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GENETIC ANALYSIS OF GRAIN YIELD AND OTHER TRAITS OF EARLY MATURING PROVITAMIN A- QUALITY PROTEIN MAIZE INBRED LINES UNDER DROUGHT AND LOW SOIL NITROGEN CONDITIONS

By

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DECLARATION

I hereby declare that with the exception of references to the work of other authors, which have been cited accordingly, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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ABSTRACT

Early maturing provitamin A (PVA) quality protein maize (QPM) hybrids with combined drought and low soil Nitrogen (low-N) tolerance are needed to address malnutrition and food insecurity problems in sub-Saharan Africa (SSA). In response to this need, PVA-QPM inbred lines with drought and low-N tolerant genetic backgrounds were developed through the concerted effort of the International Institute of Tropical Agriculture Maize Improvement Program (IITA-MIP) for hybrid development and commercialization. The objectives of the present study were to (i) identify drought and/ low-N tolerant inbred lines with elevated levels of PVA and quality protein, (ii) assess the extent of genetic variability of selected early maturing drought and low-N tolerant inbreds, (iii) study the combining ability of a set of inbreds for drought and/or low-N tolerance as well as PVA accumulation (iv) classify the set of inbred lines into heterotic groups and identify the best inbred and hybrid testers across the test environments, (v) assess yield and stability of hybrids across the contrasting environments, and (vi) validate PVA functional genes in the set of inbred lines. The genetic diversity in the inbreds were examined using the unweighted pair group method with arithmetic mean (UPGMA) clustering, the model-based structure analysis and the principal component analysis. Ninety-six hybrids generated from 24 inbreds using the North Carolina Design two (NCDII) plus four checks, as well as 70 inbreds including six checks were evaluated under drought, low-N and optimal environments in Nigeria from 2016 to 2017. Nineteen selected inbreds and 54 hybrids were assayed for PVA and tryptophan contents. Provitamin A candidate genes were validated in the inbreds using allele specific markers. Days to 50% anthesis and silking, plant and ear heights, and plant and ear aspects complemented grain yield of the set of inbred lines in identifying 33 of the 70 inbred lines for the genetic studies. Ninety-five percent of the inbreds had desirable levels of tryptophan (> 0.075%) in sample in whole grain implying that the inbreds generally met the quality standards

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of QPM genotypes. Moderate levels of PVA were recorded for the inbreds assayed indicating the need to increase the frequency of the favourable PVA alleles in the inbred lines. TZEIORQ 55 and TZEIORQ 29 combined low-N tolerance with adequate levels of tryptophan and had PVA contents of 15.38 and 12.10 µg g^{-1} respectively, while nine inbreds combined drought and low-N tolerance with adequate and moderate levels of tryptophan and PVA respectively. These inbred lines could be used in hybrid combinations to produce outstanding PVA-QPM hybrids. Five genetically distinct clusters were identified for the inbreds and the grouping was largely based on the pedigree of the set of inbred lines. Additive genetic effects were more important than non-additive for grain yield and most other agronomic traits under drought, low-N, optimal and across environments. Maternal effects were not significant for grain yield and most other agronomic traits under each and across environments, as well as the carotenoids and tryptophan quantified. The DArTseq markers were more efficient than the heterotic grouping based on general combining ability of multiple traits (HGCAMT) and identified three heterotic groups. The inbred TZEIORQ 29 was the best inbred tester either as a male or female for heterotic group I, while TZEIORQ 24 was a good combiner only as a male parent for heterotic group II. TZEIORQ 2 x TZEQI 82 was identified as the best single-cross hybrid tester across environments. The GGE-biplot analysis and the drought and low-N multiple trait base index (MI) consistently identified TZEIORQ 24 x TZEIORQ 41 as the highest yielding and most stable hybrid across stress and non-stress environments while TZEIORQ 29 x TZEIORQ 43 was the best hybrid under low-N conditions, and TZEIORQ 26 x TZEIORQ 47 was outstanding for combined drought and low-N tolerance. The combining ability study of PVA carotenoids revealed preponderance of additive genetic effects over non-additive for PVA and all measured carotenoids suggesting that superior hybrids could be produced by crossing parents with positive and significant GCA effects for PVA. All the 54 hybrids assayed had > 0.075% tryptophan per sample in whole grain and that the hybrids met

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the quality standards of QPM but none was higher than the check, "Obatanpa". The hybrid, TZEIORQ 29 x TZEIORQ 43 which had PVA content of 9.78 µg g^{-1}, was among the top hybrids including TZEIORQ 29 x TZEIORQ 40, TZEIORQ 29 x TZEIORQ 24, TZEIORQ 20 x TZEIORQ 29 and TZEIORQ 6 x TZEIORQ 29 interms of PVA levels and also combined good agronomic performance under drought, low-N and optimal conditions. These hybrids should be further tested to confirm consistency of performance and commercialized in SSA to combat the "hidden hunger" due to vitamin A deficiency and protein energy malnutrition in the sub-region. The PVA allele specific marker, *crtRB1-3'TE*, was the most polymorphic and was highly consistent with the KASP SNP (snpZM0015). The two markers identified eight inbreds containing favourable alleles of the *crtRB1* functional gene. These inbreds could serve as donor parents of favourable alleles for the *crtRB1* gene. Despite the moderate to high PVA contents of TZEIORQ 29 and TZEIORQ 55, they did not have the favourable alleles of *crtRB1* and *LcyE* genes implying that other genes were responsible for the increased levels of PVA in these inbreds. Moreover, the preponderance of additive genetic effects over non-additive in the inheritance of PVA accumulation and the significant positive GCA-male and female effects for PVA levels of TZEIORQ 29 indicated that TZEIORQ 29 and TZEIORQ 55 could contribute favourable alleles other than those of *crtRB1* and *LcyE* for improving PVA concentrations in hybrids and synthetics. The two inbreds would also be useful for the improvement of the early PVA-QPM inbred population for high PVA levels.

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DEDICATION

This work is dedicated to my dear wife Helena Obeng-Bio, our lovely son Marshall McNez Obeng-Bio and all my siblings especially, Florence Obeng-Bio, Frank Obeng-Bio and Grace Obeng-Bio. Finally, I dedicate this piece of work to my parents Mr. Francis Bio and Madam Comfort Eva Owusu of blessed memory.

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TABLE OF CONTENTS

University of Ghana http://ugspace.ug.edu.gh

University of Ghana http://ugspace.ug.edu.gh

University of Ghana http://ugspace.ug.edu.gh

University of Ghana http://ugspace.ug.edu.gh

LIST OF TABLES

University of Ghana http://ugspace.ug.edu.gh

University of Ghana http://ugspace.ug.edu.gh

University of Ghana http://ugspace.ug.edu.gh

LIST OF FIGURES

University of Ghana http://ugspace.ug.edu.gh

University of Ghana http://ugspace.ug.edu.gh

LIST OF PLATES

University of Ghana http://ugspace.ug.edu.gh

LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
AMMI	Additive main effect and multiplicative interaction
ATC	Average tester coordinate
BE	Breeding efficiency
CIMMYT	International Maize and Wheat Improvement Center
CRI	Crop Research Institute
DAP	Days after planting
DArTseq	Diversity Array Technology sequencing
DNA	Deoxyribonucleic acid
FAOSTAT	Food and Agriculture Organization Statistics
GCA	General combining ability
GD	Gene diversity
GEI	Genotype by environment interaction
GGE	Genotype plus genotype by environment interaction
GS	Genomic selection
HGCAMT	Heterotic grouping based on general combining ability of multiple traits
HPLC	High performance liquid chromatography
HSGCA	Heterotic group's specific and general combining ability
IAR	Institute of Agricultural Research
IER	Institute of Rural Economy
IGSS	Integrated Genotyping Service and Support
IITA	International Institute of Tropical Agriculture
INERA	Institute for Agricultural and Environmental Research
KASP	Kompetitive allele specific PCR
Low-N	Low soil nitrogen
MAS	Marker assisted selection
MCMC	Markov chain monte carlo
MET	Multi-environment trials
NCD II	North Carolina Design II
OPV	Open pollinated variety
PCoA	Principal component analysis

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PCR	Polymerase chain reaction
PEM	Protein energy malnutrition
PIC	Polymorphic information content
PVA	Provitamin A
QPM	Quality protein maize
QTL	Quantitative trait loci
RAPD	Randomly amplified polymorphic DNA
REML	Restricted maximum likelihood
RFLP	Restriction fragment length polymorphism
SARI	Savannah Agricultural Research Institute
SCA	Specific combining ability
SNP	Single nucleotide polymorphisms
SSA	sub-Saharan Africa
SSR	Simple sequence repeats
UPGMA	Unweighted pair group method with arithmetic average
VAD	Vitamin A deficiency
WA	West Africa
WAP	Weeks after planting
WCA	West and Central Africa
WHO	World Health Organization

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CHAPTER ONE

1.0 INTRODUCTION

Maize (*Zea mays* L.) is a major food crop in sub-Saharan Africa (SSA), serving more than 300 million people (Shiferaw *et al*., 2011; Okweche *et al*., 2013). In West Africa (WA), production of maize has been soaring over the years. In 2001-2005, 2006-2010 and 2011-2015, the average total maize production in WA was 10.2, 13.7 and 17.6 million tonnes respectively (FAOSTAT, 2016). However, the increases in the area harvested within the corresponding years were 6.8, 8.0 and 10.9 million hectares (FAOSTAT, 2016). Thus, the gradual increase in maize production was as a result of the expansion in the area under cultivation. However, yield increase is more desirable than area expansion for sustainable increase in maize production and productivity in SSA.

Considering the climatic and edaphic requirements of the maize crop, there is a greater potential for its production and productivity in the savanna belt of SSA due to the low night temperatures, low pest and diseases occurrence and high influx of solar radiation. However, grain yield of maize in farmers' fields in WA for example, has been consistently low, averaging 1.7 t ha^{-1} compared to 10.7 t ha^{-1} obtainable in North America (FAOSTAT, 2016). The low yields have been attributed to a number of biotic constraints such as *Striga hermonthica* (Del.) Benth parasitism and the fall army worm (*Spodoptera frugiperda*) invasion and abiotic factors such as recurrent drought and low-N. Studies have pointed out low-N and drought as the two major abiotic stresses reducing maize production in SSA (Kamara *et al*., 2005; Badu-Apraku *et al*., 2012a). This is because the stresses usually occur simultaneously in farmers' fields and the synergistic effects can lead to 100% yield loss (Badu-Apraku *et al*., 2011a). In SSA, maize is usually produced on small-scale basis by low

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input farmers who cannot afford to irrigate their fields. Government policies on agriculture over the years have paid little attention to irrigation and financial support to smallholder farmers. Also, the support from non-governmental organizations (NGOs) for irrigation has not been sustainable for the small-scale farmers. The sub-region, therefore, continuously experiences high yield reductions for many crops including maize. Drought at anthesis, silking or grain filling periods can result in yield losses ranging from 40 to 90% (Grant *et al*., 1989; Menkir and Akintunde, 2001). It has been proven that the yield gap between optimal conditions and that of moisture stress could be drastically reduced through genetic improvement for drought tolerance (Edmeades, 2013; Masuka *et al*., 2017; Badu-Apraku *et al*., 2018).

In SSA, maize production also occurs under low-N environments (Oikeh and Horst, 2001) by low input farmers who continuously crop maize with limited or no use of N fertilizer. This may be attributed to high cost of fertilizer relative to grain which makes it uneconomical for farmers to apply fertilizer, non-availability of fertilizer when needed most, and reduced N-uptake especially in drought prone environments due to quick mineralization of organic matter (Bänziger and Lafitte, 1997a). Farmers could mitigate the impact of low-N on maize production by applying organic fertilizers such as compost, and rotating maize with nitrogen fixing legumes. Composting may however be demanding, requiring the addition of nitrogenous base to improve the content of N produced. In view of this, very few resource poor farmers can produce adequate and quality compost for their fields (Rufino *et al*., 2006). The major challenge with rotating maize with legumes is the fallow period required to grow the legumes since farmers would want to continuously use their lands for maize production. Low-N remains a great challenge to maize production in farmers' fields. About 10 to 50% loss of maize annually resulting from the impact of low-N has been reported (Wolfe *et al*.,

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1988). Therefore, the development and use of superior maize hybrids for low-N tolerance is crucial for an increased maize production and productivity in SSA.

Improving maize for drought tolerance also led to an indirect improvement in low-N tolerance, implying that the two stresses are regulated by specific adaptive mechanisms and can be improved simultaneously (Badu-Apraku *et al*., 2011b). There is therefore, a justification to ensure that released maize varieties in SSA possess both drought and low-N tolerance to maximize production in the sub-region. In addition, the crop production system of SSA is mainly rain-fed, characterized by long dry periods. The early maize which matures relatively quicker is useful in filling the hunger gap towards the end of the long dry period in July when the main season's crop is not ready for harvest. Breeding for early maturing maize hybrids is therefore of great importance in contributing to food security and sustainability in SSA.

Vitamin A and proteins are important dietary requirements for human beings. Unfortunately, vitamin A is not synthesized by human beings and its requirement is met through external sources. Vitamin A deficiency (VAD) can cause several health disorders such as night blindness in infants. The normal maize endosperm has inadequate levels of the two essential amino acids (lysine and tryptophan) responsible for the quality protein levels in maize. Protein energy malnutrition (PEM) due to undersupply of protein in infant diets can result in many health problems including "kwashiorkor"- a fatal syndrome characterized by initial growth failure. In view of this, several approaches including the supply of dietary supplements have been employed to alleviate vitamin A and protein malnutrition among infants in SSA. However, such interventions have not been effective and sustainable because of the high cost involved (Egesel, 1997; Wurtzel, 2004). Biofortification of maize with elevated levels of provitamin A (PVA), lysine and tryptophan could be a sustainable, cheap and effective approach for combatting VAD and PEM among children in the sub-region. The

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PVA maize has the potential of supplying more than the daily dietary requirement of 15.0 µg g^{-1} DW PVA for human beings compared with about 2.0 µg g^{-1} DW in the commonly cultivated and consumed yellow maize cultivars (Pixley *et al*., 2013). Efforts at improving the PVA contents in maize have led to the identification of three genes [phytoene synthase1 (*PSY1*), lycopene epsilon cyclase (*LcyE*), and β-carotene hydroxylase1 (*crtRB1*)] which regulate the key steps involved in the accumulation of PVA carotenoids (Wurtzel *et al*., 2012). The allele *crtRB1* (*-5'TE and 3'TE*) has been the most functional for increased β-carotene levels (Yan *et al*., 2010). Also, efforts at elevating the protein content of maize has resulted in the identification of the recessive homozygous allele, the opaque-2 gene (*o2o2*), and its modifiers which is capable of doubling the lysine and tryptophan contents of QPM compared with that of the normal maize.

