12.0	2.8	76.5	79.8	1.0	3.0	150.5	233.3	82.4	12.5	62.5	10.3	93.4	328.8	13.4	35.5
7	0.0	1.7	7.9	2.6	88.4	38.8	21.3	0.0	50.8	83.5	1.0	2.0	104.0	202.5	72.0	10.1	87.5	13.93	90.5	447.8	17.6	42.2
3	0.1	1.1	7.0	2.3	153.8	29.7	10.0	2.8	77.5	86.5	1.0	1.0	151.5	209.5	73.0	20.0	62.5	11.93	51.9	355.9	17.8	33.0
28	0.1	1.8	6.3	1.3	113.3	28.8	11.5	-1.8	79.0	90.8	1.3	2.5	113.3	188.5	70.1	12.6	77.5	12.77	67.1	412.5	16.3	45.4
8	0.1	1.7	6.2	2.3	90.5	22.2	20.3	-1.3	55.3	88.3	1.0	2.3	120.8	193.5	80.5	12.4	82.5	7.449	69.7	512.4	14.7	38.4
13	0.1	2.1	6.0	0.8	128.4	29.8	12.8	1.5	75.5	86.8	1.8	2.0	129.5	241.0	87.5	10.1	72.5	8.157	75.5	325.4	11.3	40.1
36	0.0	2.1	5.2	1.4	89.6	32.6	24.3	2.8	65.3	85.8	1.0	2.5	159.5	258.8	69.9	10.0	87.5	13.71	63.4	307.1	15.2	41.9
41	0.1	2.1	4.6	0.9	129.2	48.0	14.5	2.5	75.5	85.5	1.0	2.5	100.5	229.5	74.6	10.1	37.5	9.057	117.9	383.4	15.5	38.2
27	0.0	1.2	-0.1	0.9	105.5	24.1	-10.3	-1.3	81.3	91.3	1.3	2.0	117.5	134.0	87.5	15.0	90.0	6.087	70.2	143.0	9.6	30.0
34	0.1	1.7	-0.1	1.0	118.6	26.7	15.5	2.3	77.8	84.3	1.0	2.3	129.8	174.3	77.5	10.1	82.5	7.669	74.5	155.5	12.5	39.1
35	0.0	1.3	-1.1	1.4	97.7	28.5	14.0	1.8	76.5	80.3	1.0	1.5	159.5	180.8	75.2	12.5	77.5	13.45	72.8	110.6	15.0	33.1
26	0.1	1.3	-1.9	0.7	110.3	28.0	13.3	3.3	73.5	80.8	1.3	2.0	131.8	180.3	80.0	10.0	52.5	11.86	74.7	238.7	15.8	29.1
21	0.1	1.4	-2.3	2.0	125.8	21.8	21.0	2.5	50.8	58.8	1.0	2.0	180.0	181.0	87.5	12.4	72.5	9.797	71.6	245.4	10.5	33.2

Appendix 5.1 continue….

Entry	GY			BCC	PC		ASI		DA		EPP		PH		SEN		LR		Gs		CCI	
	WS	WW	SI	BCC	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW
22	0.1	0.8	-8.7	0.7	93.9	24.7	13.5	2.8	57.0	80.3	1.0	1.0	150.0	158.3	87.9	17.5	37.5	12.19	42.1	107.9	10.5	36.0
2	0.0	1.1	-14.9	1.2	113.3	23.8	13.0	3.3	77.8	81.8	1.0	1.8	143.3	181.3	80.5	15.1	77.5	8.181	64.7	307.9	12.0	36.3
Mean	0.5	1.9		2.1	146.8	32.4	9.4	1.8	70.4	77.1	1.7	2.3	171.7	205.8	53.9	11.8	40.8	10.0	51.5	354.1	17.7	38.5
Max	1.1	2.9		4.2	329.5	55.1	24.3	3.8	83.8	91.3	3.0	3.0	280.3	271.0	87.9	20.0	90.0	13.9	117.9	581.9	29.8	47.3
Min	0.0	0.8		0.6	80.1	19.1	-10.3	-1.8	50.8	58.8	1.0	1.0	100.5	128.5	19.9	9.9	10.0	6.1	16.8	107.9	9.4	29.1

CHAPTER 6

Combining Ability Analysis and gene action of Provitamin A Maize Inbred Lines Under Water Stress and Non-Stress Environments

Abstract

Developing agronomically competitive maize cultivars that are biofortified with high concentrations of vitamin A precursors is a key approach towards alleviating vitamin A deficiency (VAD) in sub-Saharan Africa (SSA). These cultivars need to possess other important traits such as high grain yield, biotic and abiotic stress resistance, if they are to be adopted by farmers. Therefore, understanding the heritability and gene action controlling provitamin A under drought stress would be valuable towards development of provitamin A drought tolerant cultivars. This study was thus conducted to determine the combining ability effects and heritability of sixteen provitamin A inbred lines for grain yield and selected secondary traits under optimum and drought stressed environments. Sixty-four single cross hybrids were generated from an 8 x 8 North Carolina design II. These were evaluated across four optimum and two managed drought environments in South Africa and Zimbabwe during the summer and winter periods of 2017/2018. General combining ability effects attributable to males and females (GCA_m and GCA_f) and specific combining ability (SCA) were highly significant ($p \leq 0.001$) for all the traits measured, across both environmental conditions, suggesting the influence of both additive and non-additive gene action. The SCA contributed more than the GCA towards the variation in hybrids for most traits across both environments. In addition, dominance variance was greater than additive variance for most of the traits. Non-additive gene action was predominant for GY, ASI, EPP, PH and GS suggesting that potential hybrids with these attributes can be identified. Additive gene action was predominant for DA and DM under optimum conditions. This implies that genetic gain for drought tolerance can be achieved through selection for these traits. Single cross hybrids 34 (CLHP0352 X CLHP00322), 42 (CLHP00294 X CLHP00322), 4 (CLHP0312 X CLHP0310), 64 (CLHP0312 X CLHP0310), 55 (CLHP0364 X TZM113) and 46 (CLHP00294 X CML451) were identified as potential hybrids because of exhibiting significant SCA effects for grain yield and other traits. They are therefore, recommended for provitamin A screening and further testing under multi environment trials. Inbred lines 37 (TZM113), 19 (CLHP00294), 18 (CLHP0352), 19 (CLHP00294), 23 (CLHP0058), 11 (CLHP00432) and 20 (CLHP0364) exhibited desirable GCA effects for DA, ASI, DM and Gs. They are therefore considered as potential parents for

population developments. They can also be utilised in backcross breeding as donors of genes responsible for the traits they excelled in and/or quantitative trait loci mapping.

6.1 Introduction

Maize has been identified as the strategic crop to fight food security in Africa due to its widespread production and consumption in the region. However, maize production in the region is being constrained by climate change exacerbated recurrent and episodic droughts among other constraints. Genetic improvement of maize germplasm to confer drought tolerance has been identified as a sustainable way to curb the impact of drought (Campos et al., 2006).

White maize consumed by many people in SSA lacks vitamin A, placing people who largely live on the maize based diets at risk of developing vitamin A deficiency (VAD) related sickness (Nuss and Tanumihardjo, 2010). Vitamin A deficiency is associated with blindness, depressed immune response and stunted growth among children (Chandler et al., 2013). Woman and children in poor rural areas in Africa who cannot afford alternative sources of food are at high risk of developing VAD related diseases. In South Africa 33% of 6 to 71-month-old children have been reported to suffer from VAD, with Limpopo and KwaZulu-Natal Provinces leading with 43% and 38%, respectively (Faber and Wenhold, 2007). Biofortification of maize has been identified as a sustainable means of enhancing the vitamin A content of ordinary maize to meet the required quantities (Suwarno et al., 2014).

To boost maize production and seed business in Africa, most public and private seed companies are now mainstreaming hybrid maize development. Hybrids possess higher productivity in terms of grain yield potential and resilience to biotic and abiotic stresses as compared to open pollinated varieties (OPVs). This is because hybrid cultivars exhibit heterosis or hybrid vigour. In this regard several hybrids in the early, intermediate and late maturity categories have been developed. However, at present, in South Africa there are few provitamin A released cultivars and among these, none are confirmed drought tolerant, despite most of the growing regions in the country becoming increasingly suboptimal for maize production due to climate change.

The level of heterosis depends on the performance of the parental lines in the hybrid combinations (Betran et al., 2003). However, the environment (E) and/or genotype by environment interaction (GEI) effects can alter the expected heterosis (Betran et al., 2003).

This, therefore, necessitates evaluation of parental lines and the generated hybrids across multiple environments. To effectively exploit heterosis in hybrid development, it is important for the breeder to have a good understanding of the general and specific combining ability effects (GCA and SCA, respectively) of the parental lines involved (Betran et al., 2003). The GCA is the average performance of a line in a hybrid combination whilst SCA is the deviation of a hybrid's performance from the expected performance based on the average performance of the individual lines crossed (Hallauer and Miranda, 1988). Thus, information on the combining abilities is important in identifying the best parents or parental combinations for a hybridisation programme.

Furthermore, combining ability analysis enables the breeder to determine the type of gene action controlling the traits (Singh, 2015). The GCA effects are associated with the predominance of additive gene action whilst SCA are associated with non-additive gene action, which can be dominance and/or epistatic effects (Derera et al., 2007). The control of genetic tolerance to biotic and abiotic stresses such as diseases and drought have been attributed to different gene actions by different researchers. Betrán et al. (2003) reported the predominance of additive gene action for drought tolerances in contrast to Murtadha et al. (2018), who reported the predominance of non-additive gene action. In their study on Phaeosphaeria leaf spot resistance in maize, Sibiya et al. (2011) reported the predominance of additive gene action. Good general combiners are useful in breeding programmes while good specific combiners are useful in hybrid development. Combining abilities have been estimated in maize cultivar development programmes using different mating designs, among them are diallel designs (Sibiya et al., 2012) and North Carolina designs II (NCDII) (Derera et al., 2007). In this study, an NCD II design was used mainly because of its capacity to allow the assessment of many parents.

Using an integration of morpho-physiological and biochemical traits via a selection index, several promising drought tolerant provitamin A maize inbred lines were selected (herein Chapter 5). Therefore, it was necessary to assess the level of combining abilities of the selected lines under both optimum and drought conditions. The main objective of this study was to estimate combining abilities, variance components and gene action controlling grain yield and other secondary traits of provitamin A maize across optimum and drought stress environments under different environments.

6.2 Materials and methods

6.2.1 Plant materials and generation of crosses

Sixteen provitamin A inbred lines with high stress index (SI) values, were selected from the drought tolerance screening experiment in chapter 5 and crossed following an 8 * 8 NCD II as described by Hallauer and Miranda (1988). The names of parental lines are presented in Table 6.1. Five drought tolerant and one drought susceptible single cross hybrids sourced from Agriculture Research Council (ARC), South Africa were used as drought tolerant and susceptible checks, respectively (Table 6.2). Thus, a total of 70 hybrids and 16 parental lines were evaluated in this study. The crossing plan, the generated experimental 64 hybrids and the six check hybrids are presented in Appendix 6.1.

Table 6.1: List of the 16 selected parents for the hybridisation programme.

Female inbred lines				Male inbred lines			
Genotype	Entry #	Cluster	SI	Genotype	Entry #	Cluster	SI
CLHP0312	4	A	48.6267	CLHP00307	9	B	51.1468
CLHP00478	6	A	40.0173	CLHP00322	10	B	35.9401
CLHP00378	11	A	43.8182	CLHP00432	12	B	38.6382
CLHP0350	15	A	44.0099	CLHP0310	17	B	40.1479
CLHP0352	18	A	36.1369	CML486	31	B	34.4740
CLHP00294	19	A	34.8760	CML451	32	B	34.7982
CLHP0364	20	A	50.4932	TZM113	37	B	43.0560
CLHP0058	23	A	55.0090	TZM112	40	B	50.5059

Entry # - entry number, SI - selection index value, cluster - molecular cluster in chapter 4.

Table 6.2: List of six single cross hybrids used as checks.