Although a few maize varieties have been developed that separately possess some of these traits (earliness, PVA, QPM, drought, and low-N tolerance) in SSA, there is lack of an early maturing PVA-QPM hybrid that combines drought and/ or low-N tolerance. Achieving this milestone requires more commitment and intensified breeding efforts by scientist to develop inbred lines with desirable genetic backgrounds, as well as clear understanding of the combining ability and heterotic groups of the available inbred lines. The IITA-MIP has developed early maturing maize inbred lines with combined PVA and quality protein for the development of productive hybrids. Although different studies on the combining ability and heterotic grouping of maize inbred lines under varying environments have been conducted in SSA (Menkir *et al*., 2003; Badu-Apraku *et al*., 2013b; 2015b; Annor and Badu-Apraku, 2016), it is important to conduct such studies for newly developed inbred lines especially, when reliable testers are unavailable as in the West and Central Africa sub-region. This is because there have been inconsistent reports on the gene action modulating grain yield and other important agronomic traits in maize under stress and non-stress conditions. It has not been

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ascertained whether or not the PVA trait is influenced by maternal or paternal effects under drought, low-N and optimal environments. Furthermore, obtaining information on the combining ability and identification of heterotic groups of newly developed inbreds would guide breeding strategies in varying environments. Therefore, the goal of the present study was to develop early maturing, PVA-QPM single cross hybrids that are tolerant to drought and/ or low-N. The specific objectives were to:

1. identify drought and/or low-N tolerant inbreds with elevated levels of PVA and tryptophan,
2. assess the genetic variability and population structure of the inbred lines,
3. study the combining ability of selected inbred lines under drought, low-N, optimal, and across test environments,
4. classify the inbred lines into heterotic groups and identify the best inbred and hybrid testers across environments,
5. assess the yield and stability of the hybrids across test environments, and
6. determine the mode of inheritance of carotenoids, and validate PVA candidate genes in the inbreds studied.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Domestication and taxonomy of maize

Maize (*Zea mays* L.) was domesticated from its wild species ancestor, teosinte (Iltis 1983; Doebley *et al*., 1984, 1990; Norman *et al*., 1995) in the mid-elevations of South-Central Mexico, beginning with the teosinte race, Balsas (Matsuoka *et al*., 2002). The incredible genetic diversity in maize has made it highly adaptable to a wide range of climatic and soil conditions supporting the reason for its widespread use across the world. Maize was first introduced into Africa in the 16th century, most likely through the Portuguese traders, and by 1900 it was a minor food crop in Africa (Byerlee and Heisey, 1997).

Maize belongs to the family Poaceae (Grass family), subfamily Panicoideae, and the tribe Maydeae (Devos and Gale, 1997). It is a diploid (2n=20) outcrossing crop and belongs to the genus *Zea*. There are five species, *Z. mays, Z. perennis, Z. nicaraguensis, Z. luxurians* and *Z. diploperennis* (Doebley, 1990) with *Z. mays* being the only cultivated species. The other species are wild grasses (teosintes) native to the center of origin and domestication, South Central Mexico (Doebley, 1990).

2.2 Importance and production levels of maize

Maize provides food for humans, feed for poultry and livestock and also serves as a raw material for the production of a wide range of food and non-food products. It is a food security crop in the developing countries including SSA and parts of Latin America. Maize, rice and wheat together provide over 30% of the daily calories to at least 4.5 billion people globally (Shiferaw *et al*., 2011). Out of the total population of the countries that consume maize, about 900 million are poor consumers who rely on maize as the preferred staple

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(Shiferaw *et al*., 2011). The quantity of maize grain consumed directly as food was about 70% between 1995 and 1997 in SSA (Fischer *et al*., 2014). It is an undeniable fact that maize is more widely consumed than any other food crop in the households of many countries in SSA. For instance, in a period of two weeks, 94% of all households in Ghana had consumed maize as revealed by a survey conducted in 1990 (Alderman and Higgins, 1992).

Maize has the largest global production compared to all other cereals. World production of maize exceeded that of rice in 1996 and wheat in 1997 (Fischer *et al*., 2014), and recently approached 1040 Mt with the United States contributing 35%, China 21% and Brazil 8% of the total production (FAOSTAT, 2016). Maize production is increasing at twice the annual rate of rice and three times that of wheat (Fischer *et al*., 2014). In 2014, Africa produced about 78 Mt, representing 8% of the world's total maize production, with Eastern, Northern, Southern, and Western Africa producing 30, 8, 14 and 19 Mt, respectively (FAOSTAT, 2016). Grain yield in farmers' fields in West Africa has been consistently low, averaging around 1.7 t ha^{-1} compared to 4.8 and 6.7 t ha^{-1} obtainable in Southern and Northern Africa respectively (FAOSTAT, 2016). Grain yield of maize in Europe and North America is 6.7 and 10.7 t ha^{-1} respectively (FAOSTAT, 2016).

2.3 Importance of early maturing maize in the production systems of SSA

Generally, maize genotypes differ in terms of time to reach flowering or physiological maturity. Maize genotypes are classified into four maturity groups, extra-early, early, intermediate and late. Based on flowering dates under optimal environments, the extra-early group usually reaches 50% silking from 43 - 50 days after planting (DAP), the early from 51 - 55 DAP, the intermediate from 56 - 60 DAP, and the late maturing group from 61 - 70 DAP. The extra-early varieties reach maturity in 80-85 DAP, the early in 90-95 DAP, the

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intermediate varieties mature within 105 – 110 DAP and the late mature in 115-120 DAP (Badu-Apraku *et al*., 2012a).

Maize, especially the early maturing varieties, is increasingly becoming more important in the savannas of WCA. This is partly due to its ability to thrive on a wide range of soil types and many agro-ecological zones. The early maturing maize cultivars play a vital role in reducing hunger in the savanna areas of WCA due to their better response to fertilizer application and their characteristic faster maturity periods (Pswarayi and Vivek, 2008; Badu-Apraku *et al*., 2013b). Growing early maturing maize varieties may be preferred over the later varieties because they are ideal for off-season plantings and intercropping as they provide less competition for moisture, light, and nutrients. Due to their early maturity periods they easily enable multiple plantings during major and minor seasons especially, in areas with bimodal rainfall patterns (CIMMYT-Zimbabwe, 2000).

2.4 Constraints to maize production and productivity in SSA

Sub-Saharan Africa is a major hub for maize production in Africa. For example, between 2012 and 2014, SSA contributed the largest share (about 53 Mt) of maize produced in Africa (about 78 Mt) (Fischer *et al*., 2014). The increase in maize production in SSA has been primarily due to increase in area under production. Average grain yield of maize in farmers' fields in the sub-region have been very low, approximately, 1.7 t ha^{-1} (FAOSTAT, 2016). As a result, Africa continues to record the widest gaps between potential and actual yields for several crops, particularly maize (Licker *et al*., 2010; Neumann *et al*., 2010). Gibbon *et al*. (2007) identified several major factors leading to low yields in SSA. According to the study, the main agronomic factors that contribute to yield reduction are soil fertility constraints, weeds including *Striga spp.*, and drought stress. The soil fertility constraint was paramount in controlling maize yields. Other factors included late planting and disease and

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pest problems. The limited use of fertilizer (N based fertilizer) has been related to high cost of fertilizer relative to the price of grain which makes it uneconomical for farmers to purchase and apply fertilizer, non-availability of fertilizer when needed most (van Dijk *et al*., 2012), and lack of credit facilities for farmers.

Reports of other studies (Kamara *et al*., 2005; Nin-Pratt *et al*., 2011; Obeng-Bio *et al*., 2011; Badu Apraku *et al*., 2011a) have identified recurrent drought and poor soil nutrient levels as the major production contraints which limit grain yield of maize in the arid and semi-arid areas of SSA. This is because of the frequent and simultaneous occurrence of the two stresses in farmers' fields which often leads to disastrous synergistic effects (Kim and Adetimirin, 1997; Badu-Apraku *et al*., 2011a). Several decades of intensive agricultural practices without conscious efforts at regaining soil fertility have rendered most soils of SSA deficient of nutrients, degraded structure (Lal, 2006) and reduced soil moisture retention ability to support crop growth.

Changes in climatic patterns have been projected to put extra pressure on the ecosystem and agriculture in SSA (Schlenker and Lobell, 2010; Muller *et al*., 2011) through rising temperatures and erratic rainfall distribution. Many adaptation strategies have been proposed for African farmers to mitigate the effects of the changes in the future climatic patterns on maize production. However, most of these agricultural technologies were designed to fit into specific regional cropping systems and might not be effective and sustainable in other parts of the sub-region. Host plant tolerance to the major abiotic stresses such as drought and low soil nitrogen is a cost effective and sustainable approach of ensuring high and stable yields in varying environments. Thornton *et al*. (2009) and Schlenker and Lobell (2010) indicated that a combination of improved drought and low-N tolerant maize varieties forms an integral part of the preferred technologies for improving maize production in SSA, both under favourable environmental and changing climatic conditions.

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2.4.1 Impact of drought on maize production

Drought is referred to as the condition resulting from a sufficiently long period of dry weather which causes plant injury, low yields or total crop loss (Kneebone *et al*., 1992). Under optimal environmental and well-managed cropping conditions, maize produces high economic returns but it is less productive under water limited and poor crop management conditions resulting in significant yield reductions. An annual average of 15-20% of the potential global maize production was lost as a result of drought from 1980 to 2008 (Lobell *et al*., 2011). However, the degree of yield loss depends on the growth stage at which drought occurs as well as the duration and the severity of the stress. Early season drought impedes the growth of the maize plant and its subsequent development (Shaw, 1983; Heiniger, 2001), whereas mid to late season drought reduces the whole plant architecture, ear size and kernel numbers (Westgate and Boyer, 1985; Farré and Faci, 2006). When photosynthesis at flowering is reduced by drought, delayed silk growth occurs and this results in an increased anthesis-silking interval (ASI) and kernel abortion because of decreased male and female flower synchrony (Bolaños and Edmaedes, 1996). The occurrence of drought within the two weeks bracketing flowering (anthesis and silking stages) can lead to significant reductions in kernel set and kernel weight (Westgate and Bassetti, 1990; Schussler and Westgate, 1991; Bänziger *et al*., 2000; Obeng-Bio *et al*., 2011), resulting in an average yield loss of 40-90% (Grant *et al*., 1989; Nesmith and Ritchie, 1992; Menkir and Akintunde, 2001). Drought has been found to reduce grain yield by limiting invertase activities in the ovaries, leading to a reduced flux of hexose sugars accumulation, altered hormonal balance and eventually, ovary abortion (Chourey *et al*., 2010).

The impact of drought on maize could be greater regarding the timing of pollen shed and silking (Grant *et al*., 1989). Drought causes delayed silking with little effect on time of

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anthesis. For example, Moss and Downey (1971), as well as Herrero and Johnson (1980) demonstrated that the female reproductive tissues of the maize plant are more sensitive to moisture stress than the male tissues. Similarly, a study by Westgate and Boyer (1986) to compare the responses of the male and female reproductive tissues revealed a close correlation between silk water potential and changes in leaf water potential, whereas pollen water potential was constant. These findings suggest that severe drought stress is directly proportional to delayed silk growth with very little or no direct effects on tasseling and that increases ASI under drought conditions.

2.4.1.1 Adaptations of maize under drought conditions

Rainfall is highly variable and unpredictable, particularly in the tropics. The most sensitive developmental period for determining grain yield of maize centers on flowering and early grain filling (Tollenaar and Lee, 2006; Kranz *et al*., 2008). Since grain yield is of ultimate interest, drought tolerant maize cultivars are those that can produce appreciable grain yield under limited water conditions compared to yield under well-watered environments (Bänziger *et al*., 2000; Mitra, 2001). In order to differentiate between specific plant responses to drought, researchers classify drought adaptation into four categories. These are drought escape which mainly involves shortening of the lifecycle of the plant to escape the peak period of drought; drought avoidance which is associated with deeper roots, leaf wax and closing stomata; drought tolerance which involves the production of osmolytes, antioxidants, and other stress-relieving agents to withstand drought and produce economic yield; and drought recovery which is the ability of plants to resume growth and produce yield after exposure to severe drought which has caused a complete loss of turgor pressure and leaf dehydration (Yue *et al*., 2006; Luo, 2010; Lawlor, 2013). Among these adaptations, drought tolerance and to some extent drought avoidance are the most reliable mechanisms in crops (Yue *et al*., 2006).

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Upon a response to limited water availability, ABA plays crucial roles in coordinating other responses to mitigate the drought stress effect (Gill and Tuteja, 2010; Peleg and Blumwald, 2011). When soil water is limited, the root cells receive a signal which triggers a massive increase in the synthesis of ABA (Sauter *et al*., 2001). The ABA is transported mainly to the leaves as an intercellular messenger and recognized by guard cells which trigger stomatal closure via intracellular signal transduction, and reduce the metabolic activities related to plant growth (Campalans *et al.*, 1999; Boursiac *et al*., 2013). However, when drought is severe, photosynthetic membrane structures (thylakoids) are destroyed due to deficit of moisture, nutrients and energy, resulting in disruption of physiological processes (Guo *et al*., 2008). Total chlorophyll content significantly declines with decreasing soil water content, especially when there is a decrease in chlorophyll *a* (Sayed, 2003; Guo *et al*., 2008). Furthermore, maize, as a C_4 plant, is expected to possess the unique art of effectively controlling drought, since they can maintain photosynthesis by continuously fixing carbon when their stomata are closed (Lopes *et al*., 2011). Despite this physiological advantage of maize, the reproduction process of the crop is extremely impeded under drought because of poor or lack of male and female flower synchrony. That is, the male and female flower parts in maize can be up to 1 m apart, coupled with exposure of pollen and delicate stigmatic tissues to dry atmosphere for pollination to occur (Bänziger *et al*., 2000).

2.4.1.2 Breeding for drought tolerance in maize

Over the years, improving maize for drought tolerance has been slow. This is because drought tolerance is modulated by quantitative genes (Bänziger *et al*., 2000; Nguyen *et al*., 2004; Yue *et al*., 2006; Lopes *et al*., 2011), with high genotype x environment (G x E) interactions and low heritability (Bänziger *et al*., 2000) and that several mechanisms come into play to achieve a whole-plant level drought tolerance (Xiong, *et al*., 2006). Similarly,

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Bartels and Sunkar, (2005) indicated that the response of plants to drought is a complex process involving several thousands of genes and signaling pathways. In view of this, many researchers (Betrán *et al*., 2003; Melchinger *et al*., 1999) have proposed marker assisted selection (MAS) as very useful for enhancing gains in selecting for such quantitative traits through the identification of genes regulating the stress response in the metabolic pathways. However, MAS faces challenges in relation to the number of quantitative trait loci (QTL) up regulating for the trait, how the QTL allele effects are distributed, and epistasis. To increase the accuracy of molecular markers in the identification of drought tolerant genotypes, the genomic selection (GS) was proposed (Bernardo and Yu, 2007; Xu *et al*., 2012). Genomic selection offers the opportunity to identify genomic regions associated with specific traits within a wider population compared to a bi-parental mapping population which applies in most cases for MAS. These advances in GS provide the opportunities to accelerate the identification of drought tolerance genes that are exploited in cross combinations using conventional breeding methods.

Over the past decades, natural genetic variation has been exploited followed by the identification and incorporation of superior characteristics into desirable cultivars. This has been the common conventional breeding approach for drought tolerance (Xoconostle-Cazares *et al*., 2011). Also, the relative performance of genotypes under drought and well-watered conditions has been used in the identification of drought tolerant genotypes for testing under unpredictable rainfed environments (Nouri *et al*., 2011) because selection under unpredictable environments is very difficult. However, the low heritability of grain yield has rendered direct selection for the trait ineffective especially, under stress environments (Edmeades *et al*., 1999; Venuprasad *et al*., 2007; Ziyomo and Bernardo, 2013). Selection of secondary traits under induced drought and optimal environments have been effective in improving maize for drought tolerance using drought versus well-watered environments (Bänziger *et al*., 2000;

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Badu-Apraku *et al*., 2005; 2010; 2011a; 2012; 2015b; 2016b; Obeng-Bio *et al*., 2011; Edmeades, 2013; Maazou *et al*., 2016). Secondary traits are measurable indicators used to assess a crop's performance (Lafitte *et al*., 2003). Under different soil moisture regimes, high variations in heritability estimates for grain yield and secondary traits and observed decreasing heritability trends with increasing drought intensity was reported (Bolaños and Edmeades, 1996). However, the heritability estimates for ears per plant and ASI increased or remained unchanged as drought effect increased and grain yield levels declined indicating that these traits could be reliable for yield improvement under drought. Selection of genotypes under drought based on secondary traits has led to genetic gains (Bolaños and Edmeades, 1996). For example, Edmeades *et al*. (1999) recorded an average yield gain of 12.6% cycle^{-1} for the maize variety, La Posta Sequía, when evaluated under drought environments. The IITA-MIP employs a selection index combining secondary traits including ASI, plant and ear aspects, stay-green characteristic and ears per plant with grain yield to accelerate progress of improving grain yield under drought (Badu-Apraku *et al*., 2011a). Badu-Apraku *et al*. (2018) reported 348 kg ha^{-1} cycle^{-1} gain in grain yield using secondary traits under drought.