Genotype	Entry #	Pedigree	Status
WE4338	65	I-40/CML312	Drought tolerant
WE4351	66	CN07/8-224/CML312	Drought tolerant
WE4359	67	CB333/CML444	Drought tolerant
SAHTB8-360	68	CN07/244/CML312	Drought tolerant
SA4348	69	CNo7/8-193/CML312	Drought tolerant
SAHTB7-505	70	1-16/CML505	Drought susceptible

6.2.2 Study sites

Hybrids and parental inbred lines were evaluated across four optimum (OPT) and two managed drought (MD) environments during the summer and winter periods of 2017/2018. The environments comprised of Ukulinga Research farm in South Africa (29^0 40' S, 30^0 24' E; 806 masl) (Env 1) and Cedara Research station in South Africa (29^0 30' S, 30^0 19' E; 876 m masl) during summer period under optimum conditions (Env 2); Makhatini Research station in South Africa (20.32°S, 30.90°E, 555 masl) during the winter period under optimum conditions (Env 3); Department of Research and Specialists Services (DR&SS) in Harare, Zimbabwe (17.13°S, 31°E, 1 406 masl) during the summer period under optimum conditions (Env 4); Ukulinga Research farm during late summer period under drought conditions (Env 5); and Makhatini Research station during winter period under drought conditions (Env 6).

Three of the four optimum trials were carried out under rain-fed conditions with supplementary sprinkler irrigation applied at Ukulinga, Cedara and DR&SS from November 2017 to April 2018, whilst the Makhatini optimum was implemented from May to September 2018 under sprinkler irrigation. Two managed drought trials were planted at Makhatini Research station during the winter from May to September 2018 and at Ukulinga Research farm from April to August 2018 under sprinkler irrigation. The mean annual rainfall and mean temperature experienced during the respective trial periods at the environments are presented in Table 6.3.

6.2.3 Experimental design and trial establishment

Hybrid evaluation trials were laid out in a 7 X 10 alpha lattice design with two replications across all the six environments. Inbred evaluation trials were implemented in a 4 X 4 alpha lattice design across all the six environments. The plot size for both hybrid and inbred trials were two rows of 5 m long with inter-row and in-row spacings of 0.75 m and 0.25 m, respectively. Two seeds were planted per planting station and then thinned to one plant per station at three weeks after emergence. Managed drought trials involved with-holding irrigation two weeks before 50% anthesis until five weeks after 50% anthesis, after which one last irrigation was applied during the grain filling stage in accordance with CIMMYT protocols (Bänzinger et al., 2000). The 50% anthesis date (AD) was estimated from the inbred lines used in the agro-morphological diversity study in chapter 3. Optimum trials were rainfed and supplementary irrigation provided only when necessary. At Makhatini Research station, optimum conditions were achieved and maintained by 6-day interval irrigation. Weed management and other agronomic practices were done according to standard guidelines for maize production in South Africa (DAFF, 2013) and Zimbabwe, respectively and recommendations per site.

6.2.4 Data collection

Traits that were measured included: grain yield (GY), anthesis-silking interval (ASI), ears per plant (EPP), days to anthesis (DA), days to maturity (DM), plant height (PH) and stomatal conductance (G_s). Measurement procedures are same as in chapters 3 and 5.

Table 6.3: Environments rainfall and temperature data during the 2017/2018 summer and winter seasons.

Env number	Environment Name	Season	Location	Country	Mean annual rainfall (mm)	Five-month mean temperature (°C)	Type of environment
1	Ukulinga	Summer	KwaZulu-Natal	South Africa	578.56	26.79	Optimum (OPT)
2	Cedara	Summer	KwaZulu-Natal	South Africa	787.24	24.17	Optimum (OPT)
3	Makhatini	Winter	KwaZulu-Natal	South Africa	-------	25.60	Optimum (OPT)
4	DR&SS	Summer	Harare	Zimbabwe	715.00	27.23	Optimum (OPT)
5	Ukulinga	Summer - winter	KwaZulu-Natal	South Africa	--------	20.23	Managed drought (MD)
6	Makhatini	MAK-MD	KwaZulu-Natal	South Africa	--------	25.60	Managed-drought (MD)

6.3 Data analysis

Data collected were analysed using an alpha lattice procedure of the SAS (SAS, 2013). Combined analysis of variance (ANOVA) was performed following tests for normality of the data and homogeneity of variances. Genotypes were treated as fixed factors, while location, replication and incomplete blocks were treated as random factors. The following linear model (Equation 6.1) was used for analysis of variance across environments:

$$Yijk = \mu + gi + gj + Sij + ek + (ge)ik + (ge)jk + (se)ijk, \qquad 6.1$$

where $Yijk$ = the performance of the hybrid developed with *i*th *male* and *j*th *female*, in the *k*th location, μ = the overall mean; gi = the effect of the ith male; gj = the effect of the jth female; Sij = the interaction of the *i*th male with the *j*th female; ek = the effect of the kth environment; $(ge)ik$ = the interaction of the gi and ek; $(ge)jk$ = the interaction of the gj and ek; $(se)ijk$ = the interaction of Sij and ek.

The GCA effects for the parents (Equation 6.2) and SCA effects (Equation 6.3) of crosses were calculated as follows (Singh and Chaudhary, 1985):

$$GCA_m = Xm - u; \quad GCA_f = Xf - u \qquad 6.2$$

Where: GCA_m and GCA_f = *GCA* of male and female parents, respectively*; Xm* and *Xf* = mean of the male and female parents, respectively and μ = grand mean.

$$SCAij = Xij - E(Xij) = Xij - (u + GCAi + GCAj) \qquad 6.3$$

Where: *SCAij* = SCA effects of the cross; *Xij* = observed mean value of the cross; *E(Xij)* = expected value of the two parents involved; *GCAi* and *GCAj* = GCA of male and female parents, respectively.

The variance was partitioned into general combining ability of male and female variances (σ^2gca_m and σ^2gca_f, respectively), specific combining ability variance (σ^2sca). The additive (σ^2_A), dominance variance (σ^2_D) were computed from their respective mean square (MS) values as follows (Singh and Chaudhary, 1985; Dabholkar, 1992):
Covariance of the half-sib of male = σ^2gca_m (Equation 6.4)

$$\text{Cov. H. S. (male)} = \frac{MS_m - MS_{(m*f)}}{r * s * m} \qquad 6.4$$

Covariance of the half-sib of female = $\sigma^2 gca_f$ (equation 6.5)

$$\text{Cov. H. S. (female)} = \frac{MS_f - MS_{(m*f)}}{r * s * f} \quad 6.5$$

Covariance of full-sib (equation 6.6)

$$\text{Cov. F. S. (male)} = \frac{(MS_m - MS_e) + (MS_f - MS_e) + (MS_{(m*f)} + MS_e)}{3r} + \frac{6rCov.H.S - r(m+f)Cov.H.S.}{3r} \quad 6.6$$

The covariance of the average GCA was determined by the formula (equation 6.7):

$$\text{Cov. H. S.} = \frac{1}{r(2mf - m - f)} + [\frac{(m-1)(MS_m) + (f-1)(MS_f)}{m + f - 2} - MS_{(m*f)} \quad 6.7$$

Since the parents were inbred lines the inbreeding coefficient was set as one (F = 1) in the calculation of σ^2_A and σ^2_D by making σ^2_A and σ^2_D the subjects of their respective formulas (equations 6.8-6.9) (Singh and Chaudhary, 1985; Dabholkar, 1992):

$$\sigma^2_{gca} = Cov.H.S. = \left(\frac{1+F}{4}\right)\sigma^2_A \quad 6.8$$

$$\sigma^2_{sca} = Cov.F.H. = (\frac{1+F}{4})^2\sigma^2_D \quad 6.9$$

Mid-parent heterosis (MPH) and best check heterosis (standard heterosis) (SCH) were computed as follows (equations 6.10-6.11):

$$MPH = \frac{F_1 - MP}{MP} \times 100 \quad 6.10$$

$$SCH = \frac{F_1 - CC}{CC} \times 100 \quad 6.11$$

Where F_1 = the average performance of the cross, MP = the average of the two parental inbred lines and CC is mean of the best cultivar used as a check. The significance of the heterosis test was done using "t" test (Turner, 1953).

6.4 Results

6.4.1 Analysis of variance and mean performances

The combined analysis of variance across the four optimum (OPT) and two managed drought (MD) environments (Env) is presented in Table 6.4. Optimum environments were significantly different ($p \leq 0.01$; $p \leq 0.05$) for all the traits, whilst managed drought environments were

significant for most of the traits except for EPP, DA and Gs. Highly significant ($p<0.01$) differences were observed for the general combining ability for males (GCA_m) and females (GCA_f), and specific combing ability of the cross (SCA_{mf}) for all the measured traits under both environmental conditions.

The interactions between the Env and GCA_m, GCA_f, and SCA were significant ($p \leq 0.01$; $p \leq 0.05$) under both environmental conditions for GY and some of the secondary traits. Specific combining ability had higher percentage contribution of sum of squares (SSSCA%) to the hybrid sum of squares for all the traits across both environments. The percentage contributions of sum of squares due to male combining ability to the total hybrid sum of squares ($SSGCA_m$%) were higher than the percentage contributions of the female combining ability sum of squares to the total hybrid sum of squares ($SSGCA_f$%) for most of the traits across the two environmental conditions (Table 6.4). Under optimum conditions, $SSGCA_m$%, $SSGCA_f$% and SSSCA% for GY were 22.8%, 12.7% and 64.5%, respectively. In drought stressed environments, GY had $SSGCA_m$% of 20.8%, $SSGCA_f$ of 18.8% and SSSCA% of 60.3%. Among all the traits, within optimum environments, Gs had the highest SSSCA% of 73.0% whilst EPP had the highest SSSCA% of 73.3% under drought conditions.

Mean values, coefficients of variation (CVs), least significant differences (LSDs) of hybrids and parental inbred lines are presented in Tables 6.5 and 6.6 respectively. Only the top five best high yielding hybrids under drought conditions (MD) and bottom two poor yielding experimental hybrids, and four best yielding checks are presented in Table 6.5. All the 64 experimental hybrids and six check hybrids are presented in Appendix 6.2.

Table 6.4: Mean squares and significant tests of the measured traits for hybrids across optimum and drought environments.

	DF		GY		ASI		DA		DM		EPP		PH		Gs	
	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD
Env	3	1	149.2**	1.6**	1.2*	3.4*	17.7*	7.0	1218.4**	8.2	0.1	0.0	6145.2**	937.5**	2734.8**	0.2
Rep(Env)	4	1	0.2	0.0	0.9	0.9	12.9	21.1	94.0**	21.1	0.0	0.1	82.8	1.8	77.0	0.5
Hybrid	63	63	9.99**	2.04**	2.24**	9.08**	110.19**	85.25**	110.19**	85.25**	2.19*	0.94**	4679.73**	4563.95**	28978.19**	578.9**
GCA_m	7	7	20.5**	2.5**	4.4**	20.4**	201.4**	120.5**	115.6**	120.5**	4.7**	1.0**	8258.8**	6561.3**	76631.0**	699.6**
GCA_f	7	7	11.4**	2.2**	2.5**	11.7**	346.5**	195.6**	256.3**	195.6**	1.7**	0.6**	7532.6**	6599.0**	49286.8**	411.9**
SCA	49	49	8.3**	1.0**	1.9**	4.6**	63.4**	43.9**	47.3**	43.9**	1.9**	0.7**	3760.9**	2918.8**	48526.9**	297.3**
Hybrid*Env	189	63	6.6**	1.6**	1.7*	0.3	30.9**	0.7	39.0**	0.7	0.6	0.09	1033.8**	2.28*	11022.4**	1.7
GCA_m*Env	21	7	10.6**	2.3**	0.7	0.2	23.2*	0.1	74.4**	0.1	1.2*	0.1	1582.5**	0.5	5532.3**	0.1
GCA_f*Env	21	7	17.3**	1.9**	1.1*	0.2	83.1**	1.2	82.8**	1.2	0.8	0.1	1643.8**	8.5	9178.8**	4.1
SCA*Env	147	49	4.5**	0.9*	1.9*	0.1	24.5*	0.2	43.7**	0.2	1.6**	0.1	5168.0**	1112.5*	6740.3**	0.7
Error	252	63	5.5	0.0	0.6	0.3	12.0	0.9	8.9	15.9	4.3	3.2	694.4	7.3	147.4	113.1
$SSGCA_m$%			22.8	20.8	22.0	31.6	20.3	21.6	16.4	19.3	23.8	17.2	19.6	19.6	16.5	16.7
$SSGCA_f$%			12.7	18.8	12.5	18.2	34.9	29.7	36.5	31.4	8.7	9.5	17.8	19.5	10.6	33.7
SSSCA%			64.5	60.3	65.5	50.2	44.7	49.3	47.1	49.3	67.6	73.3	62.5	60.9	73.0	49.6

*, ** - significantly different at $p \leq 0.05$ and 0.01 respectively, DF - degrees of freedom, GY - grain yield, DA - days to anthesis, DM - days to maturity, EPP - ears per plant, PH - plant height, G_s - stomatal conductance, OPT - optimum environment, MD - managed drought, Env - environment, Rep - replication, GCA_m - general combining ability of the male parent, GCA_f general combining ability of the female parent, SCA - specific combining ability, $SSGCA_m$% - percentage contribution of the male combining ability sum of squares to the hybrid sum of squares, $SSGCA_f$ - percentage contribution of the female combining ability sum of squares to the hybrid sum of squares, SSSCA - percentage contribution of the specific combining ability sum of squares to the hybrid sum of squares, GCA_m*Env - male general combining ability and environment interaction, GCA_f*Env - Female general combining ability and the environment interaction and SCA - specific combining ability by the environment interaction.

There were significant differences ($p \leq 0.001$ and $p \leq 0.05$) among the hybrid performance for all the traits across all environments. The interaction between the hybrid performance and the environment (Hybrid*Env) was also significant ($p \leq 0.001$; 0.05) for GY, DM, PH and Gs across both environments for most traits but was not significant for EPP (Table 6.4). The hybrid mean GY values under optimum and drought environments were 6.60 t ha^{-1} and 3.13 t ha^{-1}, respectively, which translated to 52.6% yield difference (Table 6.1). Hybrid GY ranged from 6.06 t ha^{-1} to 9.28 t ha^{-1} and 1.41 t ha^{-1} to 4.71 t ha^{-1} across optimum and drought environments, respectively. Entry 34 (18 X 10) yielded highest across drought environments with mean GY of 4.71 t ha^{-1}, whilst entry 55 (20 X 37) had the highest yield of 9.28 t ha^{-1} across environments. Entry 34 (18 X 10) was the only experimental hybrid that yielded higher than the highest performing check 69 (SA4348) which yielded 4.61 t ha^{-1}. Similarly, under optimum environments, entry 55 (20 X 37) was the only hybrid that yielded higher than the best yielding check 69 (SA4348), which yielded 8.75 t ha^{-1}. None of the experimental hybrids yielded less than the drought susceptible check hybrid 70 (SAHTB7-505), which had 1.40 t ha^{-1} and 5.71 t ha^{-1} under drought and optimum environments, respectively.

Table 6.5: Mean performance of grain yield and secondary traits of provitamin A maize single cross hybrids.

	GY		ASI		DA		DM		EPP		PH		Gs	
Entry	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD
Top five experimental hybrids														
34	7.19	4.71	3.13	3.75	69.25	73.25	115.25	118.25	2.6	1.25	316.98	280.75	297.80	27.40
17	8.15	4.48	1.88	2.00	70.75	64.75	116.13	109.75	3.0	2.6	328.91	281.25	380.84	17.04
55	9.28	4.26	2.75	3.00	64.25	59.00	112.00	104.00	2.8	2.8	315.48	270.25	436.97	20.96
64	8.03	4.24	2.63	2.00	67.00	66.50	112.13	111.50	3.0	2.8	330.36	314.25	426.22	18.58
42	8.35	4.19	2.13	3.75	72.75	75.25	117.88	120.25	2.5	2.0	320.41	280.00	506.42	22.97
Four hybrid checks														
69	8.75	4.61	0.13	2.25	68.38	71.25	101.50	116.25	3.0	2.0	345.75	311.75	463.25	30.45
67	8.31	4.09	1.63	4.50	66.00	71.25	99.13	116.25	2.5	2.0	309.25	272.75	362.25	26.35
68	8.14	3.20	3.13	4.00	65.50	67.25	117.13	112.25	2.8	2.0	301.50	267.75	466.75	27.20
70	5.71	1.41	0.88	3.75	75.63	77.75	106.75	122.75	2.3	1.8	306.75	265.75	519.50	28.35
Bottom two experimental hybrids														
9	6.59	1.85	2.88	7.25	69.13	72.25	116.38	117.25	2.5	2.00	295.61	256.00	252.42	45.75
63	6.06	1.85	3.38	6.50	62.88	61.00	109.13	106.00	1.6	1.75	270.03	233.75	423.17	52.65
Mean	6.66	3.13	2.89	5.04	66.08	65.56	111.77	110.56	2.4	1.85	294.87	250.69	390.96	34.38
LSD	1.18	0.26	1.01	0.18	15.21	0.91	6.48	1.96	1.7	0.1	51.91	6.20	272.09	16.80
CV	16.62	3.40	26.00	12.00	5.87	1.27	3.66	7.54	23.3	16.80	8.92	0.57	18.24	3.06
Pvalue	***	***	**	*	***	**	*	*	*	*	*	*	**	***
Max	10.75	4.71	4.13	9.50	75.63	77.75	120.50	122.75	3.1	3.00	357.16	327.00	519.50	66.93
Min	4.39	1.41	0.13	2.00	58.38	57.75	99.13	102.75	1.3	1.00	247.53	170.00	252.42	17.04

*; **;*** - significantly different at $p \leq 0.05$, $p \leq 0.01$ and 0.001 respectively, GY - grain yield, ASI - anthesis silking interval, DA - days to anthesis, DM - days to maturity, EPP - ears per plant, PH - plant height, G_s - Stomatal conductance, OPT - optimum environment, MD - managed drought.

Mean ASI was 2.9 days and 5.0 days for optimum and drought environments, respectively (Table 6.5). The means for DA, DM, EPP, PH and Gs for optimum environments were 66.08 days, 111.77 days, 2.4, 294.9cm and 391 mmol m^{-2} s^{-1}, respectively, whilst the same traits across drought environments had mean values of 65.56 days, 110.56 days, 1.85, 250.69 cm and 34.38 mmol m^{-2} s^{-1}, respectively.

Parental inbred lines had mean GY of 1.6 t ha^{-1} and 0.9 t ha^{-1} across optimum and drought stressed environments, respectively. Inbred 37 (TZM113) was the highest yielding in both optimum and drought stress environments with 2.5 t ha^{-1} and 1.8 t ha^{-1}, respectively (Table 6.6). Mean values of ASI, DA, DM, EPP, PH and Gs across optimum environments were 3.4 days, 74 days, 119.3 days, 2.3, 198.3 cm and 346.1 mmol m^{-2} s^{-1}, respectively, whilst similar traits had mean values of 7.1 days, 73.1 days, 117.1, 2.3, 180.5 cm and 41.4 mmol m^{-2} s^{-1}, respectively. Majority of the traits had high significant differences ($p \leq 0.001$) except EPP which was not significant and ASI under drought stress which was significant at $p \leq 0.05$.

Table 6.6: Mean performance performances of parental inbred lines across optimum and managed drought environments.

Parental	Entry	GY		ASI		DA		DM		EPP		PH		Gs	
Type		OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD
F	4	1.2	0.7	3.5	9.0	75.3	72.0	120.3	116.0	2.8	2.8	210.1	202.5	310.5	20.9
F	6	1.0	0.5	4.0	10.8	77.9	72.8	122.9	116.8	1.9	1.8	200.4	183.8	403.4	71.3
M	9	1.7	0.7	5.0	9.8	69.8	69.8	113.8	113.8	1.8	1.8	172.5	172.5	55.0	55.0
M	10	1.2	0.7	4.0	8.5	78.4	79.0	123.4	123.0	3.0	2.5	209.8	187.3	350.2	40.8
F	11	1.9	1.1	2.5	6.3	64.3	63.3	109.3	107.3	2.5	2.3	195.8	163.3	513.7	52.1
M	12	2.1	1.4	2.8	4.5	72.6	71.5	117.6	115.5	2.5	2.5	202.1	195.3	328.7	26.6
F	15	2.4	1.3	2.6	5.8	73.0	74.0	118.0	118.0	2.9	2.8	208.0	158.3	424.5	28.8
M	17	0.6	0.4	4.3	8.8	79.1	78.0	124.1	122.0	1.1	2.0	207.3	190.3	233.5	45.0
F	18	1.9	0.9	1.6	3.5	76.5	69.3	121.5	113.3	2.6	2.5	208.5	189.0	336.7	27.6
F	19	1.0	0.7	3.8	7.5	76.5	73.8	121.5	117.8	2.1	2.0	211.0	178.3	248.7	53.2
F	20	2.2	1.4	3.0	3.5	76.8	75.3	121.8	119.3	3.0	2.5	199.6	155.3	459.9	26.6
F	23	0.9	0.6	4.0	8.3	75.8	78.3	120.8	122.3	1.5	2.0	212.4	189.5	212.1	46.2
M	31	1.6	0.6	3.3	10.3	71.6	74.0	116.6	118.0	2.1	1.8	151.6	142.8	369.8	48.5
M	32	1.9	1.0	3.4	5.0	79.1	77.0	124.1	121.0	3.0	2.5	181.0	179.0	490.2	40.5
M	40	0.9	0.7	3.9	9.5	81.1	82.0	126.1	126.0	1.8	1.8	191.6	192.3	312.6	53.2
M	37	2.5	1.8	2.6	2.8	61.4	60.3	106.4	104.3	2.8	3.0	210.9	208.3	488.4	26.2
Mean		1.6	0.9	3.4	7.1	74.3	73.1	119.3	117.1	2.3	2.3	198.3	180.5	346.1	41.4
Pvalue		***	***	*	***	***	***	***	***	ns	ns	***	***	***	***
CV (%)		12.9	15.5	22.1	13.0	10.7	25.1	6.7	15.6	25.3	27.8	7.6	19.9	8.5	8.9
LSD (5%)		0.0	0.1	0.4	0.5	0.4	1.0	0.4	1.0	0.3	0.3	0.8	1.9	16.4	1.9

*, *** - significantly different at p ≤0.05 and 0.001 respectively, ns - not significant, GY - grain yield, ASI - anthesis silking interval, DA - days to anthesis, DM - days to maturity, EPP - ears per plant, PH - plant height, G_s – Stomatal conductance, OPT – optimum environment, MD – managed drought, F – Female parental inbred line, M - Male parental inbred line, CV – coefficient of variation, LSD – least significant differences.

6.4.2 General combining ability effects

Table 6.7 presents GCA effects for all the traits understudy for the sixteen parental inbred lines evaluated across optimum and drought stress environments. Positive GCA effects are desirable for GY and EPP for both optimum and drought stress environments. Based on the results of correlation analysis in chapters 3 and 5, negative GCA effects are desirable for ASI under both optimum and drought stress environments. Negative GCA values are also desirable for Gs, DA and DM under drought stress environments, positive GCA effects for these traits are desirable under optimum conditions.

Desirable significant GCA effects were observed for the following entries: entry 37 (TZM113) with value of - 0.416 for ASI under optimum conditions, 11(CLHP00432) with value of - 0.884 for Gs under drought conditions, 18 (CLHP0352) with value of - 0.541 for DM under optimum conditions, 19 (CLHP00294) with value of - 0.558 for DA under optimum conditions and 20 (CLHP0364) with value of - 6.307 under drought conditions.

6.4.3 Specific combining ability effects

Estimates of the SCA effects of the 64 provitamin A single cross hybrids assessed across four optimum and two drought stress environments ranked in descending order of GY obtained under drought stress environments (MD) are presented in Table 6.9. All traits exhibited both positive and negative SCA values. About 50% of the entries (hybrids) exhibited significant ($p \leq 0.001$; 0.05) SCA values for at least one trait.

Hybrid 34 (18 X 10) had the highest significant SCA effects of 1.99 for GY under drought conditions whilst hybrid 42 (19 X 10) had the highest SCA effect value of 2.85 for GY under optimum conditions. Crosses with higher SCA values for GY under drought conditions were: entries 4 (4 X 17), 64 (23 X 40), 55 (20 X 37) and 46 (19 X 32) with values of 0.89, 0.68, 0.81 and 0.57, respectively whilst entries 64 (23 X 40), 34 (18 X 10) and 55 (20 X 37) had higher positive SCA values for GY and other traits under optimum conditions.