2.4.1.3 Combining ability studies under drought conditions

Combining ability of an inbred is its ability to transmit superior performance to hybrids (Panhwar *et al*., 2008). Testing inbreds in hybrid combinations is important for careful planning of crosses in a breeding program. There are two types of combining ability (i) General Combining Ability (GCA) which refers to the average contribution an inbred makes to hybrid performance in a series of hybrid combinations in comparison to other inbreds in the same series of hybrid combinations and (ii) Specific Combining Ability (SCA) referring to the contribution of an inbred to hybrid performance in a cross with a specific other inbred in relation to its contributions in crosses with an array of other inbreds (Griffing, 1956;

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Nduwumuremyi *et al*., 2013). General combining ability relates to the additive portion of genetic effects, also known as the breeding value, while SCA relates to non-additive genetic effects including dominance, overdominance, epistasis and genotype x environment interaction effects (Rojas and Sprague 1952; Falconer and Mackay, 1996). Additive and non-additive genetic effects are both useful to plant breeders. The additive genetic effect is useful during selection whilst the non-additive is important in creating good hybrids. SCA cannot be predicted and depends on how genes from each inbred line complement each other. Knowledge of the combining ability of inbreds is important for careful planning of crosses in a breeding programme. Reports on the mode of gene action controlling the inheritance of grain yield and other important traits of maize cultivars under drought stress are not only limited but also contradictory. For example, Guei and Wassom (1992) reported significant GCA and SCA effects although GCA effects were more important in the expression of flowering traits in the two maize populations studied. Similarly, Meseka *et al*. (2007) reported significant GCA and SCA effects for 24 late maturing maize inbreds, and GCA accounted for over 50% of the total genotypic variation for the measured traits under drought. Likewise, Badu-Apraku *et al*. (2004) reported the preponderance of additive genetic effects for grain yield and other measured traits of an early maturing full-sib families selected for improved grain yield under drought. In another study, additive genetic effects contributed more than 64% of the total genotypic variation for grain yield under drought environments (Oyekunle and Badu-Apraku, 2013). Furthermore, Betrán *et al*. (2003a) assessed lowland white-grained tropical maize hybrids under drought conditions and found that grain yield was conditioned by additive genetic effects and its magnitude increased with the intensity of the stress. Also, Annor and Badu-Apraku (2016) reported a greater contribution of GCA effects over SCA relative to the total variation among hybrids for grain yield and most of the measured traits under drought suggesting that additive gene effects were more important than the non-additive in the

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inheritance of the traits. However, Meseka *et al*. (2013) reported significant non-additive genetic effects for grain yield under moisture stress conditions. From the reviewed literature, it is therefore reasonable to suggeset that significant GCA and SCA effects exist in the expression of traits under drought, but GCA effect is more important, or more easily detected statiscally, than the non-additive. This suggests that recurrent selection methods that capitalize on additive gene action can be used to improve drought tolerance in maize.

2.4.2 Effects of low soil N on maize production and productivity

Nitrogen is important for plant metabolism as it is involved in protein and chlorophyll biosynthesis which are required, especially in the early phenological stages of plant development (Ceretta *et al.*, 2002). In the presence of appropriate levels of other nutrients in the soil, nitrogen provides the greatest increment to maize yield (Lemaire and Gastal, 1997). To further emphasize that N supply is a requirement to obtain appreciable yields of maize, Weber *et al*. (1996) reported that maize requires about 50 – 60 kg N and 30 Kg P ha^{-1} in plant available forms to produce a tonne of grain. Nitrogen is often the most limiting nutrient for crops produced in the tropics. Soil N varies within a range of approximately 0.06 to 0.30% for most cultivated soils of SSA and in several cases, N levels approach zero percent. The lower levels of N in tropical soils is due to ease of its immobilization, denitrification, leaching, and ammonia volatilization (Sawyer, 2008). In addition, decades of continuous cropping and removal of crop residues as feed and fuel without replenishment (Zambezi and Mwambula, 1997) aggravates soil organic matter depletion in SSA. However, the bulk of N in the soil is contained in its organic matter, with smaller amounts retained in the clay minerals. The characteristic reduced N availability and supply in soils of SSA is therefore, associated with the reduced levels of its organic matter content.

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The annual yield reduction in maize from the impact of low-N was estimated to vary between 10 and 50% (Wolfe *et al*., 1988). Nitrogen stress prior to flowering is associated with reduced rate of photosynthesis, leaf area development and number of ear spikelets while the occurrence of the low-N stress at flowering could result in kernel abortion. Furthermore, low-N effect at grain-filling can accelerate leaf death with decreased rate of photosynthesis, as well as kernel weight and size (Bänziger *et al*., 2000). Physiologically, N stress at pre-flowering period poses a great reduction in the photosynthetic capacity to produce enough assimilates, while N stress after flowering drastically limits the efficiency in both the production and supply of assimilates to the developing ears due to rapid reduction in leaf area duration.

2.4.2.1 Breeding maize for tolerance to low soil nitrogen

Low-N effects on maize could be mitigated by the application of inorganic fertilizers, compost, green manuring and also taking advantage of atmospheric nitrogen fixing legumes to increase its availability and supply in the soil. In order to significantly improve grain yield of maize in SSA, 90-120 kg N ha^{-1} fertilizer rate is recommended. Nonetheless, farmers continue to apply fertilizer at very low rates than the recommended doses because of the high cost of fertilizer and poor accessibility to the low input farmers when needed most. Also, production of compost may be demanding involving the inclusion of some nitrogenous sources to maximize its N content. A few farmers could therefore produce adequately good quality compost for their maize fields (Rufino *et al*., 2006). Furthermore, the promising alternatives of using nitrogen-fixing legumes in rotation as well as green manuring are usually restricted by fallow period for growing the leguminous crops to regain the fertility of the soil. This may not be the option for the resource poor farmers in the intensive maize production areas where the land is always used (Kaya *et al*., 2000). Additionaly, successful N fixation depends on several factors such as the use of the appropriate spp. of legume, presence of

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desirable nodulating *Rhizobia* as well as favorable edaphic and climatic conditions. In view of the challenges associated with the enhancement of N availability and supply in the soil, improving maize for low-N tolerance is the cheapest and sustainable method to increase grain yield of maize by the low-input maize producers in SSA. Laffite and Edmeades (1994a) indicated that low-N tolerant maize varieties are efficient in the uptake of N in N limiting environments and also efficiently utilizes absorbed N for increased grain production. The development and use of improved maize varieties with tolerance to low-N would therefore contribute to increased grain yield in areas prone to the low-N stress (Zaidi *et al*., 2003). Evaluation of genotypes for low-N tolerance should be conducted under both favourable and low-N environments in order to facilitate the rate of progress made within a period (Bänziger and Laffite, 1997a). This approach enhances the identification of desirable genotypes that can give appreciable yield under low-N and show much superiority under optimal environments. Thus, uniformity of screening sites for optimal and low-N conditions are prerequisite in reducing confounding effects in the identification of superior genotypes under low-N as well as showing outstanding performance under optimal conditions.

Grain yield is the main reponse variable in screening for low-N tolerant genotypes. For the low-N level to be severe enough, the grain yield reduction should range from 20 to 50% (Bänziger *et al*., 1999). Other important traits assayed for low-N tolerance include stay-green characteristics and ASI. Genotypes that record higher ASI under severe low-N conditions are considered susceptible because of high probability of missing the male female flower synchrony while genotypes that show reduced ASI and produce appreciable yields under limited N environments are classified as tolerant (Bänziger *et al*., 1999). Moreover, genotypes that exhibet delayed reduction in chlorophyll content as well as delayed senescence and produce better yields under low-N are considered tolerant to the stress (Bänziger and Lafitte, 1997b). These key secondary traits have been used to complement grain yield in the

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identification of low-N tolerant genotypes because of the low heritability of grain yield under stress conditions (Bänziger and Lafitte, 1997b; Bänziger *et al*., 1999).

2.4.2.2 Gene action controlling the inheritance of traits under low-N conditions

Knowledge of the gene action conditioning the inheritance of traits of newly developed inbreds under low-N environments is of paramount importance in improving maize for low-N tolerance as it provides the basis for devising breeding strategies. Such information on the genetic behaviour of traits under low-N is very limited and inconsistent. Contradictory findings have been reported on the mode of inheritance of traits under low-N environments. Several workers (Mosier *et al*., 2005; Miti *et al.*, 2010; Badu-Apraku *et al*., 2013a; Ifie, 2015) have reported additive genetic effects for grain yield and most traits under low-N. In addition, Adofo-Boateng *et al*. (2015) reported significant additive gene action over non-additive using intermediate maturing maize inbreds under high N environments in Ghana. In contrast, other studies (Meseka *et al*., 2006; Makumbi *et al*., 2011) have reported SCA effects to control grain yield of hybrids under limited N environments. Significant mean square and high mid-parent heterosis were observed for grain yield under low-N implying the high possibility of producing superior hybrids for areas prone to low-N stress (Meseka *et al*., 2013).

2.4.3 Relative importance of combining ability of male and female effects under drought and low-N conditions

In plant breeding and genetics, the North Carolina Design II (NCD II) (Comstock and Robinson, 1948) is very useful for assessing the type of gene action controlling quantitatively inherited traits. An important feature of the NCD II is the flexibility of dividing the variation due to hybrids into three components which include variation due to GCA-female, GCA-male and to SCA in terms of female x male interactions (Acquaah, 2012; Sarfaraz *et al*., 2014).

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Despite these advantages of the NCD II, there is limited information on the relative importance of GCA-male and GCA-female effects in tropically adapted maize particularly under low-N and drought environments. The few reports available have indicated the significance of maternal effects conditioning the inheritance of traits including grain yield under drought and favourable environments. For instance, Derera *et al*. (2008) indicated that cytoplasmic effects influenced grain yield, ear aspect, ASI and prolificacy under optimal and drought environments in maize hybrids. A similar report by Jumbo and Carena (2012) indicated significant cytoplasmic inheritance for ear height in elite early maize hybrids under favourable environments. In another study, Ifie *et al*. (2015) found a non-significant difference between additive genetic effects for male and that for female for grain yield under optimal and low-N conditions and indicated that both male and female parents equally contributed to grain yield and that there were no cases of maternal or paternal effects on the measured traits. Also, Badu-Apraku *et al*. (2016a) reported that the F test of the GCA-male to GCA-female ratio and vice versa were statistically the same for all measured traits using the early white QPM inbred lines. Moreover, information on the influence of maternal or paternal genetic effects on the control of other important agronomic traits of maize as well as nutritional traits such as PVA content remains limited.

2.4.4 Relationship between drought and low-N tolerance in maize

Direct improvement for drought tolerance in maize indirectly resulted in improved performance under limited N environments (Bänziger *et al*. 1999) implying that tolerance to low-N and drought could involve common adaptive mechanisms. Anthesis silking interval, stay-green characteristic and ears per plant that have strong correlation with grain yield under low-N and drought environments have been employed to identify low-N and drought tolerant maize genotypes (Lafitte and Edmeades, 1994a; Bänziger and Lafitte, 1997a) underscoring

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the common relationship between the two stresses. Similarly, Badu Apraku *et al*. (2015) observed an improvement in tolerance to low-N for maize cultivars derived from an early population while directly selecting for drought tolerance. This implied the high tendency for low-N and drought to occur simultaneously under field conditions which can lead to total yield loss. Furthermore, Badu-Apraku *et al*. (2012) identified common agronomic characters such as plant height, and plant and ear aspect as reliable secondary traits for the concurrent selection for improved yield under drought and low-N stresses using extra-early maturing maize cultivars confirming that tolerance of the cultivars to both stresses was influenced by similar physiological pathways. The result practically suggested the possibility of selection in one of the stress environments and being effective in the other. Senescence or stay-green characteristic was found to be a reliable trait for selection under low-N, thus confirming the findings of Lafitte and Edmeades, (1994a) that most traits used in breeding for low-N tolerance were associated with senescence. However, ears per plant was not found to be a reliable trait for selection under low-N in contrast to the report by Bänziger and Lafitte (1997a) which showed that prolificacy was among the reliable secondary traits identified for selecting for grain yield under low-N. In terms of hybrid production under low-N and drought conditions, Meseka *et al*. (2013) reported high non-additive gene action for grain yield under drought.

2.5 Improving nutrition through biofortification of maize

Over 800 million people worldwide are malnourished and many of these are in SSA and Asia (Jauhar, 2006). About 54% of mortality in infants in these less developed countries resulted from malnutrition (Monika and Mercedes, 2005). Ensuring high nutritional status for the vulnerable groups in the sub-region has not been successful. For example, protein energy malnutrition (PEM) which was described about 10 decades ago is still commonly diagnosed

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among children in SSA (Hendricks *et al*., 1995). Several interventions by the World Health Organization (WHO) such as the supply of food supplements (Bouis, and Saltzman, 2017) to curb malnutrition in SSA have not been effective and sustainable because they are expensive and a considerable proportion of the vulnerable groups in the rural areas do not have access to these interventions. There is the need to consider biofortification of the staple crops as a sustainable and cheap option to address the problem of malnutrition in SSA. Biofortification of maize with PVA and quality protein (lysine and tryptophan) involves the accumulation of these nutrients for their availability in the endosperm using plant breeding and transgenic techniques (Bouis, and Saltzman, 2017). Alleviating VAD and PEM among infants in SSA through biofortification could be achieved with maize. This is because the crop is the most widely produced and consumed staple in the sub-region and has high genetic potentials for PVA as well as lysine and tryptophan.

2.5.1 Quality protein maize and its importance

The conventional maize protein is deficient in two essential amino acids, lysine and tryptophan. As a result, consumption of conventional maize, especially when fed to infants without any balanced protein supplements, could predispose infants to childhood diseases including “kwashiorkor”- a fatal syndrome characterized by initial growth failure. The QPM has about double of its lysine and tryptophan contents than the conventional/normal maize. The contents of lysine in normal maize and QPM are about 2.0% and 4.0% respectively, in endosperm protein (Teklewold *et al*., 2015). Depending on the genetic background, the levels can be within ranges of 1.6 to 2.6% in normal maize and 2.7 to 4.5% in endosperm protein of QPM (Vivek *et al*., 2008). Similarly, the tryptophan levels in normal and QPM average 0.4% and 0.8% respectively, in endosperm protein with ranges of 0.2 to 0.6% in conventional maize compared to 0.5 to 1.1% in QPM (Vivek *et al*., 2008). The percent lysine and tryptophan

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acceptable for QPM could be expressed in terms of a given unit of samples in whole grain or endosperm. Lysine should be >0.35 and >0.32% for whole grain and endosperm, respectively, while tryptophan should be >0.075 and >0.070% for whole grain and endosperm, respectively (Vivek *et al*., 2008; Teklewold *et al*., 2015). It is worth to noting that the levels of tryptophan and lysine in maize are highly correlated (> 0.9) and, since analysis for tryptophan is less expensive, breeders typically use tryptophan levels as an indicator of the nutritive value of a QPM variety (Villegas *et al*., 1992; Nurit *et al*., 2009).

Quality protein maize was primarily developed for the regions where maize is a staple food and where availability of other protein sources is scarce (Akuamoah-Boateng, 2002) to address the protein needs of vulnerable groups. The Ghana maize programme made a breakthrough in 1992 and released a QPM variety named "Obatanpa" meaning "good nursing mother" (Badu-Apraku *et al*., 2006). Since then, several other OPVs and hybrids have been developed, not only in Ghana but in several other countries in SSA. In terms of human protein requirements, QPM is capable of supplying about 70 to 73% as compared with about 46% from normal maize (Badu-Apraku and Fakorede, 2017). Additionally, Bressani (1992) reported 80% biological value of protein (a measure of protein absorption in human beings) for QPM in comparism with 40 - 57% for conventional maize and that of eggs is 86%. The relative value of milk in the protein of QPM is 90% compared to 40% for conventional maize as reported by Badu-Apraku and Fontem-Lum (2010). Infants who were fed with QPM porridge had reduced stunting and recorded fewer sick days, and had better growth-enhancing capabilities than those fed with normal maize porridge (Akuamoah-Boateng, 2002). Beside higher protein quality, the QPM also has other nutritional advantages over conventional maize (Bressani, 1991; Prassana *et al*., 2001; Agrawal and Gupta, 2010). This could significantly improve nutritional status of vulnerable groups when QPM is grown without contamination from pollen of normal maize. For example, due to the elevated lysine content of the QPM, its

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consumption could lead to an improvement in the absorption of micronutrients such as Zn and Fe in human beings. Furthermore, apart from its usefulness in human diets, the QPM is used as animal feed in countries where meat consumption per capita is high. Several studies have indicated the overall significant impact of QPM on the weight gain of both poultry and pigs (Sofi *et al*., 2009; Panda *et al*., 2010; Mbuya *et al*., 2011). According to Krivanek *et al*. (2007) and Scott *et al*. (2009), QPM could substitute for soybean meals and synthetic lysine in feed formulations for poultry and pigs, which could result in significant savings in feed production.

2.5.1.1 Genetic basis of the quality protein maize

Knowledge of the genetic background of QPM is a prerequisite for successful development of QPM inbreds, varieties and hybrids, and seed maintenance. Research on improving maize for its protein quality commenced in the 1960s, after the discovery of several mutations (*opaque2- o2, floury2- fl2, opaque7 -o7, opaque6 -o6 and floury3 -fl3*) that resulted in a decrease in zein fraction, coupled with increased levels of other protein classes as well as increased levels of essential amino acids (McWhirter, 1971; Ma and Nelson, 1975). Of all the mutants, the recessive mutant *o2* has been the most widely studied and used as a source for improving genetic backgrounds for the QPM trait. The recessive homozygous genotypes (*o2o2*) have substantially higher lysine and tryptophan content compared with that of heterozygotes (*O2o2*) or dominant homozygotes (*O2O2*) (Crow and Kermicle, 2002). However, due to "pleiotropic effect" (one gene controlling more than one trait) of the *o2* mutation, the endosperm of the QPM becomes soft, suscebtible to cracking, ear rot and storage pests. The genes conditioning the soft and starchy texture of *o2* endosperm were discovered and designated as opaque2 modifiers (*Opm*) and they have proved to be genetically complex in nature, because they are polygenes, each with very little effect (Babu *et al*., 2015). Breeding for high quality protein maize requires the manipulation of three distinct genetic systems

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(Krivanek *et al*., 2007) including (i) the opaque-2 gene (*o2o2*) recessive allele (ii) enhancers of the endosperm containing the *o2*-gene for increased levels of tryptophan and lysine and (iii) modifying genes responsible for the hardness of the *o2*-induced soft endosperm (Twumasi-Afriyie *et al*., 2016). Intensive breeding efforts by IITA and CIMMYT have resulted in the release of hard texture endosperm QPM for commercialization (Vivek *et al*., 2008; Teklewold *et al*., 2015; Badu-Apraku *et al*., 2016a). Varadaraju and Joel (2017) reported high lysine and tryptophan levels in hybrids to be primarily related to the contributions of maternal inbred parents, indicating that quality protein content was under maternal genetic control in the set of inbreds used. Annor and Badu-Apraku (2016) reported 55 - 87% contribution of GCA effects for grain yield and other traits among the extra-early QPM hybrids under drought, low-N, optimal and across environments indicating that additive genetic effects were more important than the non-additive in the inheritance of the traits.

2.5.1.2 Isolation requirements of QPM varieties

Recessive genes regulate lysine and trytophan levels of QPM and therefore if QPM is grown in areas where normal maize is grown without isolation, the recessive genes could be masked by dominant genes from the normal maize nearby. The occurrence of such a phenomenon could contaminate and deterioriate the levels of lysine and tryptophan in QPM with time, especially when farmers continue to recycle their seeds for next growing seasons in the case of open pollinated varieties (OPVs). The need to isolate QPM from normal maize either by distance or time could limit the dissemination and adoption of QPM varieties especially hybrids (Tandzi *et al*., 2017). Ghana dominates the production of QPM in Africa with the cultivation of "Obatanpa" (an OPV) on nearly 70,000 hectares (Tandzi *et al*., 2017). Southern Africa allocates only 1% of the total area for maize production to QPM (FAOSTAT, 2012). Several studies have been carried out to establish the levels of outcrossing between

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QPM and non-QPM varieties at different distances and reports have been inconsistent. For example, Twumasi-Afriyie *et al*. (1996) found no significant contamination of a QPM by a non-QPM at a distance of approximately 12 m. However, Machida *et al*. (2012) estimated outcrossing levels as high as 63 – 83% at the borders of QPM field 10 m away from normal maize field. Burris (2001) found an outcrossing lelevel of 1.11% at a distance of 200m. The difference in the results of these studies could be due to the variation in the environmental conditions when the flowering periods overlap. Ideally, an isolation distance of 200 – 400 m depending on the environmental conditions is recommended. In terms of isolation by time, the early maize (90 – 95 days to maturity) could play a vital role through very early or very late planting to avaoid flowering synchrony which often occurs at the peak season between QPM and normal maize varieties.

2.5.2 Improvement of maize for provitamin A carotenoids

Vitamin-A, an essential micronutrient, is needed by human beings for improved vision and skin health, enhanced immune system, gene transcription and protein synthesis especially, in infants and pre-school children. Unfortunately, vitamin-A cannot be synthesised by humans and its requirements are met through external sources. Inadequate supply of vitamin-A compromises the immune system, increase the risk of child mortality resulting from infectious diseases (Rice *et al*., 2004). Vitamin-A deficiency (VAD) affects about one-third of preschool children worldwide and 1% of these develop night blindness (Ramalho *et al*., 2002; West 2002; HarvestPlus 2004; Howe and Tanumihardjo, 2006; WHO 2009). Approaches to combat VAD through regular dietary supplementation with vitamin-A have not been sustainable in the developing world (Egesel, 1997; Wurtzel, 2004). Maize as a food security crop in Africa with high biologically useful concentrations of PVA has been targeted for biofortification to alleviate VAD and its associated health problems in the sub-region. Although initial cost of

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developing biofortified maize with elevated levels of PVA could be high, after a one-time investment in developing the biofortified seeds, recurrent costs are low and the approach becomes highly sustainable.

Maize grain carotenoid concentrations are among the highest produced in cereals (Howitt and Pogson, 2006), and exhibit considerable diversity in the composition of grain carotenoid profiles with respect to the predominant carotenoids (lutein and zeaxanthin), PVA carotenoids (α-carotene, β-carotene and β-cryptoxanthin) and other non-PVA carotenoids (zeinoxanthin) (Harjes *et al*. 2008). The normal yellow maize contains considerable levels of β-carotene, a source of vitamin-A (Menkir *et al*., 2008), and has natural variation for carotenoids (Mishra and Singh, 2010; Harjes *et al*., 2008; Pfeiffer and McClafferty, 2007; Weber, 1987). However, kernels of typical normal yellow maize varieties can only provide 0.5 to 1.5 μg g^{-1} PVA (Egesel *et al*., 2003) which is extremely inadequate to prevent VAD in diets dominated by maize compared to the current breeding target of 15 μg g^{-1} dry weight (DW) of PVA set by HarvestPlus (HarvestPlus 2004; Ortiz-Monasterio *et al*., 2007; Harjes *et al.*, 2008). This challenge therefore led to more research into the normal yellow maize to increase the PVA content. For example, in several improved populations and inbreds, Babu *et al*. (2012) found the that the levels of PVA in whole grain had reached 15 - 20 μg g^{-1}. Considering the potentials of maize for accumulating high levels of PVA, improving maize for increased levels of PVA through breeding is a feasible approach to alleviate VAD in SSA.

2.5.2.1 Genetics of provitamin A maize

The maize carotenoid biosynthetic pathway is well known and the functional genes involved in the regulation of the pathway have been characterized (Fig. 2.1) (Vallabhaneni and Wurtzel, 2009; Yan *et al*., 2010; Cuttriss *et al*., 2011; Wurtzel *et al*., 2012). Three loci, β-

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carotene hydroxylase1 (crtRB1), phytoene synthase (PSY) and Lycopene epsilon cyclase (LcyE) have been shown to control the accumulation of PVA carotenoids in the endosperm.

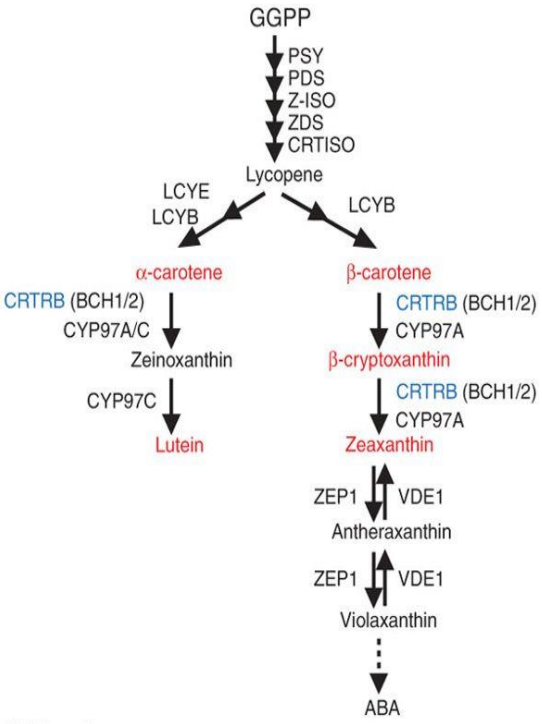

Figure 2. 1 The maize carotenoid biosynthetic pathway (Yan *et al*., 2010)

The crtrRB1 is the most functional for increased β-carotene levels, and favourable alleles at that locus have resulted in higher levels of PVA compared to the wild type alleles (Vallabhaneni and Wurtzel, 2009; Yan *et al*., 2010; Babu *et al*., 2013). However, other studies including Owens *et al*. (2014) have identified several other genes that are involved in PVA accumulation in maize. Several of these genes were found within QTL intervals associated with visual scores of relative orange endosperm colour intensity (Chandler *et al*., 2013). Wong *et al*. (2004) in their QTL mapping study of maize grain carotenoids revealed the proximity of some of the identified QTLs to two of the three carotenoid biosynthetic genes of phytoene synthase (y1 and zds1) that had been cloned at the time. The identification of possible QTL association with carotenoid biosynthetic genes prompted efforts to identify and characterize

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alleles of genes in the carotenoid biosynthetic pathway that may be associated with quantitative levels of carotenoids. This suggested that the PVA trait could be quantitatively inherited and therefore it is worth to study its combining ability (Egesel *et al*., 2003; Senete *et al*., 2011; Li *et al*., 2013; Suwarno *et al*., 2015). Moreover, the discovery of the natural genetic variability in carotenoids of yellow maize inbreds and hybrid varieties in the temperate zone (Wong, 1999; Maziya-Dixon *et al*., 2000; Menkir and Maziya-Dixon, 2004; Egesel *et al*., 2003) and in the tropics (Pfeiffer and McClafferty, 2007; Burt, 2010; Suwarno *et al*., 2015) has prompted research on the genetics of carotenoids in yellow maize. For example, medium (0.55) to high (0.90) heritability estimates and primarily additive over the non-additive gene effects influencing PVA concentrations in maize have been widely reported (Egesel *et al*., 2003; Suwarno, 2012; Suwarno *et al*., 2015; Menkir *et al*., 2014; Owens *et al*., 2014), suggesting that application of recurrent selection methods should be effective (Coors, 1999). Other studies have indicated the importance of non-additive gene action over that of additive in controlling the PVA carotenoids. For instance, Halilu *et al*. (2016), found SCA effects to be higher than GCA effects for all the carotenoids measured including PVA, suggesting a predominance of non-additive gene action over that of additive. Also, in their study, higher broad-sense heritability (H^2) estimate (> 0.60) was obtained for, plant height, days to anthesis and zeaxanthin, moderate (0.30 – 0.60) for grain yield and lutein, and low (< 0.30) PVA, β-carotene, β-cryptoxanthin and α-carotene. Grain yield did not significantly correlate with carotenoid concentrations, indicating that these traits can be improved independently (Halilu *et al*., 2016). Maize naturally produces PVA carotenoids as intermediate products and alleles such as *LcyE* and *crtRB1* with substantially reduced transcript levels increase accumulation of β-branch carotenoids and decrease hydroxylation of β-carotene, respectively, resulting in higher PVA levels in kernels (Harjes *et al*., 2008; Yan *et al*., 2010). Visual scores of deep orange kernel colour may not necessarily correlate with high PVA levels in maize (Chandler

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et al., 2013). However, in crops like cassava, the PVA genes are naturally rare, and thus the presence of deep orange flesh colour of an improved PVA cassava automatically suggests high PVA levels. Thus, selecting for increased levels of PVA in cassava is easier and straightforward unlike maize.

Furthermore, positive correlations among individual carotenoids (Kurilich and Juvik 1999; Menkir *et al*., 2008; Owens *et al*., 2014), and between grain yield and PVA content (Menkir *et al*., 2014; Suwarno *et al*., 2015) have been reported. These findings have confirmed that simultaneous increases in accumulation of PVA and other carotenoids may be effectively accomplished without compromising grain yield potential and related important agronomic traits (Pfeiffer and McClafferty, 2007; Bouis and Welch 2010; Menkir *et al*. 2014). Furthermore, Pixlley *et al*. (2013) observed higher chances of improving both PVA and QPM traits in a breeding programme as it was hypothesized that selection for high PVA could enhance lysine and tryptophan levels. For the white and orange QPM studied, the authors found higher levels of lysine and tryptophan to be associated with the PVA biofortified maize varieties relative to the white types.

2.6 Assessment of genetic diversity in maize using molecular markers

Knowledge and clear understanding of the genetic variation and population structure of breeding materials is crucial for the success of a hybrid programme because it facilitates judicious and effective planning of crosses and utilisation of resources in the breeding programme. In maize breeding, genetic diversity has been exploited to select diverse parents to maximise heterosis in the development of hybrids (Betrán *et al*., 2003; Melchinger, 1999). This is because maize has a complex genome (Schnable *et al*., 2009) with incredible genetic diversity which is considered a major factor in heterosis (Gore *et al*., 2009). According to Semagn *et al*. (2012), high genetic variation in a source population enhanced the development

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and identification of desirable inbreds to produce superior hybrids. Moreover, studies have revealed tremendous variability among tropical maize germplasm. For example, Zhang *et al*. (2016), estimated genetic diversity among tropical and temperate maize populations and reported higher diversity in the tropical than the temperate group. Nevertheless, the highest heterosis found in maize is in temperate hybrids involving crosses between stiff stalk and non-stiff stalk inbreds that are not very diverse genetically (Barata and Carena, 2006).

Genetic diversity studies are routinely conducted using various techniques including morphological markers, biochemical characterization, and DNA (molecular) markers. The most widely utilized technology is the molecular markers method because they are abundant, and unaffected by environmental factors as well as the stage of development of plants (Winter and Kahl, 1995). Marker-based genetic distance estimates may be useful in preventing crosses involving closely related inbreds (Lu *et al*., 2009). This is very important in cases of unavailability of well-established heterotic groups and when pedigree information is also lacking. Molecular makers such as rapid amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), restriction fragment length polymorphisms (RFLPs) (Mohan, *et al*., 1997), simple sequence repeat (SSR) or microsatellite, expressed sequence tags (EST) based-SSR as well as single nucleotide polymorphisms (SNPs) have been used to investigate the diversity and the population structure in tropical maize.