Table 6.7: General combining ability effects of parental inbred lines evaluated across drought and optimum environments.

Parent entry	GY		ASI		DA		DM		EPP		PH		Gs	
Males	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD
40	0.098	0.015	-0.090	-0.858	1.391	1.481	0.009	1.481	0.359***	0.056	7.618	16.289	0.001	5.100***
12	0.043	0.215	0.064	-0.551	-0.638	1.525	0.000	1.525	0.062	0.013	-2.609	-0.796	0.000	2.926
9	0.362	0.010	-0.356	-0.834	1.883	1.773	0.001	1.773	0.166	0.132	7.572	8.819	0.002	-2.913
31	-0.118	0.003	0.031	0.274	-1.235	-1.113	0.003	-1.113	-0.029	-0.076	0.523	0.481	0.000	0.870
32	-0.168	-0.006	0.108	1.217	-1.896	-2.255	0.004	-2.255	-0.154	-0.042	-5.056	-11.479	0.000	3.376
37	0.131	0.011	-0.416***	0.087	-0.828	-1.292	0.001	-1.292	-0.064	-0.044	-1.595	-3.668	0.001	4.324
10	-0.037	-0.013	0.053	0.180	0.049	-0.580***	0.000	-0.058	-0.015	0.046	-6.034	-12.383	0.000	1.677
17	-0.049	-0.014	0.102	0.156	-0.002	-0.061	0.000	-0.061	-0.126	-0.085	-0.419	2.737	0.000	0.693
Females														
4	0.048	0.165	-0.006	-0.472	0.861	-1.507	-0.489	-1.507	0.007	0.001	0.166	1.921	0.002	-8.735
6	0.001	0.036	-0.008	-0.395	0.289	2.030	0.425	2.030	0.001	0.009	4.621	3.793	0.000	-1.446
11	0.003	0.005	0.000	-0.104	2.589	4.103	-0.079	4.103	0.001	0.001	6.911	16.880	0.003	-0.884
15	0.005	-0.003	-0.022	-0.453	-1.493	-1.970	-0.220	-1.970	0.000	0.000	-0.968	-3.599	0.000	-4.892
18	0.023	-0.017	0.022	-0.220	-2.404	-3.614	-0.541***	-3.614	0.003	0.001	0.383	4.551	0.021	-3.672
19	0.070	-0.021	0.013	0.652	-0.558***	-0.020	0.427	-0.558***	0.000	0.000	-5.905	-11.509	0.060	12.116
20	0.003	-0.028	0.001	0.380	0.297	1.676	0.303	1.676	0.000	0.002	-4.935	-14.325	0.000	-6.307***
23	0.050	-0.036	0.000	0.613	-0.120	-0.160	0.174	-0.160***	0.000	0.000	-0.273	2.289	0.001	-0.562

*** - significantly different at $p \leq 0.001$ respectively, GY - grain yield, DA - days to anthesis, DM - days to maturity, EPP - ears per plant, PH - plant height, G_s - stomatal conductance, OPT - optimum environment, MD - managed drought.

Most hybrids had non-significant SCA values for ASI under optimum conditions whilst under drought conditions many entries had significant ($p \leq 0.001$; 0.05) SCA values (Table 6.8). Under drought conditions, entry 55 (20 X 37) had a desirable negative SCA value for ASI of -2.83 whilst under optimum conditions entry 32 (15 X 40) had a negative SCA value of -1.16 for ASI. Hybrid 4 (4 X 17) had a desirable negative SCA value of -6.40 for both DA and DM under drought stressed conditions. Hybrid 55 (20 X 37) had a desirable positive SCA value of 0.77 for EPP across drought conditions whilst hybrid 4 (4 X 17) had the highest SCA value of 0.49 for EPP under optimum conditions. For Gs, hybrid entry 55 (20 X 37) had a desirable negative SCA value of - 23.29 under drought conditions whilst hybrid entry 34 (18 X 10) had the highest SCA value under optimum environments.

Table 6.8: Specific combining ability effects of evaluated across drought and optimum environments.

Hybrid	GY		ASI		DA		DM		EPP		PH		Gs	
	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD
18 X 10	1.11**	1.99***	0.07	-0.44	1.50	4.42***	-0.31	4.42**	0.11	-0.56**	15.47	28.23	-59.97***	-3.37
4 X 17	0.46	0.89**	-0.41	-0.31	-4.06	-6.40**	-0.29	-6.40**	0.49***	0.21	4.46	6.52	29.24	-10.18**
23 X40	2.01**	0.68*	0.01	-1.05	-0.18	-4.24	-0.44	-4.24**	0.14	0.71**	9.00	8.00	32.83	-7.69
20 X 37	1.77**	0.81*	-0.21	-2.83**	-0.08	0.51	0.07	0.51	0.31	0.77**	26.72	60.65	22.44	-23.29**
19 X 32	1.47*	0.57	0.13	0.73	-0.96	-4.24***	-0.62	-4.24**	-0.07	-0.70*	-0.61	13.01	27.92	18.48**
11 X 40	0.48	0.37	-0.45	-1.76**	1.13	-0.74	-0.45	-0.74	0.28	0.03	19.73	21.53	0.35	-6.62
6 X 17	0.63*	0.37	-0.27	-1.97**	1.34	1.40	-0.26	1.40	0.11	-0.47**	13.66	24.18	8.10	-8.06
23 X 31	0.15	0.16	0.04	-1.10	-0.25	-0.19	-0.28	-0.19	0.09	0.19	14.35	32.33	12.32	-12.90**
11 X 9	-0.05	0.06	-0.09	-1.06	1.61	2.08	0.78	2.08	-0.49**	-0.77	12.06	32.86	-2.11	-4.38
18 X 37	0.57**	0.05	-0.48	-0.15	3.27	6.47**	-0.52	6.47**	-0.09	0.08	8.65	10.68	74.54	-5.64
20 X 32	0.28	0.05	-0.08	-1.66**	0.80	0.11	0.48	0.11	0.45**	0.19	-4.32	-29.79	29.19	-3.94
15 X 40	0.25	0.04	-1.16	-1.78**	3.43	7.47**	0.30	7.47**	0.19	0.22	15.69	36.78	54.72	-7.37
18 X 32	-0.55	0.04	-0.04	-0.65	-0.22	-1.67	0.45	-1.67	0.24	0.17	13.17	19.79	47.63	-6.75
6 X 10	-0.09	0.03	-0.05	0.01	0.84	3.71**	0.23	3.71**	0.12	0.36	5.01	25.44	61.42**	-5.16
4 X 10	0.61	0.03	-0.04	-1.19**	0.54	2.96	0.05	2.96	0.30	0.36	13.23	17.83	10.06	-15.14**
11 X 37	0.36	0.03	0.10	0.64	1.93	9.16**	0.38	9.16**	0.29	0.12	-31.36**	-66.32	-5.37	4.19
19 X 17	-0.41	0.02	-0.16	-0.08	2.58	7.41**	0.70	7.41**	0.09	0.24	29.41**	60.22	25.63	-5.30
20 X 10	-0.16	0.02	-0.13	0.68	-1.28	0.64	0.34	0.64	-0.26	-0.08	-22.98***	-27.22	-7.71	3.55
11 X 10	-0.20	0.02	0.30	-0.60	0.15	-2.34	-0.49	-2.34	0.39	0.14	6.19	26.33	29.59	-0.24
11 X 12	0.49	0.02	-0.26	-1.04	-0.07	-5.87**	-1.06	-5.87**	0.49**	0.25	13.02	33.52	17.60	-5.15
6 X L7	-0.12	0.02	-0.07	-0.65	-0.22	-5.65**	0.47	-5.65**	0.21**	0.31	-5.51	-9.76	-45.10	-7.20
20 X 40	-0.27	0.01	0.04	0.71	-0.70	-2.62	-0.51	-2.62	-0.27	0.04	-7.50	-1.03	-27.34	13.89**
15 X 12	-0.07	0.01	0.24	0.61	-1.10	-2.56	-0.46	-2.56	-0.09	0.19	6.09	5.94	-50.77	-2.83
15 X 31	0.21	0.01	-0.09	-0.40	-1.14	0.12	-0.21	0.12	0.14	-0.51**	-7.83	-13.09	-20.43	-2.29

Table 6.9 continued…

Hybrid	GY		ASI		DA		DM		EPP		PH		Gs	
	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD
11 X 31	-0.42	0.01	-0.03	1.04	-2.07	-2.68	-0.86	-2.68	-0.25	-0.04	-19.82	-37.11	-59.29**	1.07
18 X 9	0.08	0.01	0.24	-0.19	-4.75**	-5.83**	-0.13	-5.83**	-0.10	0.31	-1.54	-2.81	46.11	4.90
19 X 37	-0.19	0.01	0.46	0.52	-0.13	-1.08	-0.28	-1.08	0.09	-0.79**	27.96**	44.83	-1.83	0.24
4 X 12	-0.29	0.01	0.35	1.70**	0.46	2.47	0.43	2.47	-0.75**	-0.66**	-24.95**	-36.62	-59.73**	16.82**
15 X 37	-0.53	0.00	-0.06	-0.33	-2.61	-3.61**	-0.19	-3.61**	0.00	0.06	5.08	10.00	50.04	3.10
19 X 40	-0.05	0.00	-0.24	-0.22	-0.12	2.09	0.33	2.09	0.17	0.04	34.98**	48.89	19.95	-1.08
23 X 12	-0.32	0.00	0.05	-0.33	-0.95	-0.35	0.28	-0.35	0.45	0.23	19.17	18.94	-8.21	-7.65
18 X 31	0.40	0.00	-0.29	-1.41**	0.58	2.32	0.61	2.32	0.43	1.07**	11.09	17.79	30.96	-10.51**
23 X 37	-0.16	0.00	0.43	-0.08	-0.57	-1.83	-0.06	-1.83	-0.04	0.08	16.01	43.31	36.85	-1.98
23 X 9	-0.02	-0.01	-0.24	-1.07	1.79	-0.43	-0.27	-0.43	-0.34	0.11	-8.77	-13.39	45.72	-13.66**
15 X 9	0.55	-0.01	-0.12	-1.32**	1.35	2.38	0.21	2.38	0.18	0.98	6.96	18.10	-62.11**	-10.49**
15 X 17	0.44	-0.01	-0.49	-0.46	-0.10	-1.01	-0.30	-1.01	0.39	0.17	-2.04	-8.07	-26.58	-3.78
11 X 17	-0.77**	-0.01	0.34	-0.68	-0.54	-3.83**	-0.20	-3.83**	-0.19	0.01	-11.70	-24.42	-64.94**	-1.85
19 X 12	0.56	-0.01	0.02	-1.40**	2.63	8.35**	-0.13	8.35**	0.10	0.24	5.99	43.38	-33.73	-8.02
19 X 10	2.85**	-0.01	-0.36	-0.49	2.40	3.81**	0.21	3.81**	0.19	0.15	2.83	-6.12	48.95	-7.77
15 X 10	-0.09	-0.01	0.11	0.33	-0.83	-5.08**	0.16	-5.08**	0.39	0.34	-11.59	-25.44	30.92	2.68
4 X 9	-0.18	-0.01	0.13	0.25	1.82**	2.49	0.86	2.49	0.29	0.11	-10.13	-24.50	-18.93**	10.05**
6 X 12	-0.50	-0.02	0.00	1.00	-2.01	-4.60**	0.86	-4.60**	-0.65**	-0.69**	-15.85	-33.14	1.65	2.61
11 X 32	-0.24	-0.02	-0.26	1.33**	-0.97	1.06	0.61	1.06	0.04	-0.46**	13.17	25.75	-38.27	-4.91
20 X 31	-0.48	-0.02	0.00	2.33**	0.00	-1.60	0.36	-1.60	-0.42	0.20	-13.09	-24.44	-33.04	11.97**
4 X 40	0.16	-0.02	0.23	0.26	-0.75	-1.34	-0.33	-1.34	0.19	0.02	-30.51**	-59.74	4.31	-14.10**
6 X 9	-0.17	-0.02	0.03	1.21**	0.23	4.24**	0.09	4.24**	0.39**	0.31	15.91	31.15	33.09	10.16**
18 X 40	0.33	-0.02	0.16	-0.41	0.00	2.26	0.56	2.26	0.29	0.66**	10.75	6.93	1.52	-8.11