Simple sequence repeat markers have been broadly employed for the characterization of germplasm of crops due to their co-dominant nature, relatively low price, the large number of alleles per locus that provides higher level of polymorphism and are easy to use (Heckenberger *et al*., 2002; Park *et al*., 2009). Also, SNPs have received greater attention in recent times because of their frequent occurence in the genome compared to SSRs (Van Inghelandt *et al*., 2010; Govindaraj *et al*., 2015). Although, SNPs are less informative compared with SSRs, SNPs can be used for similar studies as SSRs because their higher

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density of occurence in the genome is associated with lower rates of genotyping errors. SNPs can be easily automated with high-throughput techniques (Rafalski, 2002). Dao *et al*. (2014) used 1057 SNPs to investigate the variation in the backgroungs of maize inbreds from INERA, IITA, CIMMYT and temperate lines and further examined how the inbreds were related. The SNP markers were more informative in revealing the genetic differences between IITA, INERA and temperate inbreds but not the CIMMYT inbreds, confirming the efficiency of the SNPs used. The record of lower levels of heterozygosity for the INERA inbred lines suggested considerable progress made in the INERA lines than the other inbreds used in the study. Lu *et al*. (2009) as well as Semagn *et al*. (2012) earlier reported a similar finding that SNPs could provide useful genetic information for characterizing tropical maize germplasm.

The diversity array technology (DArT) is another molecular technique which provides a whole genome fingerprinting tool (Jaccoud *et al*., 2001; Huttner *et al*., 2005; Semagn *et al*., 2012; Govindaraj *et al*., 2015). Successful application of DArT for the analysis of DNA polymorphisms was demonstrated using the rice genome as a model (Jaccoud *et al*., 2001) and the technology was later used for other crops including maize (Huttner *et al*., 2005). The initial DArT procedure implemented the microarray platform using hybridization and fluorescent labelling of representations to dedicated DArT arrays. Thus, the initial DArT markers are independent of DNA sequence (Jaccoud *et al*., 2001; Huttner *et al*., 2005). In 2010, the DArT procedure was upgraded as DArTseq method which uses sequencing of the representations adopting the platform of the Next Generation Sequencing (NGS). This transition has allowed dramatic increase in the number of genomic fragments analysed, with a corresponding increase in the number of reported markers. The change from the microarray platform of DArT to the NGS platform of DArTseq has enabled scalable and cost-effective genotyping (https://www.diversityarrays.com/index.php/technology-and-resources/dartseq/).

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2.7 Heterotic grouping and establishment of heterotic patterns in maize

Heterosis, the superior performance of a hybrid over the average of its parents referred to as mid-parent heterosis, or over the high parent called heterobeltiosis (Miranda, 1999), is a useful concept in hybrid maize development programmes. The degree of expression of heterosis mostly relies on the genetic diversity of the inbred parents, type of gene action conditioning the trait of interest, type of testers used to assess the parental lines, and environmental factors (Hallauer and Miranda, 1988). Specifically, the expression of heterosis depends primarily on the specific combining ability of the inbred parents. A heterotic group is a group of unrelated or related germplasm from different or the same population, displaying similar heterotic response and combining ability when crossed with germplasm from other genetically unique sources (Melchinger and Gumber, 1998), while a heterotic pattern involves specific pair of heterotic groups, either lines or populations, that show in their cross combination high degree of heterosis and hence high hybrid vigour (Carena and Hallauer, 2001; Acquaah, 2012). In commercial practice, a heterotic pattern is the degree of SCA that an inbred has with a series of other inbreds. Although hybrid testing over locations and years is expensive and takes more time, especially if breeders use 2 or 3 generations of advance per year, identifying parents that would produce oustanding hybrids is equally time-consuming and an expensive phase in a hybrid breeding programme. Several authors have indicated that crosses among inbred lines derived from unrelated heterotic groups have better grain yield performance than those crosses among lines belonging to the same group (Moll *et al*., 1965; Hallauer and Miranda, 1988; Melchinger, 1999; Suwarno *et al*., 2015; Badu-Apraku *et al*., 2016a). The development of successful maize hybrids requires identification of heterotic groups and subsequently, establishment of different heterotic interactions for inbred lines which would show low or high SCA with other inbreds. The most exploited heterotic patterns are crosses between Lancaster Sure Crop2 inbreds and Iowa Stiff Stalk Synthetic (BSSS)

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inbreds in the US corn belt (Barata and Carena, 2006). However, there are differential patterns among inbreds from both groups. For example, some B73 related lines show considerable heterosis with B37 lines. Both lines are from the BSSS. It is therefore, much more useful to look at crosses between different inbreds and then classify the inbreds as being complementary or opposite to each other. Another reported heterotic pattern is the "European flint" x "Corn Belt Dent" in Europe which was identified on the basis of types of endosperm (Acquaah, 2012).

In instances where common testers are available, heterotic groups and patterns can be established by a cross between the new germplasm and reliable testers from opposing heterotic patterns or by crossing the genotypes in possible combinations depending on the mating design used. Heterotic groups are defined by testers and they can be developed and improved using testers to select new inbreds that combine well with testers. US maize breeders have been most successful in developing new inbreds and hybrids by recombining the most elite genetic material available. The recycling of inbred lines within separate heterotic families has resulted in the accumulation of genes within separate heterotic families that maximize hybrid yield.

Mating designs including the NCDII, diallel, and the line x tester (Ifie *et al*., 2015; Badu-Apraku *et al*., 2016a; Hayman, 1954; Fan *et al*., 2009) have been useful in identifying heterotic groups. However, morphological traits, especially quantitative traits are affected by differences in environments and have shortfalls in revealing minute variations among genotypes that are closely related (Smith and Smith, 1992). Molecular markers could be useful for the classification of lines into heterotic groups (Barata and Carena, 2006). This method could be helpful in instances of new inbred sets with no pedigree information. Reports by Makumbi (2005) and Legesse *et al*. (2007) have indicated that genetic distances have limitations in predicting the performance of hybrids, SCA and heterosis of specific crosses.

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However, several studies by Lee *et al*. (1989), Smith *et al*. (1990), Betrán *et al*. (2003a), Xu *et al*. (2004), Makumbi, (2005) and Kiula *et al*. (2008) have demonstrated the existence of high correlation between hybrid performance and genetic distance. The general underlying explanation for this phenomenon is that although molecular markers are very efficient for mapping the location of genes in the genome, they only provide little information on the physiological functions and interactions of gene products. Heterosis is based on dominance, over dominance and epistatic interactions of gene products which DNA markers cannot measure. Due to the shortfalls of both morphological traits and molecular markers in classifying inbred lines into heterotic groups, any of the approaches could be used in combinations to complement the other in a genetic improvement programme.

Different methods of identifying heterotic groups have been proposed. The use of SCA effect of grain yield (Fan *et al*., 2004) is recognized as the oldest method. Using this method to assign inbreds into different heterotic groups could be biased by genotype x environment interactions which could result in inconsistent grouping of inbreds in different studies (Badu-Apraku *et al*., 2013b). The SCA and the GCA effects of grain yield were therefore combined in a method called heterotic group's specific and general combining ability effects of grain yield (HSGCA) to provide more efficiency in grouping maize inbreds (Fan *et al*., 2009). Unfortunaletly, the HSGCA and SCA methods are based only on grain yield making them less efficient. This is because grain yield has low heritability especially under stress as indicated by Bolaños and Edmeades (1993), and hence directly selecting for grain yield alone under stress may delay progress. For these reasons, Badu-Apraku *et al*. (2013b) proposed the Heterotic grouping based on GCA of multiple traits (HGCAMT). Only the measured traits which had significant genotypic variances are employed in this method, and it becomes the method of choice to classify inbreds lines into heterotic groups in the NCD II arrangements where the crosses are restricted to specific sets of inbreds. Furthermore, it is the most

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appropriate method for grouping inbred lines when the breeding objective is to develop resistance or tolerance to multiple stresses involving the measurement of several traits.

Several authors have assessed the efficiency of the different grouping methods. Results so far have been contradictory. Akinwale *et al*. (2014) found the HSGCA method as the most efficient after comparing the SCA, HSGCA and SNP marker methods. Likewise, Badu-Apraku *et al*. (2015b) ranked the different methods in decreasing order of efficiency as follows; HSGCA > HGCAMT > SNP markers > SCA method in a study that compared the efficiencies of these four grouping methods. In another study, the grouping based on HSGCA was more efficient over the SCA method when grouping the extra-early maturing white maize inbred lines (Amegbor *et al*., 2017). Conversely, Badu-Apraku *et al*. (2013a) and (2016b) compared the grouping methods ranked them in the following order; the HGCAMT > HSGCA > SNP markers. The contradictory reports emanating from the different studies were principally ascribed to the differences in the inbreds used, their responses to the differences in environments with respect to the SCA, HSCGA and HGCAMT methods, as well as the number of markers used with respect to the SNP-marker based method.

2.8 Genotype by environment interactions in testing of maize hybrids

Prior to the commercialization of promising genotypes, their performance across variable environmental conditions is assessed by evaluation across multiple environments, commonly referred to as multi-environment trials (MET), including farmers' fields. Genotype x environment interaction (GEI) exists if a genotype performs differently in varying environments. Once a significant GEI is detected, the source of variation is decomposed into its components to identify those genotypes that have superior and stable yielding ability across environments. Significant GEI is advantageous when the objective is to develop location specific varieties. However, it is not desirable when the objective is to develop cultivars

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adapted to a wide range of production environments (Badu-Apraku and Fakorede, 2017). Several statistical packages are available for analyzing and interpreting multi-location trials (METs) data. For example, the Genotype and Genotype x Environment (GGE) biplot software (Yan *et al*., 2000) as well as the Additive Main Effects and Multiplicative Interaction (AMMI) package (Gauch and Zobel, 1997) are commonly used depending on the objectives of the researcher.

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CHAPTER THREE

3.0 Performance of early maturing provitamin A-quality protein maize inbred lines under drought, low soil Nitrogen and optimal conditions

3.1 Introduction

Several biotic and abiotic constraints limit the production of maize in SSA. Among the abiotic constraints, drought and low-N are the two most important (Badu-Apraku *et al*., 2011a) and have been found to be influenced by similar adaptive mechanisms (Bänziger *et al*., 1999). This explains the high tendency for the two stresses to occur simultaneously under field conditions which aggravates yield reduction (Badu-Apraku *et al*., 2011a). Moreover, scarce use of hybrid maize varieties and inadequate levels or lack of N-based fertilizer application by the resource poor farmers are major factors that reduce the production levels of maize in the sub-region (Fisher *et al*., 2014). The development and commercialization of low-N and drought tolerant hybrids is crucial to address food insecurity in SSA. Achieving this goal requires the identification of low-N and drought tolerant inbreds for hybrid development under low-N, drought and optimal environments.

The normal endosperm maize has a major defect as food for human beings, especially for infants, because the protein is deficient in lysine and tryptophan, the two essential amino acids responsible for the quality protein levels in maize. Compared to the normal endosperm maize, QPM has the potential to provide more than twice the levels of lysine and tryptophan to reduce protein energy malnutrition. The protein of QPM has 80% biological value compared to 40-57% for normal maize (Bressani, 1992). The kernels of normal, yellow maize provide 0.5 to 1.5 μg g^{-1} PVA (Egesel, 2003) which is low compared to the breeding target of 15 μg g^{-1} dry weight (DW) set by HarvestPlus to prevent vitamin A deficiency (VAD) in diets dominated by maize. Maize with more than 15 μg g^{-1} dry weight (DW) of PVA would

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contribute to the combat againt VAD and its health-related problems such as night blindness and depressed immune response (Pixley *et al*., 2013).

Successful performance of maize hybrids under stress and optimal conditions depends on the genetic potentials of the inbred parents that constitute the hybrid especially when the inbreds combine superior performance with high heritability estimates for important agronomic traits (Betrán *et al*., 2003a). Between 2007 and 2015, 70 inbreds with varying levels of drought and low-N tolerance as well as PVA and quality-protein were developed by the IITA-MIP. It was important to evaluate and select inbreds with combined drought and low-N tolerance and elevated levels of PVA and quality protein to develop drought and low-N tolerant, PVA-QPM hybrids and synthetics. The objectives of this study were to:

1. identify drought and low-N tolerant, early PVA-QPM inbreds using the multiple trait base index,
2. determine the PVA and tryptophan contents of the inbreds and their relation with grain yield, and
3. identify inbreds with combined drought and low-N tolerance and elevated levels of PVA and tryptophan.

3.2 Materials and Methods

3.2.1 Background of genetic materials

Sixty-four early maturing PVA-QPM inbred lines and six inbred checks were used in the present study (Tables 3.1a and b). Four of the checks, TZEQI 85, TZEQI 91, TZEQI 74 and TZEQI 82 are early maturing, yellow, QPM inbred lines, while the remaining two, TZEI 129 and TZEI 24 are early maturing, normal endosperm yellow inbred lines. These checks

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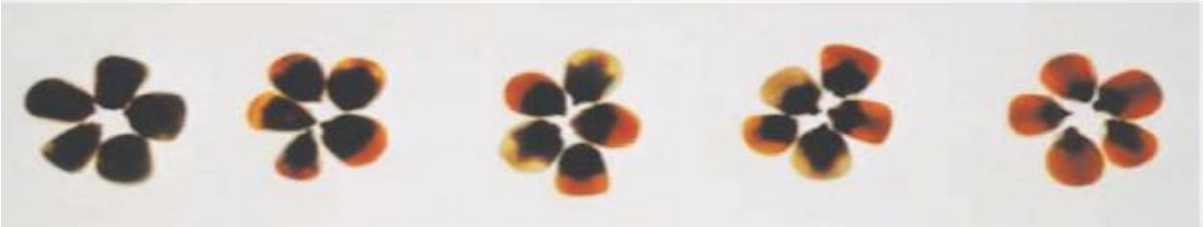
were used because early maturing PVA QPM inbred checks were not available. The development of the inbred lines was initiated in 2007 by crossing a drought and *Striga* resistant early QPM variety, TZE-Y Pop DT STR QPM with an intermediate maturing (105-110 days to maturity) high PVA maize [Syn KU1409/DES/1409 (OR2)] from the IITA-MIP to introgress genes for high β-carotene into the QPM variety. This was followed by one cycle of backcrossing to the recurrent parent to recover earliness. In 2008, the BC_1F_1 lines with deep orange colour (for PVA) and/or appropriate endosperm modifications were selected and advanced to the F_2 and the F_3 generations. In 2009, the F_3 lines were selected based on their reactions to *Striga* and drought, and recombined to constitute the early PVA-QPM variety, 2009 TZE-OR2 DT STR QPM. Subsequent evaluations of this variety for *Striga* resistance and drought tolerance in 2010 showed superior performance. In addition, as part of another programme initiated in 2011 to extract the first generation of early maturing inbreds from different high PVA sources, S_1 lines from the PVA-QPM variety 2009 TZE - OR2 DT STR QPM were advanced through inbreeding to the S_6 generation from 2011 to 2014. In the course of the inbreeding programme, the inbred lines were screened at the S_2 to the S_5 generations to select for kernels with deep orange colour for the PVA trait and the appropriate endosperms modification ranging from 25 – 50% opaqueness for the QPM trait (Plate. 3.1). In 2015, a set of 73 early PVA-QPM inbreds were selected on the basis of their reactions to drought and low-N, and 64 were used in the present study.



1 2 3 4 5

Plate 3. 1 Varying degrees of opaqueness indicating different levels of endosperm modification for tryptophan and lysine contents. 1= opaque; 2= 25% modification; 3= 50% modification; 4= 75% modification; and 5= 100% modification (Vivek *et al*., 2008).

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Table 3. 1 Designations and pedigrees of the 64 early maturing provitamin A quality protein maize inbreds and six inbred checks

S/N	Inbred Designation	Pedigree	Reaction to Drought	Low-N
1	TZEIORQ 2	2009 TZE OR2 DT-STR QPM S_6 Inb 2-2/3-1/3-1/3-1/2-1/1	T	T
2	TZEIORQ 3	2009 TZE OR2 DT-STR QPM S_6 Inb 2-2/3-1/3-3/3-3/3-1/1	-	-
3	TZEIORQ 5	2009 TZE OR2 DT-STR QPM S_6 Inb 2-2/3-2/3-1/4-3/3-1/1	T	S
4	TZEIORQ 6	2009 TZE OR2 DT-STR QPM S_6 Inb 2-2/3-2/3-2/4-1/5-1/1	T	S
5	TZEIORQ 7	2009 TZE OR2 DT-STR QPM S_6 Inb 2-2/3-2/3-3/4-1/3-1/1	T	S
6	TZEIORQ 8	2009 TZE OR2 DT-STR QPM S_6 Inb 2-2/3-2/3-4/4-3/3-1/1	T	S
7	TZEIORQ 9	2009 TZE OR2 DT-STR QPM S_6 Inb 2-2/3-3/3-1/5-1/2-1/1	S	T
8	TZEIORQ 10	2009 TZE OR2 DT-STR QPM S_6 Inb 7-1/3-1/2-1/2-1/4-1/1	S	-
9	TZEIORQ 11	2009 TZE OR2 DT-STR QPM S_6 Inb 7-1/3-1/2-1/2-4/4-1/1	T	T
10	TZEIORQ 12	2009 TZE OR2 DT-STR QPM S_6 Inb 7-1/3-1/2-2/2-2/3-1/1	S	-
11	TZEIORQ 13	2009 TZE OR2 DT-STR QPM S_6 Inb 7-1/3-1/2-2/2-3/3-1/1	-	-
12	TZEIORQ 14	2009 TZE OR2 DT-STR QPM S_6 Inb 7-2/3-1/2-2/4-2/2-1/1	-	-
13	TZEIORQ 15	2009 TZE OR2 DT-STR QPM S_6 Inb 7-2/3-1/2-3/4-1/3-1/1	T	S
14	TZEIORQ 16	2009 TZE OR2 DT-STR QPM S_6 Inb 7-2/3-1/2-4/4-1/3-1/1	S	-
15	TZEIORQ 17	2009 TZE OR2 DT STR QPM S_6 Inb 22-1/2-1/1-2/2-3/3-1/1	S	S
16	TZEIORQ 18	2009 TZE OR2 DT STR QPM S_6 Inb 22-2/2-1/3-2/3-2/2-1/1	-	-
17	TZEIORQ 19	2009 TZE OR2 DT STR QPM S_6 Inb 22-2/2-1/3-3/3-1/1-1/1	-	-
18	TZEIORQ 20	2009 TZE OR2 DT STR QPM S_6 Inb 26-1/1-1/2-1/6-1/2-1/1	T	T
19	TZEIORQ 21	2009 TZE OR2 DT STR QPM S_6 Inb 26-1/1-1/2-3/6-1/4-1/1	T	T
20	TZEIORQ 22	2009 TZE OR2 DT STR QPM S_6 Inb 26-1/1-1/2-3/6-3/4-1/1	T	T
21	TZEIORQ 23	2009 TZE OR2 DT STR QPM S_6 Inb 26-1/1-1/2-4/6-1/3-1/1	T	S
22	TZEIORQ 24	2009 TZE OR2 DT STR QPM S_6 Inb 26-1/1-1/2-4/6-2/3-1/1	T	T
23	TZEIORQ 25	2009 TZE OR2 DT STR QPM S_6 Inb 26-1/1-1/2-5/6-1/3-1/1	T	T
24	TZEIORQ 26	2009 TZE OR2 DT STR QPM S_6 Inb 26-1/1-1/2-6/6-2/3-1/1	T	S
25	TZEIORQ 27	2009 TZE OR2 DT STR QPM S_6 Inb 26-1/1-2/2-3/3-1/2-1/1	T	-
26	TZEIORQ 28	2009 TZE OR2 DT STR QPM S_6 Inb 28-1/1-1/2-2/2-1/3-1/1	-	-
27	TZEIORQ 29	2009 TZE OR2 DT STR QPM S_6 Inb 28-1/1-2/2-1/2-1/2-1/1	T	S
28	TZEIORQ 30	2009 TZE OR2 DT STR QPM S_6 Inb 28-1/1-2/2-1/2-2/2-1/1	T	S
29	TZEIORQ 31	2009 TZE OR2 DT STR QPM S_6 Inb 31-1/2-1/2-2/4-1/1-1/1	-	-
30	TZEIORQ 32	2009 TZE OR2 DT STR QPM S_6 Inb 31-1/2-1/2-3/4-1/1-1/1	-	-
31	TZEIORQ 33	2009 TZE OR2 DT STR-QPM S_6 Inb 31-1/2-4/4-1/2-1/1-1-1	S	T
32	TZEIORQ 34	2009 TZE OR2 DT STR QPM S_6 Inb 31-2/2-1/2-1/2-1/2-1/1	S	S
33	TZEIORQ 35	2009 TZE OR2 DT STR QPM S_6 Inb 32-1/1-1/2-1/3-2/5-1/1	-	-
34	TZEIORQ 36	2009 TZE OR2 DT STR QPM S_6 Inb 32-1/1-1/2-1/3-3/3-1/1	S	S
35	TZEIORQ 37	2009 TZE OR2 DT STR QPM S_6 Inb 32-1/1-1/2-2/3-2/2-1/1	T	T
36	TZEIORQ 39	2009 TZE OR2 DT STR QPM S_6 Inb 35-2/3-1/3-3/5-2/3-1/1	T	T
37	TZEIORQ 40	2009 TZE OR2 DT STR QPM S_6 Inb 35-2/3-2/3-1/2-2/2-1/1	S	T
38	TZEIORQ 41	2009 TZE OR2 DT STR QPM S_6 Inb 35-2/3-3/3-1/4-3/3-1/1	-	-
39	TZEIORQ 42	2009 TZE OR2 DT STR QPM S_6 Inb 35-2/3-3/3-2/4-2/2-1/1	T	T

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Table 3. 1 Continuation

S/N	Inbred Designation	Pedigree	Reaction to Drought	Low-N
40	TZEIORQ 43	2009 TZE OR2 DT STR QPM S_6 Inb 35-2/3-3/3-3/4-1/2-1/1	T	S
41	TZEIORQ 44	2009 TZE OR2 DT STR QPM S_6 Inb 35-2/3-3/3-4/4-1/4-1/1	T	S
42	TZEIORQ 45	2009 TZE OR2 DT STR QPM S_6 Inb 35-2/3-3/3-4/4-3/4-1/1	S	T
43	TZEIORQ 46	2009 TZE OR2 DT STR QPM S_6 Inb 35-3/3-3/3-1/3-1/2-1/1	-	-
44	TZEIORQ 47	2009 TZE OR2 DT STR QPM S_6 Inb 35-3/3-3/3-1/3-2/2-1/1	S	T
45	TZEIORQ 48	2009 TZE OR2 DT STR QPM S_6 Inb 41-1/2-1/3-1/2-3/3-1/1	S	-
46	TZEIORQ 52	2009 TZE OR2 DT STR QPM S_6 Inb 41-2/2-1/2-1/3-1/1-1/1	-	-
47	TZEIORQ 53	2009 TZE OR2 DT STR QPM S_6 Inb 41-2/2-1/2-2/3-2/2-1/1	S	S
48	TZEIORQ 54	2009 TZE OR2 DT STR QPM S_6 Inb 41-2/2-1/2-3/3-1/1-1/1	S	T
49	TZEIORQ 55	2009 TZE OR2 DT STR QPM S_6 Inb 42-2/2-2/2-1/1-1/1-1/1	-	-
50	TZEIORQ 57	2009 TZE OR2 DT STR QPM S_6 Inb 50-2/2-1/3-1/3-3/3-1/1	-	-
51	TZEIORQ 58	2009 TZE OR2 DT STR QPM S_6 Inb 50-2/2-1/3-2/3-1/2-1/1	S	S
52	TZEIORQ 59	2009 TZE OR2 DT STR QPM S_6 Inb 50-2/2-1/3-2/3-2/2-1/1	T	T
53	TZEIORQ 60	2009 TZE OR2 DT STR QPM S_6 Inb 50-2/2-1/3-3/3-1/2-1/1	-	-
54	TZEIORQ 61	2009 TZE OR2 DT STR QPM S_6 Inb 50-2/2-2/3-1/2-1/2-1/1	T	T
55	TZEIORQ 62	2009 TZE OR2 DT STR QPM S_6 Inb 50-2/2-2/3-2/2-1/1-1/1A	-	-
56	TZEIORQ 63	2009 TZE OR2 DT STR QPM S_6 Inb 50-2/2-2/3-2/2-1/1-1/1B	-	-
57	TZEIORQ 65	2009 TZE OR2 DT STR QPM S_6 Inb 50-2/2-3/3-3/4-1/1-1/1	S	S
58	TZEIORQ 66	2009 TZE OR2 DT STR QPM S_6 Inb 51-2/3-1/2-2/3-1/1-1/1	S	S
59	TZEIORQ 68	2009 TZE OR2 DT STR QPM-S_6 Inb 57-2/2-1/2-1/4-2/2-1/1	S	T
60	TZEIORQ 69	2009 TZE OR2 DT STR QPM S_6 Inb 57-2/2-2/2-1/1-1/2-1/1	T	T
61	TZEIORQ 70	2009 TZE OR2 DT TR QPM S_6 Inb 60-2/2-1/2-1/3-1/4-1/1	S	T
62	TZEIORQ 71	2009 TZE OR2 DT STR QPM S_6 Inb 60-2/2-1/2-2/3-1/1-1/1	S	S
63	TZEIORQ 72	2009 TZE OR2-DT STR QPM S_6 Inb 62-2/2-2/2-1/2-1/2-1/1	S	S
64	TZEIORQ 73	2009 TZE OR2-DT STR QPM S_6 Inb 62-2/2-2/2-2/2-1/1-1/1	-	-
65	C-1 TZEQI 85	TZE COMP5-Y C6S6 Inb 25 x Pool 18 SR QPM BC2S6 4-5-1-1-3-5	T	T
66	C-2 TZEQI 91	TZE Y Pop STR C0 S6 Inb 142 x Pool 18 SR QPM BC2S6 4-35-5-8-4-8	T	T
67	C-3 TZEQI 74	TZE COMP5-Y C6S6 Inb 10 x Pool 18 SR QPM BC2S6 2-2-1-1	T	T
68	C-4 TZEQI 82	TZE COMP5-Y C6S6 Inb 25 x Pool 18 SR QPM BC2S6 2-3-1-1-6-6	T	T
69	C-5 TZEI 129	TZE Y Pop STR Co S6 Inbred 16-1-3	T	T
70	C-6 TZEI 24	TZE Y Pop STR Co S6 Inbred 142-2-2	T	T

Entries 65 to 70 are checks; T= tolerance; S= susceptibility; - = data not available.

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3.2.2 Experimental sites and stress management

3.2.2.1 Evaluation of inbreds lines under induced drought conditions

The inbreds were planted under managed drought conditions at Ikenne (6º 53'N, 30º 42'E, 60 m altitude, 1200 mm annual rainfall) during the 2016/2017 and 2017/2018 dry seasons. The induced drought at Ikenne was achieved by supplying 17 mm of irrigation water per week until 25 days after planting when irrigation water was withdrawn. The inbred lines depended on stored soil moisture until physiological maturity. The managed drought trials received fertilizer rates of 60 kg ha^{-1} each of N, P and K (15-15-15) at planting. Additionally, top-dressing was done at 2 WAP using 60 kg ha^{-1} of urea. Weeds were controlled by applying pre- and post-emergence herbicides (atrazine and gramozone) at 5 l ha^{-1} each of primextra and paraquat, followed by manual weeding.

3.2.2.2 Evaluation of inbred lines under low-N conditions

The inbreds were assessed under low-N (30 kg ha^{-1}) conditions at Ile-Ife (7° 28' N, 4° 33' E, and 244 m above sea level, 1200 mm annual rainfall) and Mokwa (9º18'N, 5º 4'E, 457 m altitude, 1100 mm annual rainfall) in 2016 and 2017 in Nigeria. The soil at Mokwa is a luvisol while that of Ile-Ife is Alfisol (Soil Survey Staff, 1999). The experimental fields at both locations were depleted of N by continuous planting of maize and removing the stover after harvest. Before the low-N fields were prepared, soil samples from 0 to 15 cm depth were taken for analysis at the IITA analytical services laboratory, Ibadan, Nigeria. The Kjeldahl digestion and colorimetric method (Bremner and Mulvaney, 1982) was use for the analysis of the soil samples to determine the levels of nitrogen (N), phosphorus (P) and potassium (K). The soil from the low-N experimental field at Mokwa contained 0.085 % of N, 6.32 ppm of P and 0.20 cmol kg^{-1} of K, while that of Ile-Ife had 0.084 % of N, 2.05 ppm of P and 0.358 cmol kg^{-1} of K. Based on the soil tests, NPK fertilizer was formulated using urea, single superphosphate and muriate of potash, respectively. The NPK fertilizer was applied at 2

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weeks after planting (WAP) immediately after thinning to bring the levels of the total available basal N to 15 kg ha^{-1}. The levels of the single superphosphate (P_2O_5) and the muriate of potash (K_2O) fertilizers supplied were 60 kg ha^{-1} each of P and K. An additional 15 kg ha^{-1} of urea was top-dressed at 4 WAP to bring the total available N to 30 kg ha^{-1}. Weed control was carried out as described for the drought trials in section 3.2.2.1.

3.2.2.3 Assessment of inbred lines under optimal environments

The inbreds were also assessed under optimal-conditions in the 2016 and 2017 growing seasons at Ikenne, Ile Ife and Mokwa. For the experiments under optimal conditions, N P K (15:15:15) was applied at 2 WAP to supply 60 kg ha^{-1} each of N, P and K and top-dressed with an additional 30 kg N ha^{-1} at 4 WAP. Weed control was done for the optimal trials as described for the drought and low-N trials.

3.2.3 Field layout

All the inbred experiments under drought, low-N and optimal environmentss were laid-out using a 7 × 10 alpha lattice design with two replications. Each experimental unit was a one-row plot, 3 m long with inter-row and intra-row spacing of 0.75 and 0.40 m, respectively. In all the experiments, three seeds were planted per hill and the seedlings were later thinned to two at 2 WAP to obtain a plant population density of approximately 66,666 ha^{-1}.

3.2.4 Data collection

Data were recorded on plot basis for all measured traits for the three sets of experiments (drought, low N and optimal) as follows:

Days to silking, DS = number of days from planting to 50% silk emergence.

Days to anthesis, DA = number of days from planting to 50% pollen shed.

Anthesis silking interval, ASI = DA - DS.

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Plant height PLHT, (cm) = distance from the base of the plant to the first tassel branch (mean of 10 plants selected randomly).

Ear height EHT, (cm) = distance from the base of the plant to the height of the node bearing the upper ear (mean of 10 plant selected randomly).

Root lodging, RL = % plants leaning more than 30° from the vertical.

Stalk lodging, SL = % plants broken at or below the highest ear node.

Husk cover = measure of how tight or loose the ear tip was, rated on a 1-9 scale where:

1 = husks tightly arranged and extended beyond the ear tip,

9 = tips of ears exposed completely.

Ear aspect = the assessment of the overall appearance of the ears without the husks. The ear aspect was rated on a scale of 1 – 9 based on the ear size; uniformity of size, colour and texture; extent of grain filling, insect and disease damage, where:

1 = excellent ears with no disease/insect damage, large cobs, uniform ears and fully filled grains,

2 = very good ears with no disease/insect damage and fully filled grains, one or two irregularities in cob size,

3 = good ears with no disease/insect damage and fully filled grains, one or two irregularities in cob size,

4 = ears with little insect damage, no disease, fully filled grains, one or two irregularities in cob size,

5 = ears with mild disease/insect damage and fully filled grains, one or two irregularities in cob size,

6 = ears with severe disease/insect damage and fully filled grains, smaller cobs, non-uniform cob size,

7 = ears with severe disease/insect damage, scanty grain filling, few ears, non-uniformity of cobs,

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8 = ears with severe disease/insect damage, scanty grain filling, very few ears, and

9 = only one or no ears.

Number of ears per plant was computed by dividing the total number of ears harvested per plot by the number of plants in a plot at harvest.