Table 6.10 continued…

Hybrid	GY		ASI		DA		DM		EPP		PH		Gs	
	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD	OPT	MD
6 X 31	-0.34	-0.02	0.17	2.13**	0.41	-0.94	-1.17	-0.94	-0.43	-0.75**	-10.25	-16.84	-41.01	7.02
19 X 31	-0.07	-0.03	0.33	0.21	-0.55	-4.27**	-0.14	-4.27**	-0.16	-0.68**	-10.07	-14.23	-23.07	10.07**
23 X 17	-0.49	-0.03	0.50**	1.22**	-1.65	-0.28	-0.34	-0.28	-0.71**	-0.02	-5.03	-0.67	-26.42	11.42**
19 X 9	0.25	-0.04	-0.19	0.25	-2.28	-3.50**	-0.26	-3.50	0.56**	-0.55**	-36.94**	-83.41	20.70	-4.80
18 X17	-0.11	-0.04	-0.08	0.44	0.56	-0.85	0.12	-0.85	-0.28	-0.46**	-23.57	-53.06	-35.94	13.77**
4 X 32	-0.06	-0.04	0.05	0.03	2.01	3.65**	0.15	3.65**	-0.15	-0.05	5.78	12.51	-3.69	9.42**
18 X 12	0.10	-0.04	-0.03	1.03	-0.53	-2.87	0.33	-2.87	-0.46	-0.70**	1.79	-3.60	-44.78	12.75**
15 X 32	-0.34	-0.05	0.32	1.31**	-1.07	-1.84	-0.08	-1.84	-0.73**	-0.07	-19.87	-46.94	36.43	10.97
4 X 31	-0.22	-0.05	0.26	0.22	-0.85	-3.26	0.49	-3.26	-0.24	-0.05	4.43	-19.15	31.04	15.33**
6 X 32	0.74**	-0.05	0.04	-1.15	-1.78	-3.87**	-0.22	-3.87**	0.25	0.38**	0.33	-2.66	-50.83	-14.55**
20 X 12	-0.19	-0.06	-0.01	-1.18**	1.56	5.14**	-0.19	5.14**	0.23	0.02	-7.84	-10.60	41.96	-4.47
20 X 17	-0.31	-0.07	0.69**	2.51**	-0.65	-1.64	0.13	-1.64	-0.07	-0.68**	-1.97	-1.57	3.75	9.06
23 X 10	0.84**	-0.07	0.32	0.34	-2.01	-1.01	-0.66	-1.01	-0.90**	-0.53	-24.26**	-44.23	-83.99**	8.32
20 X 9	-0.63	-0.07	0.43	2.37**	0.32	-1.71	0.43	-1.71	-0.56**	0.11	-14.78	-38.65	10.43	18.05**
4 X 37	-0.25	-0.08	-0.48	0.76	1.24	2.93	-0.57	2.93	0.10	0.11	-0.54	12.71	19.03	0.71
23 X 32	0.04	-0.08	0.14	1.09	0.50	0.78	0.35	0.78	-0.48**	-0.05	-17.50	-15.57	22.34	16.61**
6 X 40	-0.26	-0.09	0.14	2.18**	1.03	5.38**	0.45	5.38**	-0.09	0.00	-5.41	-3.93	-84.16**	14.02**

*, **,*** - significantly different at $p \leq 0.05$, $p \leq 0.01$ and ≤ 0.001 respectively, GY - grain yield (t ha^{-1}), ASI - anthesis silking interval, DA - days to anthesis (days), DM - days to maturity (days), EPP - ears per plant (count number), PH - plant height (cm), G_s - Stomatal conductance, OPT - optimum environment, MD - managed drought.

6.4.4 Variance components and heritability estimates

The estimates of variance components and heritability for the seven phenotypic traits of the 64 provitamin A maize hybrids evaluated across four optimum and two drought stressed environments are presented in Table 6.9. Variance for male general combining ability (σ^2GCA_m) were greater than that attributed to the females (σ^2GCA_f) for ASI and EPP under both environmental conditions. Conversely, higher σ^2GCA_f than σ^2GCA_m were observed under optimum conditions for DA and DM. Specific combining ability variance σ^2SCA was higher than pooled GCA variance for all traits under both environmental conditions except for DM and DA under optimum conditions. Most traits had a Bakers' ratio [Pooled σ^2GCA /(σ^2SCA + pooled σ^2GCA)] of less than 0.5 across both conditions except DA and DM which had ratios of 0.54 and 0.509, respectively, across optimum conditions (Baker, 1978). The degree of dominance $[\sigma^2_D/\sigma^2_A]^{0.5}$ ratio was greater than a unit for all the traits across both environments except for AD and DM, which had 0.937 and 0.933, respectively, under optimum conditions.

Broad sense heritability (H^2) ranged from 0.317 to 0.795 under optimum conditions and from 0.122 to 0.681 under drought conditions. Narrow sense heritability (h^2) varied from 0.024 to 0.410 and from 0.017 to 0.195 under optimum and drought conditions, respectively among the measured traits. Grain yield exhibited low h^2 of 0.140 under optimum conditions and very low h^2 of 0.024 under drought conditions. Most secondary traits exhibited greater heritability values under drought, conditions as compared to under optimum conditions except for DA and DM.

6.4.5 Heterosis estimations

Heterosis analysis revealed that all the 64 crosses had positive significant ($p \leq 0.001$) mid parent heterosis (MPH) across both optimum and drought environments (Table 6.10). Higher mean MPH of 347.25% was observed under optimum conditions than under drought conditions (265.33%). The MPH ranged from 129.63% to 879.11% and from 76.09% to 595.21% under optimum and drought conditions, respectively. Hybrid 44 (19 X 17) exhibited highest heterosis (879.11%) under optimum conditions whilst hybrid 13 (6 X 31) had the highest heterosis under drought conditions. Only one cross 55 (20 X 37) exhibited positive standard heterosis (best check heterosis) under optimum conditions whilst the rest had negative values. Similarly, hybrid 34 (18 X 10) had the only positive standard heterosis across drought environments (Table of results not presented).

Table 6.11: Variance components and heritability estimates for the measured traits assessed on 64 hybrids across drought and optimum environments.

	GY		ASI		DA		DM		EPP		PH		Gs	
	OPT	DM	OPT	DM	OPT	DM	OPT	DM	OPT	DM	OPT	DM	OPT	DS
σ^2_{GCAm}	0.191	0.004	0.042	0.667	2.359	3.556	5.615	3.556	0.043	0.016	58.658	168.511	439.126	19.128
σ^2_{GCAf}	0.048	0.011	0.003	0.366	3.482	7.912	3.292	7.912	0	0	46.712	173.838	11.873	54.707
σ^2GCApooled	0.239	0.015	0.045	1.033	5.842	11.468	8.907	11.468	0.043	0.016	105.37	342.349	450.999	73.835
σ^2_{SCA}	0.448	0.029	0.15	1.649	4.811	16.555	8.582	16.555	0.178	0.214	361.981	1097.453	2322.247	112.021
$[\sigma^2GCA_{pooled}/(\sigma^2_{SCA}+\sigma^2GCA_{pooled})]$	0.348	0.341	0.231	0.385	0.548	0.409	0.509	0.409	0.195	0.070	0.225	0.238	0.163	0.397
σ^2_A	0.217	0.012	0.017	0.051	2.142	0.179	4.238	0.179	0.012	0.001	36.918	26.31	128.857	0.594
σ^2_D	0.518	0.049	0.024	0.102	1.88	1.019	3.686	1.019	0.02	0.039	43.386	32.691	1001.371	6.771
σ^2_P	1.555	0.5	0.13	0.238	5.226	4.888	9.963	4.863	0.101	0.063	208.75	134.988	2508.025	10.819
$[\sigma^2_D / \sigma^2_A]^{0.5}$	1.545	2.021	1.189	1.416	0.937	2.387	0.933	2.387	1.329	5.874	1.084	1.115	2.788	3.377
σ^2_e	0.82	0.439	0.089	0.085	1.204	3.69	2.039	3.665	0.069	0.023	128.446	75.987	1377.797	3.454
H^2	0.473	0.122	0.317	0.642	0.77	0.245	0.795	0.246	0.317	0.629	0.385	0.437	0.451	0.681
h^2	0.140	0.024	0.131	0.214	0.41	0.037	0.425	0.037	0.115	0.018	0.177	0.195	0.051	0.055

GY - grain yield, ASI - anthesis silking interval, DA - days to anthesis, DM - days to maturity, EPP - ears per plant, PH - plant height, G_s - Stomatal conductance, OPT - optimum environment, MD - managed drought environment, Env - environment, σ^2_{gcam} - GCA general combining ability variance attributable to males, σ^2_{gcaf} - general combining ability variance attributable to females, σ^2GCA_{pooled} - pooled GCA variance combined from the male and female GCA variances; σ^2sca - variance of the specific combining ability (SCA), σ^2_A – additive variance, σ^2_D - dominance variance, σ^2_e – environmental variance, [σ²gca/ (σ²gca+σ²sca)] - GCA over SCA ratio, $[\sigma^2_D/\sigma^2_A]^{0.5}$ - degree of dominance, H^2 - broad sense heritability and h^2 - narrow sense heritability

Table 6.12: Estimates of mid-parent heterosis for grain yield of provitamin A maize experimental hybrids.

Hybrid Entry	Full cross name	Entry cross	GY (t ha^{-1})		MPH	
			OPT	MD	OPT	MD
1	CLHP0312 X CLHP00307	4 X 9	7.64	2.87	427.18**	309.37**
2	CLHP0312 X CLHP00322	4 X 10	6.12	2.55	409.90**	264.69**
3	CLHP0312 X CLHP00432	4 X 12	8.08	3.62	389.55**	244.35**
4	CLHP0312 X CLHP0310	4 X 17	5.87	2.82	551.70**	413.21**
5	CLHP0312 X CML486	4 X 31	7.42	4.16	429.95**	540.29**
6	CLHP0312 X CML451	4 X 32	5.71	2.19	268.51**	157.37**
7	CLHP0312 X TZM113	4 X 37	6.18	2.26	489.01**	223.44**
8	CLHP0312 X TZM112	4 X 40	6.23	2.20	236.74**	76.09**
9	CLHP00478 X CLHP00307	6 X 9	6.59	1.85	387.98**	209.07**
10	CLHP00478 X CLHP00322	6 X 10	5.99	2.44	444.24**	306.35**
11	CLHP00478 X CLHP00432	6 X12	6.34	3.59	308.87**	277.83**
12	CLHP00478 X CLHP0310	6 X 17	5.22	2.49	551.95**	454.44**
13	CLHP00478 X CML486	6 X 31	7.60	3.82	484.74**	595.21**
14	CLHP00478 X CML451	6 X 32	5.35	2.50	268.91**	233.45**
15	CLHP00478 X TZM113	6 X 37	7.86	2.07	727.38**	244.21**
16	CLHP00478 X TZM112	6 X 40	6.41	3.35	266.50**	191.48**
17	CLHP00378 X CLHP00307	11 X 9	8.15	4.48	352.80**	397.55**
18	CLHP00378 X CLHP00322	11 X 10	6.17	3.96	298.25**	340.45**
19	CLHP00378 X CLHP00432	11 X 12	5.95	3.90	197.61**	211.81**
20	CLHP00378 X CLHP0310	11 X 17	7.36	3.44	489.04**	358.34**
21	CLHP00378 X CML486	11 X 31	4.39	3.34	150.86**	292.97**
22	CLHP00378 X CML451	11 X 32	5.11	3.52	168.71**	235.20**
23	CLHP00378 X TZM113	11 X 37	5.58	3.06	298.53**	239.77**
24	CLHP00378 X TZM112	11 X 40	7.42	4.02	237.43**	177.44**
25	CLHP0350 X CLHP00307	15 X 9	7.96	3.79	288.33**	279.07**
26	CLHP0350 X CLHP00322	15 X 10	7.86	2.77	336.48**	177.18**
27	CLHP0350 X CLHP00432	15 X 12	6.55	3.14	190.90**	132.85**
28	CLHP0350 X CLHP0310	15 X 17	6.43	3.06	328.55**	260.15**
29	CLHP0350 X CML486	15 X 31	7.45	2.99	272.44**	214.45**
30	CLHP0350 X CML451	15 X 32	6.79	3.17	215.60**	175.87**
31	CLHP0350 X TZM113	15 X 37	5.62	2.34	240.38**	133.64**
32	CLHP0350 X TZM112	15 X 40	5.65	3.36	130.67**	116.81**
33	CLHP0352 X CLHP00307	18 X 9	8.32	3.17	362.09**	296.65**
34	CLHP0352 X CLHP00322	18 X 10	6.97	3.24	349.37**	304.44**
35	CLHP0352 X CLHP00432	18 X 12	7.19	4.71	259.35**	309.94**
36	CLHP0352 X CLHP0310	18 X 17	6.99	2.51	459.00**	286.18**
37	CLHP0352 X CML486	18 X 31	6.39	2.86	265.12**	280.72**
38	CLHP0352 X CML451	18 X 32	7.45	3.17	291.86**	233.17**