Plant aspect was rated on a scale of 1- 9 based on the assessment of overall architecture of plants in a plot as they appeal to sight, where:

1 = excellent overall phenotypic appeal,

2 = very good overall phenotypic appeal,

3 = good overall phenotypic appeal,

4 = relatively good overall phenotypic appeal,

5 = acceptable,

6 = relatively poor overall phenotypic appeal,

7 = poor overall phenotypic appeal,

8 = very poor overall phenotypic appeal,

9 = extremely poor overall phenotypic appeal.

Data were recorded on stay-green characteristics for the drought and low N trials at 70 DAP (10 WAP) using a scale of 1-9 on the basis of the percentage of dead leaf area, where,

1 = 0-10% of dead leaf area

2 = 11-20% of dead leaf area

3 = 21-30% of dead leaf area

4 = 31-40% of dead leaf area

5 = 41-50% of dead leaf area

6 = 51-60% of dead leaf area

7 = 61-70% of dead leaf area

8 = 71-80% of dead leaf area

9 = 81-100% of dead leaf area

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For the drought and low-N trials, all harvested ears from each plot were shelled and grain weight measured. The amount of moisture in the grains was determined using Kett moisture tester PM-450. Grain yield in kg ha^{-1} was computed from the shelled grain weight, adjusted to 15% moisture content. However, for the optimal trials, a shelling percentage of 80% was assumed for inbred lines per plot and grain yield (obtained from ear weight and converted to kg ha^{-1}) was adjusted to 15% moisture content.

3.2.5 Data analysis

Data recorded for stalk lodging, root lodging and ear rot were transformed using the square root transformation procedure. Each location-year combination was considered as a test environment while the low-N, induced drought and the optimal growing conditions were regarded as research conditions (treatments). Analysis of variance (ANOVA) was carried out separately on data collected under low-N, drought, and optimal conditions with the general linear model procedure (PROC GLM) in Statistical Analysis System (SAS) using a random statement with test option (SAS Institute, 2012). Subsequently, combined ANOVA was performed across the eight test environments. In the ANOVA for each and across environments, the environments, replications within environment, and incomplete blocks within replications × environments interaction were considered as random factors whereas the entries (inbreds) were regarded as a fixed factor. The statistical model corresponding to the experimental layout was:

$$y_{klmi} = \mu_i + E_{ki} + R(E)_{kli} + G_{mi} + GE_{kmi} + \epsilon_{klmi}$$

Where y_{klmi} is the observed measurement of trait *i* with mean effect μi. E_{ki} is the effect of environment k on trait *i*, $R(E)_{kli}$ is the effect of replication *l* within environment *k* on trait *i*, G_{mi} is the effect of genotype *m* on trait *i*, GE_{kmi} is the effect of the interaction between genotype *m* and environment *k* on trait *i*, and $\epsilon_{klmi}$is the experimental error effect associated with genotype *m* and replication *l* within environment *k* on trait *i*. The entry means were

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adjusted for block effects, according to the lattice design (Cochran and Cox, 1960) and means were separated using standard error (S.E). Genetic and phenotypic variance components of the inbreds were estimated with the restricted maximum likelihood (REML) method using PROC Varcomp in SAS to compute the broad sense heritability (H^2) for each of the measured trait. Broad sense heritability was calculated as:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{e} + \frac{\sigma_e^2}{re}}$$

where σ_g^2 is the genotypic variance, σ_{ge}^2 is the genotype x environment and σ_e^2 is the error variance, e is the number of environments, and r the number of replicates per environment (Fehr, 1991). Furthermore, the relationship between grain yield and other agronomic traits under drought, low-N and optimal environments were examined by estimating the genetic correlation coefficients for the traits using the meta menus program in SAS version 9.4 (SAS Institute, 2012).

3.2.6 Selection of inbred lines under drought and low-N conditions using the multiple trait base index

Drought and low-N tolerant inbred lines were selected using the multiple trait base index (MI) for combined drought and low-N proposed by Badu-Apraku *et al*. (2011a). The traits employed in the drought and low-N MI were grain yield, number of ears per plant, stay-green characteristics, plant aspect, ear aspect and ASI. The MI was calculated as:

MI = [(2 x Yield) + EPP –ASI – PASP – EASP – STGR]

Where:

Yield = grain yield under drought or low-N environments,

EPP = number of ears per plant under drought or low-N environments,

EASP = ear aspect under drought or low-N environments,

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PASP = plant aspect under drought or low-N environments,

SGR = stay green characteristics under drought or low-N environments,

ASI = anthesis-silking interval under drought or low-N environments.

Each of the parameters used in the MI was standardized with standard deviation of 1 and a mean of zero to minimize the effect of different scales. A positive value of MI was an indicator of drought or low-N tolerance, while a negative value indicated susceptibility of an inbred line. Also, the MI was applied to data across drought and low-N environments to assess the reactions of the inbred lines to the two stresses.

3.2.7 Assessment of provitamin A carotenoids and tryptophan contents of the inbreds

Comparison of levels of PVA carotenoids and tryptophan for the early maturing PVA-QPM inbred population was necessary to measure the progress made in the improvement of the PVA and quality protein traits over the years. Among the two essential amino acids (lysine and tryptophan) responsible for quality protein levels in maize, only tryptophan was assayed in this study to assess the quality protein trait in the inbreds. This is because lysine and tryptophan contents in the maize grain are highly correlated (> 0.9) and, because tryptophan analysis is less expensive, researchers use tryptophan contents as an indicator of the quality protein content of a QPM cultivar (Villegas *et al*., 1992; Nurit *et al*., 2009). Also, due to the high cost involved in the analysis of maize carotenoids using the HPLC technique, a set of 19 inbreds were selected and assayed for carotenoids and including Obatanpa for tryptophan to describe the inbred population with respect to these two nutritive values. The selection of inbreds was based on available pedigree information, kernel colour, endosperm modification and existing data on the reactions of the inbreds to drought and low-N conditions.

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3.2.8 Production of inbred kernel samples for carotenoids and tryptophan analyses

The 18 selected inbred lines plus one check for tryptophan content, "Obatanpa", were planted under well-watered growing conditions in January, 2018 at IITA Ibadan, to produce kernel samples for carotenoids and tryptophan analyses. The inbred lines were planted in single rows, 1 m long with row and hill spacing of 0.75 and 0.20 m, respectively. Two seeds were sown per hill and later thinned to one plant to provide at least five plants per each inbred line. Seed samples were produced by controlled self-pollination of all plants in each plot. Ears of each self-pollinated line in each plot were harvested, well dried under ambient temperature with minimal exposure to direct sun light and shelled separately. Samples of 100 kernels were collected and sent to the IITA nutritional laboratory for carotenoids and tryptophan analyses. In addition, kernel colour was scored following the standard colour scale for estimating total carotenoid content, and this was converted to a scale of 1 to 12 (Plate. 3.2) representing shades of colours from pale yellow to darkest orange as observed within the early maturing PVA-QPM inbred set evaluated.

Plate 3. 2 Standardized colour scale that included representative kernels from the early maturing, PVA-QPM inbreds evaluated in 2016 and 2017. The ordinal colour scale ranges from 1 (pale yellow) to 12 (darkest orange) (Chandler *et al*., 2013).

3.2.9 Analysis of provitamin-A carotenoids

Carotenoids were extracted and quantified by HPLC at the IITA nutritional laboratory, Ibadan, Nigeria. The protocol for extraction and carotenoid analysis was based on the procedure described in Howe and Tanumihardjo (2006). Finely ground 0.6 g sample of each

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entry was transferred into a 50 ml glass centrifuge tube to which 6 ml of Ethanol plus 0.1% butylated hydroxyl toluene were added. After vortexing for 15 seconds tubes were placed in 85°C water bath for 5 min and 500 µl of 80% potassium hydroxide (w:v) was added. The samples were vortexed for 15 seconds, and put back in the water bath for 10 min with vortexing at 5 min interval approximately. They were then immediately placed on ice and 3 ml ice cold deionized water was added, vortexed for 15 seconds, followed by addition of 200 µl of the internal standard β-Apo-8'-carotenal and 4 ml hexane. After vortexing and centrifugation, the top hexane layer formed was transferred into a new test tube. The hexane extraction was repeated twice, adding 3 ml hexane each time. Samples were allowed to dry down completely under nitrogen gas using a Turbovap LV concentrator and reconstituted in 500 µl of 50:50 Methanol: Dichloroethane. Following vortexing and centrifuging, the extracts were transferred to HPLC vials placed in the autosampler tray and 50 µl aliquots of each extract were injected into an HPLC system. The Waters HPLC system was operated with Empower 1 software and included a 717 Plus auto sampler with temperature control set at 5°C, a Waters 1525 binary HPLC pump, and a 2996 photodiode array detector for carotenoid quantification. Carotenoids were separated on a 3 µm C30 YMC Carotenoid Column (4.6 × 250 mm) eluted with a mobile phase of methanol/water (92:8 v/v) with 10 mM ammonium acetate as solvent A, and 100 % methyl tertiary butyl ether as solvent B. The gradient was applied for 30 minutes from 70% solvent A:30% solvent B, to 40% solvent A:60% solvent B. The flow rate was 1.0 mL/min. To maximize detection of carotenoids, the absorbance was measured at 450 nm. Beta-carotene (*cis* and *trans* isomers), α-carotene, β-cryptoxanthin, zeaxanthin, and lutein were assayed based on calibrations using the respective external standards. Total carotenoids were computed as the sum of concentrations of α-carotene, β-carotene, lutein, Zeaxanthin and β-cryptoxanthin. PVA was computed as the sum of β-carotene, and half of each of β-cryptoxanthin and α-carotene contents, since β-cryptoxanthin and α-carotene contribute half (50%) of the value of β-carotene as PVA (US institute of

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medicine, 2001). Values of all carotenoids for each sample were obtained from two independent measurements to allow for statistical analysis.

3.2.10 Analysis of tryptophan content

The selected inbred lines plus the open-pollinated QPM variety standard check (Obatanpa) were analysed for tryptophan levels in the whole grain flour. Tryptophan was quantified by the colourimetric method (Herbabdes and Bates, 1969). Degermed kernels were ground and defatted in a sexlet extractor with hexane, and finely ground using an amalgamater. A 100 mg flour sample with 4 ml papain enzyme was incubated at 65°C overnight. One millilitre of the hydrolysed sample was transferred into a test tube containing 'reagent C' and incubated at 65°C for 15 mins for colour development. The optical density (OD) values of the samples were read using spectrophotometer (Systronic117) at a wavelength of 545 nm. The tryptophan content was determined using a standard curve of a known check. Values of tryptophan levels for each sample were obtained from two independent measurements to allow for statistical analysis. The tryptophan (%) was expressed in terms of a given unit of samples (Vivek *et al*., 2008; Teklewold *et al*., 2015).

3.2.11 Statistical analysis of carotenoids and tryptophan of selected inbreds

PVA carotenoids and tryptophan data of inbred lines were transformed using natural logarithm as the ratios were not expected to follow a normal distribution curve. The ANOVA for PVA carotenoids and tryptophan of the inbred lines were performed using SAS statistical package version 9.4 (SAS Institute, 2012), and means were separated using standard error (SE). Phenotypic correlations between PVA and other carotenoids, kernel colour, tryptophan, and grain yield were estimated to assess the relationships among the traits. Phenotypic correlation coefficients were computed using the Spearman rank correlation method implemented in SAS version 9.4 (SAS Institute, 2012).

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3.3 Results

The combined ANOVA across the two drought environments revealed significant ($P< 0.05$ or $P< 0.01$) differences among environments (E) and inbred lines (G) for measured traits except the E effects for ASI, plant aspect, ear aspect and ears per plant and the G effects for ear rot (Table 3.2). Significant ($P < 0.05$) inbred x environments interactions (GEI) effects were detected for grain yield, ASI and root lodging. Broad sense heritability (H^2) estimates on plot mean basis ranged from 17% for plant aspect to 83% for plant height and grain yield had 56% while that of root lodging could not be estimated because of zero genotypic variance recorded for the trait.

Grain yield under drought when compared to the corresponding yield performance of the inbreds under optimal environments revealed varying yield reductions from 25% for TZEIORQ 8 to 94% for TZEIORQ 11 with a mean of 72% (Table 3.3). Generally, the inbred lines that recorded more days to anthesis, had increased anthesis-silking interval, poor plant and ear aspects, fewer ears per plants, and high percentage grain yield reduction and also had low grain yield and negative selection indices under drought. The inbred lines showed varying reactions to the two years of induced drought (Appendix 1). Thirty-two out of the seventy inbred lines had positive base indices indicating drought tolerance. Also, 46 inbreds used in the present study were included in previous evaluations under drought in the IITA-MIP. Comparing the results of the present study to that of the previous evaluation in 2015 under drought (Table 3.1 a and b), 14 and 12 of the 46 inbred lines were confirmed tolerant and susceptible respectively, while 20 of the lines showed inconsistent reactions to drought as revealed by the drought base index.

Under the three low-N environments, the combined ANOVA showed significant ($P< 0.05$ or $P< 0.01$) differences among mean the squares for E, G and GEI for all measured traits with the exception of GEI effects for ear height, and root and stalk lodging (Table 3.4). The H^2 ranged from a very low estimate of 10% for ears per plant to a moderately high estimate

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of 64% for days to 50% anthesis and grain yield recorded 41%. H^2 could not be estimated for husk cover due to the negative genotypic variance recorded for the trait.

Grain yield reduction due to effect of the low-N ranged from 7% for TZEIORQ 42 to 84% for TZEQI 74 with a mean of 37% (Table 3.5). Generally, higher days to anthesis, increased anthesis-silking interval, reduced plant aspect, fewer ears per plants, reduced ear aspects and high percentage yield reduction were observed among the inbred lines which recorded low grain yield and negative base indices under low-N. Thirty-seven (53%) of the inbreds evaluated under low-N had positive base indices, an indication of low-N tolerance.

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Table 3. 2 Mean squares and heritability estimates of 70 early maturing provitamin A quality protein maize inbred lines evaluated under drought conditions at Ikenne during the 2016/2017 and 2017/2018 dry seasons

Source	DF	YIELD	DA	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP	STGR
Env	1	480952.72**	278.01**	284.01**	0.03	19881.61**	9165.73**	5.23**	9.54**	425.11**	2.41	0.01	68.76**	0.07	38.63**
Rep (Env)	2	11038.47	2.8	3.21	11.51*	294.78*	66.58	0.16	1.1	2.70*	0.46	2.35	12.43	0.03	5.16**
Block(Env*Rep)	36	65311.25**	17.35**	21.68**	3.93	330.43**	109.03**	0.31	0.98	2.15**	1.92**	3.60**	6.48	0.06**	1.71**
Inbred	69	131623.07**	14.85**	21.2**	5.92**	475.15**	165.85**	0.58**	1.43**	1.84**	1.86**	4.29**	6.78	0.08**	2.33**
Env*Inbred	69	55852.2*	3.9	7.15	4.96*	91.73	59.43	0.55**	0.71	0.78	0.64	1.11	4.6	0.02	0.92
Error	102	34510.18	6.81	10.62	3.45	93	54.13	0.32	0.85	0.68	0.62	1.07	5.61	0.03	0.64
Heritability	**-**	**0.56**	**0.65**	**0.61**	**0.18**	**0.83**	**0.65**	**-**	**0.47**	**0.61**	**0.71**	**0.78**	**0.17**	**0.75**	**0.65**

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; Env = environment; Rep=replication; YIELD = Grain yield; DA = days to 50% anthesis; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; RL = root lodging; SL = stalk lodging; HC = husk cover; PASP = plant aspect; EASP = ear plant aspect; EROT = ear rot; EPP =ears per plant; STGR = stay-green characteristic.