Table 6.13 continued…

Hybrid Entry	Full cross name	Entry cross	GY (t ha^{-1})		MPH	
			OPT	MD	OPT	MD
39	CLHP0352 X TZM113	18 X 37	5.34	3.62	281.21**	352.69**
40	CLHP0352 X TZM112	18 X40	8.35	4.19	279.54**	210.50**
41	CLHP00294 X CLHP00307	19 X 9	7.31	3.28	441.12**	368.19**
42	CLHP00294 X CLHP00322	19 X 10	7.14	2.46	548.92**	250.96**
43	CLHP00294 X CLHP00432	19 X 12	8.66	3.19	458.95**	203.34**
44	CLHP00294 X CLHP0310	19 X 17	7.83	2.76	879.11**	401.16**
45	CLHP00294 X CML486	19 X 31	5.50	3.47	323.00**	433.12**
46	CLHP00294 X CML451	19 X 32	6.18	2.67	325.97**	214.41**
47	CLHP00294 X TZM113	19 X 37	5.39	3.91	467.14**	458.06**
48	CLHP00294 X TZM112	19 X 40	6.46	3.49	269.23**	179.01**
49	CLHP0364 X CLHP00307	20 X9	6.28	3.33	221.99**	216.84**
50	CLHP0364 X CLHP00322	20 X 10	4.65	1.86	173.82**	77.56**
51	CLHP0364 X CLHP00432	20 X 12	5.86	3.48	172.67**	148.72**
52	CLHP0364 X CLHP0310	20 X 17	5.59	2.02	298.94**	124.91**
53	CLHP0364 X CML486	20 X 31	5.22	2.16	174.54**	116.14**
54	CLHP0364 X CML451	20 X 32	4.71	2.66	129.63**	121.98**
55	CLHP0364 X TZM113	20 X 37	6.50	3.43	319.58**	226.59**
56	CLHP0364 X TZM112	20 X 40	8.03	4.24	241.81**	164.89**
57	CLHP0058 X CLHP00307	23 X 9	9.28	4.26	614.00**	555.48**
58	CLHP0058 X CLHP00322	23 X 10	6.10	2.69	480.78**	313.51**
59	CLHP0058 X CLHP00432	23 X 12	4.45	2.34	196.52**	134.32**
60	CLHP0058 X CLHP0310	23 X 17	5.44	2.78	625.39**	455.88**
61	CLHP0058 X CML486	23 X 31	4.89	2.66	291.57**	342.90**
62	CLHP0058 X CML451	23 X 32	6.27	3.70	348.06**	362.17**
63	CLHP0058 X TZM113	23 X 37	6.06	1.85	573.50**	184.64**
64	CLHP0058 X TZM112	23 X 40	6.13	3.21	260.47**	167.48**
Mean			6.50	3.10	347.25	265.33
Max			9.28	4.71	879.12	595.21
Min			4.39	4.71	129.63	76.08

GY - grain yield, MPH - mid-parent heterosis, *** - significant at $p \leq 0.001$, OPT – optimum environment, MD – managed drought environment.

6.5 Discussion

The presence of significant genetic variability indicates the potential value of the breeding programme. High significance of hybrid mean square values observed in this study for all the traits across both environments indicates the presence of high variation among the experimental hybrids and the differential response of the hybrids in the different environments.

On the other hand, the observed significant interaction between hybrids and the environment for GY, DA, DM, PH and Gs under both conditions indicates changes in rank order of the hybrids in each environment. This was also confirmed by the significance of mean of squares for GCA_m*Env, GCA_f*Env and SCA*Env. The environmental influence could be largely due to the water-stress treatments imposed in addition to soil fertility and temperature variations. The presence of genotype x environment interaction (GEI) complicates selections and recommendations of superior genotypes as breeders have to decide whether to breed for specific or broad adaptability (Badu-Apraku et al., 2011). This, therefore, necessitates the implementation of multi-environment trials (MET) to dissect the GEI effect and breeding for multiple trait tolerance, that is, drought, heat and low soil fertility tolerance (Bänziger and Cooper, 2001; Cairns et al., 2013). The presence of GEI observed in this study is consistent with findings by Makumbi et al. (2011).

The high significance of GCA_m, GCA_f and SCAs mean squares for all the measured traits across both optimum and drought environments indicated the importance of both additive and non-additive gene action in controlling the traits under both environmental conditions. Additionally, the significance of mean squares for GCA_m and GCA_f showed a significant genetic difference among the male and female parents, respectively. On the other hand, the significance of SCA mean squares infers that non-additive gene action is important for this set of germplasm. These observations are in line with findings in previous researches in maize drought tolerance breeding (Betran et al., 2003; Derera et al., 2007).

Variance component analysis showed that dominance variance (σ^2_D) was greater than additive variance (σ^2_A) for most traits under both environmental condition except for DA and DM under optimum conditions. This indicates the predominance of non-additive gene action in controlling GY, ASI, EPP, PH and Gs under both optimum and drought stressed environments whilst DA and DM under optimum conditions were predominantly controlled by additive gene action. The presence of few parental inbred lines with significant GCA effects as compared to the number of hybrids with significant SCA effects indicates that the GCA of the lines cannot be used to predict the performance of a hybrid; the cross has to be made. These findings are in contrast with some of the previous research on abiotic stress tolerance (Betran et al., 2003; Derera et al., 2007; Makumbi et al., 2011), who reported the predominance of additive gene action for most traits including grain yield under drought stress. Our findings agree with Mhike et al. (2011) and Murtadha et al. (2018) who reported the predominance of non-additive gene action

in the control of GY and some secondary traits (ASI, EPP and PH) under both optimum and drought conditions.

The predominance of dominance variance over additive variance observed in the current study could be attributed to high heterozygosity of the single cross hybrids brought about by mating genetically divergent parental inbred lines, given that the parents were drawn from different molecular clusters as described in chapter 5. This is also reflected by the observed high MPH and the generally high grain yield. The observed MPH is far higher than the one reported by Makumbi et al. (2011).

According to heritability classifications given by Dabholkar (1992), the majority of heritabilities observed in this study falls within low to moderate except for DA and DM that exhibited higher heritability under optimum conditions of 0.770 and 0.795, respectively. The observed very low to moderate low broad sense and narrow sense heritability values for GY, ASI, EPP, and PH under both optimum and drought stress conditions could be attributed to the influence of non-additive gene action for these traits. On the other hand, the observed higher heritability estimates for DA and DM under optimum conditions could be due to the influence of additive gene action as these traits exhibited lower degree of dominance values. Broad sense heritability estimates of 0.473 and 0.122 were observed in the current study for GY under optimum and drought conditions, respectively, while the narrow sense heritability was 0.140 and 0.024 under optimum and drought conditions, respectively. This shows great influence of the environment on the expression of these traits. These values are lower than values reported by Bolaños and Edmeades (1996) of 0.6 and 0.4 under optimum and drought conditions, respectively.

The deviation of our results from some of the earlier reported findings may be attributed to differences in the genetic constitution of the germplasm used and the environment in which they were evaluated. For instance, as pointed earlier on, the parental lines used in this study are widely genetically divergent as they were selected from different molecular clusters in chapter 5. Yields observed in the current study by the experimental hybrids especially under optimum conditions are higher than most of the reported yields (Magorokosho et al., 2003; Bankole et al., 2017; Masuka et al., 2017). Apart from genetic superiority of the materials used herein emanating from high parent divergence, the difference could be attributed to environmental differences. For instance, environments like DR&SS and Makhatini are highly productive due to high rainfall and high soil fertility status, respectively.

6.6 Conclusion

Predominantly non-additive gene action controlled GY, ASI, PH, Gs and EPP under both conditions whilst AD and DM was predominantly controlled by additive gene effect under optimum conditions and by non-additive gene effect under drought stress conditions. Crosses, 34 (CLHP0352 X CLHP00322), 42 (CLHP00294 X CLHP00322), 4 (CLHP0312 X CLHP0310), 64 (CLHP0312 X CLHP0310), 55 (CLHP0364 X TZM113) and 46 (CLHP00294 X CML451), were identified as promising hybrids by consistently exhibiting significant SCA effects and MPH heterosis for grain yield and other secondary. They are therefore, recommended for screening for provitamin A content. They can also be utilised in the development of three-way hybrids.

The lines that exhibited high significant GCA effects should be considered as suitable potential parental inbred lines for population development in drought tolerant breeding programmes. These are 37 (TZM113), 19 (CLHP00294), 18 (CLHP0352), 19 (CLHP00294), 23 (CLHP0058), 11 (CLHP00432) and 20 (CLHP0364). They can also be utilised as donor lines in backcross breeding programmes donating genes responsible for the traits they had high GCA effects. Thus, line 37 (TZM113) can be utilised as donor parent for reduced ASI while lines 19 (CLHP00294), 18 (CLHP0352) and 23 (CLHP0058) can be used for donating earliness.

6.7 References

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6.8 Appendices

Appendix 6.1: The complete 8*8 NCD II crossing plan with 6 checks.

Female entry #	Female name	Male entry #	Male name	Entry crosses	Hybrid	Hybrid Entry#
4	CLHP0312	9	CLHP00307	4 X 9	CLHP0312 X CLHP00307	1
4	CLHP0312	10	CLHP00322	4 X 10	CLHP0312 X CLHP00322	2
4	CLHP0312	12	CLHP00432	4 X 12	CLHP0312 X CLHP00432	3
4	CLHP0312	17	CLHP0310	4 X 17	CLHP0312 X CLHP0310	4
4	CLHP0312	31	CML486	4 X 31	CLHP0312 X CML486	5
4	CLHP0312	32	CML451	4 X 32	CLHP0312 X CML451	6
4	CLHP0312	37	TZM113	4 X 37	CLHP0312 X TZM113	7
4	CLHP0312	40	TZM112	4 X 40	CLHP0312 X TZM112	8
6	CLHP00478	9	CLHP00307	6 X 9	CLHP00478 X CLHP00307	9
6	CLHP00478	10	CLHP00322	6 X 10	CLHP00478 X CLHP00322	10
6	CLHP00478	12	CLHP00432	6 X 12	CLHP00478 X CLHP00432	11
6	CLHP00478	17	CLHP0310	6 X 17	CLHP00478 X CLHP0310	12
6	CLHP00478	31	CML486	6 X 31	CLHP00478 X CML486	13
6	CLHP00478	32	CML451	6 X 32	CLHP00478 X CML451	14
6	CLHP00478	37	TZM113	6 X 37	CLHP00478 X TZM113	15
6	CLHP00478	40	TZM112	6 X 40	CLHP00478 X TZM112	16
11	CLHP00378	9	CLHP00307	11 X 9	CLHP00378 X CLHP00307	17
11	CLHP00378	10	CLHP00322	11 X 10	CLHP00378 X CLHP00322	18
11	CLHP00378	12	CLHP00432	11 X 12	CLHP00378 X CLHP00432	19
11	CLHP00378	17	CLHP0310	11 X 17	CLHP00378 X CLHP0310	20
11	CLHP00378	31	CML486	11 X 31	CLHP00378 X CML486	21
11	CLHP00378	32	CML451	11 X 32	CLHP00378 X CML451	22
11	CLHP00378	37	TZM113	11 X 37	CLHP00378 X TZM113	23
11	CLHP00378	40	TZM112	11 X 40	CLHP00378 X TZM112	24
15	CLHP0350	9	CLHP00307	15 XL9	CLHP0350 X CLHP00307	25
15	CLHP0350	10	CLHP00322	15 X 10	CLHP0350 X CLHP00322	26
15	CLHP0350	12	CLHP00432	15 X 12	CLHP0350 X CLHP00432	27
15	CLHP0350	17	CLHP0310	15 X 17	CLHP0350 X CLHP0310	28
15	CLHP0350	31	CML486	15 X 31	CLHP0350 X CML486	29
15	CLHP0350	32	CML451	15 X 32	CLHP0350 X CML451	30
15	CLHP0350	37	TZM113	15 X 37	CLHP0350 X TZM113	31
15	CLHP0350	40	TZM112	15 X 40	CLHP0350 X TZM112	32
18	CLHP0352	9	CLHP00307	18 X 9	CLHP0352 X CLHP00307	33
18	CLHP0352	10	CLHP00322	18 X 10	CLHP0352 X CLHP00322	34
18	CLHP0352	12	CLHP00432	18 X12	CLHP0352 X CLHP00432	35
18	CLHP0352	17	CLHP0310	18 X 17	CLHP0352 X CLHP0310	36
18	CLHP0352	31	CML486	18 X 31	CLHP0352 X CML486	37
18	CLHP0352	32	CML451	18 X 32	CLHP0352 X CML451	38

Appendix 6.1 continued….

Female entry #	Female name	Male entry #	Male name	Entry crosses	Hybrid	Hybrid Entry#
18	CLHP0352	37	TZM113	18 X 37	CLHP0352 X TZM113	39
18	CLHP0352	40	TZM112	18 X 40	CLHP0352 X TZM112	40
19	CLHP00294	9	CLHP00307	19 X 9	CLHP00294 X CLHP00307	41
19	CLHP00294	10	CLHP00322	19 X 10	CLHP00294 X CLHP00322	42
19	CLHP00294	12	CLHP00432	19 X 12	CLHP00294 X CLHP00432	43
19	CLHP00294	17	CLHP0310	19 X 17	CLHP00294 X CLHP0310	44
19	CLHP00294	31	CML486	19 X 31	CLHP00294 X CML486	45
19	CLHP00294	32	CML451	19 X 32	CLHP00294 X CML451	46
19	CLHP00294	37	TZM113	19 X 37	CLHP00294 X TZM113	47
19	CLHP00294	40	TZM112	19 XL40	CLHP00294 X TZM112	48
20	CLHP0364	9	CLHP00307	20 X9	CLHP0364 X CLHP00307	49
20	CLHP0364	10	CLHP00322	20 X 10	CLHP0364 X CLHP00322	50
20	CLHP0364	12	CLHP00432	20 X 12	CLHP0364 X CLHP00432	51
20	CLHP0364	17	CLHP0310	20 XL17	CLHP0364 X CLHP0310	52
20	CLHP0364	31	CML486	20 X 31	CLHP0364 X CML486	53
20	CLHP0364	32	CML451	20 X 32	CLHP0364 X CML451	54
20	CLHP0364	37	TZM113	20 X 37	CLHP0364 X TZM113	55
20	CLHP0364	40	TZM112	20 X 40	CLHP0364 X TZM112	56
23	CLHP0058	9	CLHP00307	23 X 9	CLHP0058 X CLHP00307	57
23	CLHP0058	10	CLHP00322	23 X 10	CLHP0058 X CLHP00322	58
23	CLHP0058	12	CLHP00432	23 X 12	CLHP0058 X CLHP00432	59
23	CLHP0058	17	CLHP0310	23 X 17	CLHP0058 X CLHP0310	60
23	CLHP0058	31	CML486	23 X 31	CLHP0058 X CML486	61
23	CLHP0058	32	CML451	23 X 32	CLHP0058 X CML451	62
23	CLHP0058	37	TZM113	23 X 37	CLHP0058 X TZM113	63
23	CLHP0058	40	TZM112	23 X 40	CLHP0058 X TZM112	64
Drought tolerant check 1					WE4338	65
Drought tolerant check 2					WE4351	66
Drought tolerant check 3					WE4359	67
Drought tolerant check 4					SAHTB8-360	68
Drought tolerant check 5					SA4348	69
Drought susceptible check 6					SAHTB7-505	70

Appendix 6.2: Mean performance of 64 single cross experimental hybrids evaluated across four optimum and two drought stress environments.

Entry	GY		ASI		DA		DM		EPP		PH		Gs	
	OPT	DS	OPT	DS	OPT	DS	OPT	DS	OPT	DS	OPT	DS	OPT	DS
1	7.6	2.9	3.0	5.0	66.5	67.3	112.3	112.3	2.9	2.0	257.1	184.0	392.7	24.3
2	6.1	2.6	3.3	6.0	69.3	69.3	114.1	114.3	2.8	2.0	267.5	198.0	359.5	52.9
3	8.1	3.6	3.0	3.8	67.6	71.3	114.4	116.3	2.9	2.3	301.9	252.0	402.3	23.0
4	5.9	2.8	3.6	7.5	67.1	69.3	114.1	114.3	1.3	1.0	255.0	201.0	290.8	58.8
5	7.4	4.2	2.4	5.5	58.4	59.3	107.6	104.3	3.0	2.0	294.7	242.0	427.8	32.1
6	5.7	2.2	3.5	7.0	62.9	61.3	111.3	106.3	1.9	1.8	287.5	204.0	430.0	60.0
7	6.2	2.3	3.1	6.0	68.6	69.3	115.0	114.3	2.1	1.8	293.6	244.0	382.7	54.9
8	6.2	2.2	2.1	5.5	69.4	71.3	116.0	116.3	2.8	2.0	296.2	264.0	419.8	36.7
9	6.6	1.9	2.9	7.3	69.1	72.3	116.4	117.3	2.5	2.0	295.6	256.0	252.4	45.8
10	6.0	2.4	3.1	7.3	65.9	69.3	110.9	114.3	2.9	2.3	307.1	269.5	433.2	46.5
11	6.3	3.6	3.0	5.3	68.0	70.3	115.0	115.3	2.6	2.3	297.2	275.5	474.9	26.5
12	5.2	2.5	3.1	7.0	62.4	60.3	109.9	105.3	1.4	1.0	272.5	220.5	378.7	37.7
13	7.6	3.8	2.6	4.0	66.6	65.3	112.3	110.3	2.5	1.3	312.0	275.5	390.0	27.1
14	5.3	2.5	3.4	9.3	64.4	61.8	110.1	106.8	1.6	1.0	274.3	222.3	314.8	44.9
15	7.9	2.1	3.1	5.0	62.0	59.8	112.1	104.8	2.6	2.3	292.3	244.3	305.3	24.3
16	6.4	3.4	2.8	4.3	66.4	60.8	111.8	105.8	2.9	2.3	295.7	257.3	316.6	22.1
17	8.2	4.5	1.9	2.0	70.8	64.8	116.1	109.8	3.0	2.0	328.9	281.3	380.8	17.0
18	6.2	4.0	2.9	3.8	69.4	65.8	114.9	110.8	1.8	1.0	302.8	271.3	379.3	23.7
19	6.0	3.9	3.5	3.5	68.0	62.8	113.3	107.8	3.0	2.0	299.3	276.3	426.2	23.1
20	7.4	3.4	2.6	3.8	66.6	57.8	111.9	102.8	2.9	2.0	310.5	287.3	402.7	22.0
21	4.4	3.3	3.5	4.3	65.0	58.8	113.4	103.8	2.1	1.8	279.9	227.0	278.6	25.6
22	5.1	3.5	3.0	7.0	61.6	58.8	109.9	103.8	1.9	1.8	262.8	202.0	286.7	30.9
23	5.6	3.1	2.6	6.5	64.8	63.5	111.3	108.5	2.4	1.3	309.6	273.0	324.2	25.7
24	7.4	4.0	3.0	4.5	71.6	74.5	118.0	119.5	3.0	2.0	262.7	201.0	376.8	25.6

Appendix 6.2 continued….

Entry	GY		ASI		DA		DM		EPP		PH		Gs	
	OPT	DS	OPT	DS	OPT	DS	OPT	DS	OPT	DS	OPT	DS	OPT	DS
25	8.0	3.8	0.8	2.0	71.6	72.5	115.8	117.5	2.9	2.3	322.4	291.0	469.5	20.0
26	7.9	2.8	2.8	3.5	66.6	65.5	113.4	110.5	2.6	3.0	294.7	251.0	293.8	21.4
27	6.5	3.1	3.1	4.5	63.3	59.5	110.5	104.5	3.0	2.3	274.6	219.0	434.1	30.0
28	6.4	3.1	3.4	5.5	62.6	60.5	108.4	105.5	2.1	2.0	300.4	253.8	304.5	28.1
29	7.4	3.0	2.1	4.5	62.8	61.0	110.0	106.0	2.9	2.0	290.7	237.8	342.8	27.4
30	6.8	3.2	2.9	5.5	60.3	61.0	106.8	106.0	2.4	1.3	276.6	220.8	351.7	31.3
31	5.6	2.3	3.5	6.5	61.4	60.0	106.3	105.0	1.4	1.8	265.0	194.8	443.8	45.7
32	5.7	3.4	2.8	3.5	61.0	61.0	109.4	106.0	2.6	2.0	308.5	271.8	467.0	28.3
33	8.3	3.2	2.9	3.5	67.9	71.3	112.5	116.3	3.0	2.8	322.7	268.8	390.5	22.7
34	7.0	3.2	3.4	4.8	58.6	61.3	107.1	106.3	2.3	2.3	290.7	237.8	460.7	40.3
35	7.2	4.7	3.1	3.8	69.3	73.3	115.3	118.3	2.6	1.3	317.0	280.8	297.8	27.4
36	7.0	2.5	3.0	6.0	65.3	64.3	112.6	109.3	1.6	1.0	301.8	252.3	315.7	47.3
37	6.4	2.9	2.9	5.5	66.1	65.3	112.5	110.3	2.0	1.3	270.2	200.3	330.7	48.3
38	7.4	3.2	2.6	4.5	65.4	67.3	112.3	112.3	2.8	3.0	308.3	259.3	432.0	26.2
39	5.3	3.6	3.0	4.5	65.0	64.3	112.8	109.3	2.6	2.0	315.0	269.3	462.9	31.2
40	8.3	4.2	2.1	3.8	72.8	75.3	117.9	120.3	2.5	2.0	320.4	280.0	506.4	23.0
41	7.3	3.3	2.3	4.0	70.9	73.3	117.3	118.3	2.9	2.0	357.2	323.0	419.7	32.0
42	7.1	2.5	2.8	5.5	65.8	65.8	111.3	110.8	3.1	1.3	247.5	170.0	423.0	32.7
43	8.7	3.2	2.5	4.0	73.8	74.8	120.5	119.8	2.8	2.0	303.4	259.0	464.7	25.2
44	7.8	2.8	3.1	3.8	73.6	77.8	117.9	122.8	2.4	2.0	310.3	312.0	333.5	28.5
45	5.5	3.5	2.8	5.3	72.3	75.8	116.5	120.8	2.5	2.0	341.7	326.0	425.5	31.6
46	6.2	2.7	3.6	6.5	66.4	62.8	110.9	107.8	2.0	1.0	283.6	240.0	350.8	49.4
47	5.4	3.9	3.3	6.3	66.9	63.8	115.1	108.8	2.3	1.0	300.0	275.0	434.0	58.8
48	6.5	3.5	3.6	4.8	70.3	69.8	116.4	114.8	2.8	1.0	348.4	327.0	391.2	31.2

Appendix 6.2 continued…...

Entry	GY		ASI		DA		DM		EPP		PH		Gs	
	OPT	DS	OPT	DS	OPT	DS	OPT	DS	OPT	DS	OPT	DS	OPT	DS
49	6.3	3.3	2.8	5.8	66.3	63.8	114.0	108.8	2.3	2.0	285.9	245.3	345.7	58.2
50	4.7	1.9	3.8	8.5	66.0	62.8	112.8	107.8	1.6	2.0	260.1	186.3	405.5	66.9
51	5.9	3.5	2.9	6.0	64.4	66.8	110.3	111.8	2.1	1.8	253.5	209.3	376.5	47.6
52	5.6	2.0	3.1	4.8	68.3	69.8	116.0	114.8	2.5	1.8	275.9	229.3	446.8	43.5
53	5.2	2.2	4.1	8.8	63.6	61.8	111.9	106.8	2.3	1.0	285.0	236.3	390.2	57.1
54	4.7	2.7	3.1	9.5	64.1	60.8	111.9	105.8	1.6	2.0	263.9	201.3	333.7	62.4
55	6.5	3.4	3.0	4.5	66.4	63.5	112.9	108.5	3.0	2.0	278.9	204.3	434.0	47.7
56	8.0	4.2	2.6	2.0	67.0	66.5	112.1	111.5	3.0	2.8	330.4	314.3	426.2	18.6
57	9.3	4.3	2.8	3.0	64.3	59.0	112.0	104.0	2.8	2.8	315.5	270.3	437.0	21.0
58	6.1	2.7	2.8	4.0	65.4	61.0	113.5	106.0	1.9	2.0	275.7	227.3	458.8	19.4
59	4.4	2.3	3.6	4.8	60.4	62.0	107.3	107.0	1.3	1.3	260.2	208.3	260.0	37.0
60	5.4	2.8	3.3	4.8	61.3	61.0	107.6	106.0	2.8	2.0	318.9	275.0	370.1	24.5
61	4.9	2.7	3.9	6.5	58.9	60.0	106.1	105.0	1.4	1.8	288.4	253.0	343.9	43.8
62	6.3	3.7	3.3	5.0	60.5	59.0	109.9	104.0	2.3	2.0	307.0	274.0	402.4	21.7
63	6.1	1.9	3.4	6.5	62.9	61.0	109.1	106.0	1.6	1.8	270.0	233.8	423.2	52.7
64	6.1	3.2	3.6	4.0	63.4	61.0	108.8	106.0	2.5	2.0	324.5	312.8	447.8	24.3
Checks														
65	8.6	3.8	3.0	4.0	68.5	67.3	102.0	112.3	3.0	2.0	288.0	268.8	406.6	22.7
66	8.9	3.7	2.4	3.8	66.6	64.3	100.9	109.3	3.0	2.0	268.6	232.8	473.5	26.3
67	8.3	4.1	1.6	4.5	66.0	71.3	99.1	116.3	2.5	2.0	309.3	272.8	362.3	26.4
68	8.1	3.2	3.1	4.0	65.5	67.3	117.1	112.3	2.8	2.0	301.5	267.8	466.8	27.2
69	8.7	4.6	0.1	2.3	68.4	71.3	101.5	116.3	3.0	2.0	345.8	311.8	463.3	30.5
70	5.7	1.4	0.9	3.8	75.6	77.8	106.8	122.8	2.3	1.8	306.8	265.8	519.5	28.4
Mean	6.7	3.1	2.9	5.0	66.1	65.6	111.8	110.6	2.4	1.9	294.9	250.7	391.0	34.4

Appendix 6.2 continued….

Entry	GY		ASI		DA		DM		EPP		PH		Gs	
	OPT	DS	OPT	DS	OPT	DS	OPT	DS	OPT	DS	OPT	DS	OPT	DS
LSD	0.26	0.03	0.18	0.14	0.91	0.20	0.96	0.20	0.13	0.07	6.20	0.33	16.80	0.25
std	2.0	0.6	0.7	0.5	4.2	1.2	9.3	1.2	0.6	0.2	25.2	2.2	107.8	1.3
CV	16.6	3.4	26.0	12.0	5.9	1.3	3.7	7.5	23.3	16.8	8.9	0.6	18.2	3.1
Pvalue	***	***	**	*	***	**	*	ns	**	**	*	*	***	***
Max	10.7	4.7	4.1	9.5	75.6	77.8	120.5	122.8	3.1	3.0	357.2	327.0	519.5	66.9
Min	4.4	1.4	0.1	2.0	58.4	57.8	99.1	102.8	1.3	1.0	247.5	170.0	252.4	17.0

CHAPTER 7

General Overview and Implications of the Study

7.1 Introduction

The purpose of this chapter is to give an overview of the study, highlight the main findings and the implications for provitamin A maize and drought tolerance breeding. The following research hypotheses were tested:

I. There is adequate agro-morphological and molecular diversity among the available provitamin A inbred lines, which can be exploited to generate drought tolerant materials.
II. The available provitamin A inbred lines respond differently to drought stress.
III. Grain yield and secondary traits of the available inbred lines and their hybrid combinations are controlled by both additive and non-additive gene action when produced under optimum and drought stress conditions.

7.2 Summary of major research findings

7.2.1 Diversity analysis of provitamin A maize inbred lines using agro-morphological traits and β-carotene content

Forty-six elite provitamin A inbred lines sourced from CIMMYT and IITA were evaluated for agro-morphological performance and β-carotene content. Eleven traits were evaluated, which were: grain yield, β-carotene content, anthesis silking interval, days to anthesis, number of ears per plant, plant height, shelling percentage, root lodging, stem lodging and 100 seed kernel weight. The major findings were as follows:

- Provitamin A inbred lines exhibited high diversity with respect to β-carotene, grain yield and other agro-morphological traits.
- Inbred lines CLHP00306, CML451, CLHP0350 and CLH0364 exhibited both high β-carotene and grain yield.
- Phenotypic cluster analysis apportioned the genotypes into three clusters with cluster A dominated by genotypes with high β-carotene, cluster B had late maturing genotypes and cluster C was associated with early anthesis and few days to maturity.

- High heritability values (> 50%) were observed for grain yield, β-carotene and plant height whilst anthesis silking interval, number of ears per plant, plant stand, and shelling percentage had moderate heritability values (50% <H^2>30%). Stem lodging, root lodging, 100 kernel weight and days to anthesis had low heritability.
- There was no significant correlation between β-carotene and other traits.
- Grain yield was positively correlated to plant height, days to maturity, plant stand and shelling percentage but negatively correlated to anthesis silking interval.
- The highest observed phenotypic distance was 8.37 between inbred lines TZM114 and CLHPO331.

7.2.2 Diversity analysis of provitamin A maize inbred lines using single nucleotide polymorphism markers

Agro-morphological diversity analysis described above was complemented by molecular marker-based diversity analysis in which 3046 single nucleotide polymorphism (SNP) markers were used. The major findings were as follows:

- An average of 1.615 effective alleles per locus were detected with mean polymorphic information content of 0.433 ranging from 0.429 to 0.441.
- The mean gene diversity was 0.363 ranging from 0.351 to 0.372.
- Total variation was apportioned as 78% among individual genotypes, 12% among populations and 10% within individuals.
- Cluster analysis detected two distinct clusters, which were largely in accordance with the sources of genotypes.
- The average genetic distance observed was 0.59 ranging from 0.07 to 0.68.

7.2.3 Screening of provitamin A maize inbred lines for drought tolerance using β-carotene content, morpho-physiological and biochemical traits

Fifty inbred lines, which were made of the 46 provitamin A inbred lines and four drought tolerant checks (non-provitamin A) were screened for drought tolerance in the greenhouse and field in a total of three environments. Traits that were measured were: grain yield (GY), β-carotene (BCC), anthesis silking interval (ASI), plant height (PH), chlorophyll content (CC), leaf senescence (SEN), stomatal conductance (Gs), proline content (PC) and leaf rolling (LR). Screening was done under optimum and drought stress conditions. Drought stress was imposed at three weeks before 50% anthesis date until five weeks after 50% anthesis date

when one irrigation was provided. Analysis of variance (ANOVA), Pearson's correlation coefficient, principal component (PC) and stress index (SI) were computed. The major findings were as follows:

- The applied morpho-physiological and biochemical traits were effective in discriminating among genotypes and identifying promising drought tolerant provitamin A inbred lines.
- Proline content significantly increased to higher levels under drought conditions in tolerant genotypes indicating that it can be used in maize drought stress screening.
- There were significant correlations between GY and most of the traits.
- Principal component analysis showed that EPP, ASI, G_s and PH were the most influential traits.
- Twenty inbred lines with higher SI were selected as parents for the hybridisation programme.

7.2.4 Combining ability and variance components of provitamin A maize inbred lines under water stress and non-stress environments

Sixteen selected parents were crossed using North Carolina design II (NCD II). The F_1 hybrids were evaluated across two managed drought and four optimum field environments during the winter and summer periods of 2017/2018 seasons for combining ability, gene action and heterosis. The main findings were as follows:

- Inbred lines with desirable GCA values for the respective traits were: 37 (TZM113) for ASI under optimum conditions, 11 (CLHP00432) for Gs under drought conditions, 18 (CLHP0352) for DM under optimum conditions, 19 (CLHP00294) for DA under optimum conditions and 20 (CLHP0364) for DA under drought conditions.
- Crosses 34 (CLHP0352 X CLHP00322), 42 (CLHP00294 X CLHP00322), 4 (CLHP0312 X CLHP0310), 64 (CLHP0312 X CLHP0310), 55 (CLHP0364 X TZM113) and 46 (CLHP00294 X CML451) had high desirable SCA values for GY across both drought and optimum conditions.
- Both additive and non-additive gene action were important.
- Non-addititive gene action predominantly controlled GY, PH, EPP, Gs and ASI across both environments, and DM and DA under drought conditions only.

- Additive gene action was predominant for DA and DM under optimum conditions.
- Low to moderately low broad sense heritability (H^2) values were observed for GY and most of the other traits across both environmental conditions except for DA and MD under optimum conditions.
- All the experimental hybrids had higher positive mid parent heterosis (MPH), which ranged from 129.63% to 879.11% and from 76.09% to 595.21% across drought stressed and optimum environments, respectively.
- Hybrid 44 (CLHP00294 X CLHP0310) and 13 (CLHP00478 X CML486) exhibited the highest MPH across optimum and drought stressed environments, respectively.
- Hybrids 55 (CLHP0364 X TZM113) and 34 (CLHP0352 X CLHP00322) were the only ones with positive best check heterosis values under optimum and drought conditions, respectively.

7.3 Implications of findings for drought tolerance breeding of provitamin A maize and recommendations

Developing drought tolerant provitamin A maize has a potential to significantly complement other strategies in curbing vitamin A deficiency (VAD) among maize consumers and at the same time cushioning farmers from the negative impacts of drought. The detected high-level of genetic diversity at both phenotypic and molecular levels indicates the presence of high genetic variation that can be exploited by breeders in developing provitamin A maize with diverse traits. Furthermore, the genetic relationships determined by cluster analysis can assist breeders in identifying divergent parents and designing an effective hybridisation programme.

Additionally, the observed high heritability of grain yield and β-carotene of the inbred lines implies greater opportunity for selection of high yielding genotypes that also exhibit high β-carotene content. However, the expression of these traits except β-carotene is affected by environments. It is therefore, recommended to evaluate these materials over different seasons and across different locations to identify stable, best performing genotypes. Molecular cluster analysis results can also assist in selection of parents and grouping genotypes into heterotic groups for hybrid development programmes.

Plants adapt to drought stress through morphological, physiological and biochemical structures. The current study demonstrated that screening of maize inbred lines for drought

tolerance using a combination of morphological, physiological and biochemical based selection methods can increase selection efficiency, which can lead to higher genetic gain. This was evidenced by selection of parents that generated high yielding hybrids with high heterosis under both optimum and drought stress conditions. The significant correlation between grain yield and proline content under drought stressed conditions indicates that proline content might be an effective trait in maize drought-tolerance screening.

Information on the combining ability of the available breeding materials is important to hybrid maize breeders as it assists in important decisions on the materials. The observed significance of both GCA and SCA mean squares under both drought and optimum environments imply that superior lines and hybrids can be selected from the materials. Hybrids that exhibited high SCA values are recommended for provitamin A screening and stability testing. On the other hand, inbred lines, which were good general combiners for various traits can be used as parental lines in the hybrid breeding programme. Furthermore, good general combiners can also be utilised in backcross programmes in which they act as donors for genes responsible for the traits they had high desirable and significant GCA effects. Thus, line 37 (TZM113) can be utilised as donor parent for reduced ASI, which is an important aspect in ensuring good anthesis-silking synchronisation since it exhibited high negative GCA for ASI. Likewise, lines 19 (CLHP00294), 18 (CLHP0352) and 23 (CLHP0058) can be used for earliness breeding as they exhibited desirable negative GCA effects for AD and DM under drought conditions. Lines 11 (CLHP00432) and 20 (CLHP0364), which exhibited significant negative GCA effects for G_s can be utilised as donors of genes responsible for low Gs under drought conditions as an adaptation to reduce water loss under drought conditions. They can also be utilised in QTL mapping for drought tolerance.