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Table 3. 3 Performance of 25 early maturing provitamin A-quality protein maize inbred lines plus six checks evaluated under drought conditions at Ikenne during the 2016/2017 and 2017/2018 dry seasons

Inbred	Grain yield (kg ha^{-1})	Days to Anthesis	Anthesis Silking Interval	Plant Height (cm)	Plant aspect (1 – 9)	Ear aspect (1 – 9)	Ears Per plant	STGR	Yield reduction (%)	Drought Base Index
TZEIORQ 8	895	57	3	87.17	4	5	0.72	3	25.00	16.75
TZEIORQ 25	737	54	3	87.71	4	4	0.60	4	33.68	14.14
TZEIORQ 6	702	55	4	87.68	4	5	0.55	4	40.51	12.46
TZEIORQ 5	519	56	2	77.32	4	5	0.65	4	56.55	12.01
TZEIORQ 7	596	57	2	87.21	5	5	0.57	4	46.03	11.25
TZEIORQ 9	523	57	3	97.83	4	5	0.55	4	70.36	9.81
TZEIORQ 24	519	58	4	83.77	5	5	0.56	4	29.09	8.44
TZEIORQ 37	463	57	4	88.50	5	5	0.45	3	74.33	7.87
TZEIORQ 28	568	56	4	84.01	5	5	0.39	4	72.65	7.34
TZEIORQ 23	445	59	3	82.87	6	6	0.39	3	55.93	6.97
TZEIORQ 26	342	56	3	84.39	5	6	0.37	4	73.86	5.51
TZEIORQ 73	446	58	5	81.18	5	6	0.39	4	62.69	5.01
TZEIORQ 27	702	56	4	88.61	5	7	0.22	5	63.07	4.87
TZEIORQ 40	227	54	3	68.89	5	6	0.60	5	84.40	4.05
C5-TZEI 129	336	53	5	102.96	5	6	0.31	5	81.77	2.64
C3-TZEQI 74	299	57	4	72.11	6	7	0.40	4	60.67	1.57
C6-TZEI 24	350	55	7	56.61	6	7	0.33	5	81.77	-1.09
TZEIORQ 29	297	61	6	56.82	6	7	0.24	4	73.90	-2.02
C4-TZEQI 82	109	60	6	67.60	6	8	0.13	4	90.82	-4.64
C1-TZEQI 85	105	56	6	73.43	6	7	0.10	5	93.50	-6.00
TZEIORQ 32	197	62	6	67.64	6	8	0.08	5	81.49	-6.11
TZEIORQ 69	214	62	5	60.46	7	9	0.04	5	86.24	-6.13
TZEIORQ 72	506	61	7	65.69	7	9	0.03	5	47.31	-6.14
TZEIORQ 15	157	59	5	67.74	7	8	0.12	6	91.01	-6.43
TZEIORQ 45	169	55	5	57.90	7	8	0.18	6	89.50	-6.52
TZEIORQ 11	122	58	5	72.62	6	9	0.13	6	94.42	-6.64
C2-TZEQI 91	145	61	6	61.19	7	8	0.15	6	91.40	-6.95
TZEIORQ 39	130	57	7	62.59	7	8	0.15	6	91.81	-7.66
TZEIORQ 70	156	56	5	63.57	7	8	0.12	6	92.19	-8.31
TZEIORQ 10	128	54	6	80.54	6	8	0.01	6	89.64	-8.47
TZEIORQ 41	100	55	7	63.58	7	8	0.06	6	91.60	-9.40
Mean	**361.36**	**57**	**5**	**75.49**	**6**	**7**	**0.31**	**5**	**71.52**	
Sed	**105.21**	**1.50**	**1.05**	**5.46**	**0.5**	**0.6**	**0.09**	**0.5**		

C1, C2, C3, C4, C5 and C6 = Check 1, 2, 3, 4, 5 and 6 respectively; STGR= stay-green characteristic (1-9).

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Table 3. 4 Mean squares and heritability estimates of 70 early maturing provitamin A quality protein maize inbred lines evaluated under low-N conditions at Ile-Ife and Mokwa during the 2016 and 2017 growing seasons

Source	DF	YIELD	DA	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP	STGR
Env	2	33597047.68**	41.40**	318.44**	115.78**	6370.30**	474.82**	7.22**	1.77**	25.4**	56.12**	24.99**	384.47**	4.35**	170.53**
Rep (Env)	3	673976.31**	4.03	3.47	0.05	1170.16**	946.18**	5.64**	0.07	3.84**	3.13**	3.25	20.51**	0.06	0.37
Block(Env*Rep)	54	302824.7**	2.15	2.54	0.96	277.44**	108.01*	0.70	0.24	1.23*	1.61**	2.60**	5.09	0.04*	0.72*
Inbred	69	526260.25**	9.38**	13.03**	4.75**	749.38**	186.99**	0.85*	0.59**	5.22**	1.28**	3.18**	17.41**	0.11**	1.57**
Env*Inbred	138	307000.74**	3.49**	5.88**	3.01**	295.84**	80.45	0.65	0.40	5.52**	1.05**	3.14**	9.68**	0.08**	0.94**
Error	153	143160.7	1.85	2.45	0.83	110.53	70.74	0.61	0.33	0.78	0.62	1.36	5.04	0.03	0.50
Heritability	**-**	**0.41**	**0.64**	**0.55**	**0.33**	**0.59**	**0.58**	**0.24**	**0.31**	**-**	**0.17**	**0.20**	**0.55**	**0.10**	**0.43**

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; Env = environment; Rep=replication; YIELD = Grain yield; DA= days to 50% anthesis; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; RL = root lodging; SL = stalk lodging; HC = husk cover; PASP = plant aspect; EASP = ear plant aspect; EROT = ear rot; EPP =ears per plant; STGR= stay-green characteristic.

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Table 3. 5 Grain yield and other agronomic traits of 25 early maturing provitamin A quality protein maize inbred lines plus six checks evaluated under low-N conditions at Ile-Ife and Mokwa during the 2016 and 2017 growing seasons

Inbred	Grain yield (kg ha^{-1})	Days to anthesis	Anthesis Silking Interval	Plant Height (cm)	Plant Aspect (1 – 9)	Ear aspect (1 – 9)	Ears Per Plant	STGR	Yield Reduction (%)	Low-N Base Index
TZEIORQ 12	1882	56	0	133.15	4	3	0.88	3	13.96	12.67
TZEIORQ 48	1773	57	0	130.82	4	4	0.86	4	9.63	9.03
C6-TZEI 24	1438	57	1	103.18	4	5	0.75	3	25.14	7.59
TZEIORQ 27	1321	58	0	126.33	3	5	0.82	3	30.47	7.30
TZEIORQ 70	1163	55	0	115.80	3	4	0.81	3	41.68	6.49
TZEIORQ 3	1415	59	2	120.40	4	4	0.73	3	20.08	6.16
TZEIORQ 45	1152	55	0	117.24	4	4	0.76	3	28.26	5.62
TZEIORQ 15	1301	57	1	119.63	5	4	0.83	3	25.45	5.40
TZEIORQ 17	1351	56	1	112.53	5	4	0.83	3	14.87	4.90
TZEIORQ 33	882	59	0	104.03	4	5	0.77	3	61.34	4.30
TZEIORQ 37	1329	58	1	120.96	5	4	0.70	3	26.27	4.24
C5-TZEI 129	1273	57	0	138.10	4	5	0.57	3	30.92	4.05
TZEIORQ 55	1227	58	0	130.08	4	4	0.84	4	9.35	4.05
TZEIORQ 42	1172	55	1	118.83	4	4	0.67	4	7.09	3.50
TZEIORQ 29	994	56	0	109.83	4	4	0.65	3	37.23	3.44
TZEIORQ 10	1136	57	0	109.88	5	4	0.78	3	8.23	3.41
TZEIORQ 11	845	58	2	130.54	5	3	1.08	4	61.40	3.08
TZEIORQ 39	858	59	1	95.88	4	5	0.80	3	24.68	1.57
TZEIORQ 23	850	57	0	120.17	5	5	0.64	5	15.88	-3.02
TZEIORQ 73	710	58	1	111.47	5	5	0.58	4	40.57	-4.32
C4-TZEQI 82	854	61	2	109.69	5	6	0.65	4	36.40	-4.36
C2 -TZEQI 91	987	60	2	111.29	6	6	0.45	4	41.41	-4.90
TZEIORQ 20	572	58	0	112.32	5	5	0.66	4	45.84	-5.52
TZEIORQ 2	607	60	1	121.94	5	6	0.51	4	58.49	-5.79
TZEIORQ 52	599	58	1	109.74	5	6	0.50	5	61.30	-7.31
TZEIORQ 62	588	59	2	115.45	5	6	0.38	4	54.18	-7.53
TZEIORQ 53	573	59	2	121.09	6	6	0.51	4	21.64	-7.61
TZEIORQ 41	443	57	3	102.45	5	6	0.31	4	62.79	-8.97
TZEIORQ 46	458	57	2	87.18	5	6	0.50	5	67.17	-9.39
C1-TZEQI 85	298	61	3	93.63	5	7	0.25	4	81.49	-12.82
C3-TZEQI 74	124	60	5	73.06	6	8	0.30	4	83.68	-18.70
Mean	**973.40**	**57**	**1.8**	**114.77**	**5**	**5.0**	**0.65**	**4**	**36.99**	
Sed	**175.01**	**0.72**	**0.42**	**4.86**	**0.42**	**0.54**	**0.07**	**0.33**		

C1, C2, C3, C4, C5 and C6 = Check 1, 2, 3, 4, 5 and 6 respectively; STGR= stay-green characteristic (1-9).

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The combined ANOVA across three optimal environments revealed significant ($P< 0.05$ or $P< 0.01$) variation among E, G and GEI mean squares for all measured traits except GEI effects for days to 50% anthesis, plant height, ear aspect and ear rot (Table 3.6). H^2 estimates ranged from 13% for root lodging to 87% for days to 50% for anthesis and grain yield had a moderately high estimate of 58%. Plant height and ear aspect were equally highly heritable (81%) under optimal environments. The multiple trait base index was used to rank the inbred lines under optimal conditions since the focus of the research was on drought and low-N tolerance. The best inbred check under optimal conditions, TZEI 24 was ranked 12th among the 15 best performing inbred lines (Table 3.7).

The combined ANOVA across eight environments involving the three research conditions (drought, low-N and optimal) showed significant ($P< 0.05$ or $P< 0.01$) E, G and GEI effects for all measured traits except the GEI mean squares for days to 50% anthesis and stalk lodging. Although the mean squares for inbred x research condition interactions was not significant for the major response variable (grain yield) and most of the measured traits, significant variations were detected for a few other traits notably, anthesis silking interval, stalk lodging, husk cover, ear aspect and stay green characteristic (Table 3.8). Across research conditions, grain yield had a moderately high H^2 estimate of 56% with ASI recording the lowest estimate of 18% while the highest (83%) was observed for plant height. Grain yield ranged from 386 kg ha^{-1} for TZEQI 74 (check 3) to 1457 kg ha^{-1} for TZEIORQ 27 with a mean of 977 kg ha^{-1} (Table 3.9) In summary, 32 inbred lines displayed tolerance to drought alone using the drought base index, 37 to low-N alone using the low-N base index, 16 to combined drought and low-N while 33 inbred lines showed tolerance across the three contrasting environments using the multiple trait base index, with the optimal environments included as a check (Table 3.10).

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Table 3. 6 Mean squares and heritability estimates of 70 early maturing provitamin A quality protein maize inbred lines evaluated under optimal conditions at Ile-Ife, Ikenne and Mokwa in Nigeria during the 2016 and 2017 growing seasons

Source	DF	YIELD	DA	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP
Env	2	134313818.6**	101.29**	362.52**	81.52**	45577.87**	20840.08**	2.11**	4.99**	52.22**	118.98**	173.25**	360.75**	7.55**
Rep (Env)	3	21773.4	0.54	1.57	0.93	694.99**	420.69**	0.20*	0.12	7.05**	10.25**	9.62**	29.68**	0.13**
Block(Env*Rep)	54	103579.7	5.55**	7.96**	1.00**	311.75**	118.27**	0.08	0.11	1.32	1.33*	1.31	5.54**	0.05*
Inbred	69	768236.7**	14.95**	18.25**	1.43**	633.41**	220.14**	0.13**	0.16**	2.51**	1.68**	2.53**	7.86**	0.06**
Env*Inbred	138	490480.8**	2.18	3.05*	1.02**	141.65	85.31*	0.11**	0.12*	1.92**	1.12*	1.22	4.31	0.04*
Error	153	73194.1	1.86	2.26	0.58	111.5689	60.91	0.07	0.08	1.07	0.83	1.03	3.35	0.03
Heritability	**-**	**0.58**	**0.87**	**0.86**	**0.54**	**0.81**	**0.65**	**0.13**	**0.21**	**0.34**	**0.55**	**0.81**	**0.39**	**0.52**

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; Env = environment; Rep=replication; YIELD = Grain yield; DA= days to 50% anthesis; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; RL = root lodging; SL = stalk lodging; HC = husk cover; PASP = plant aspect; EASP = ear plant aspect; EROT = ear rot; EPP =ears per plant.

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Table 3. 7 Performance of 25 early maturing provitamin A-quality protein maize inbred lines plus six checks evaluated under optimal environments at Ile-Ife, Ikenne and Mokwa in Nigeria, during the 2016 and 2017 growing seasons

Inbred	Grain yield (kg ha^{-1})	Days to anthesis	Anthesis Silking Interval	Plant Height (cm)	Plant Aspect (1 – 9)	Ear aspect (1 – 9)	Husk Cover (1-9)	Ears Per Plant	Drought and Low-N Base index
TZEIORQ 12	1652	55	1	132.07	3	3	4	0.81	9.90
TZEIORQ 48	1618	56	1	127.28	4	4	3	0.75	8.26
TZEIORQ 6	1180	56	0	115.72	5	5	3	0.71	8.26
TZEIORQ 8	1194	54	1	123.42	5	5	4	0.73	8.01
TZEIORQ 37	1803	55	1	126.93	4	4	4	0.80	7.97
TZEIORQ 25	1112	55	1	114.22	5	4	5	0.56	7.89
TZEIORQ 7	1218	56	1	117.46	4	4	3	0.91	7.79
TZEIORQ 27	1899	56	0	122.96	4	5	5	0.79	7.50
TZEIORQ 5	1194	55	0	121.42	5	5	4	0.67	7.03
TZEIORQ 9	1765	54	1	123.71	5	4	5	0.80	6.20
TZEIORQ 28	2075	56	0	124.04	3	4	5	0.64	5.70
C6-TZEI 24	1920	54	0	107.04	4	4	3	0.82	5.17
TZEIORQ 17	1351	55	0	117.79	4	5	4	0.64	4.79
TZEIORQ 40	1457	53	1	118.15	4	4	3	0.86	4.69
TZEIORQ 26	1310	54	0	124.17	4	4	3	0.71	4.65
C5-TZEI 129	1843	53	1	144.78	4	4	5	0.74	4.57
TZEIORQ 29	1139	60	1	95.52	5	4	4	0.77	-0.20
TZEIORQ 20	1055	55	1	124.90	5	5	3	0.62	-3.59
TZEIORQ 13	2171	54	0	114.24	3	3	4	0.78	-3.85
TZEIORQ 72	960	57	2	119.72	5	5	4	0.56	-4.03
TZEIORQ 55	1427	58	2	129.69	4	5	3	0.73	-4.45
TZEIORQ 62	1283	58	1	125.85	5	4	4	0.84	-4.65
C4-TZEQI 82	1342	57	2	107.09	4	5	3	0.63	-5.21
TZEIORQ 2	1462	58	1	130.05	4	5	3	0.88	-5.52
TZEIORQ 66	732	54	1	132.55	5	5	4	0.85	-6.49
TZEIORQ 52	1548	55	2	131.56	4	4	4	0.72	-7.26
C2 -TZEQI 91	1684	60	0	123.50	4	4	3	0.75	-7.49
TZEIORQ 46	1396	53	1	106.30	4	4	3	0.84	-10.89
TZEIORQ 41	1191	54	1	119.54	5	5	3	0.83	-11.82
C1-TZEQI 85	1610	57	2	114.32	3	4	3	0.69	-13.22
C3-TZEQI 74	760	58	1	95.57	6	6	4	0.50	-13.26
Mean	**1421.31**	**56**	**1**	**120.37**	**4**	**4.3**	**4**	**0.74**	
Sed	**125.15**	**0.63**	**0.35**	**4.88**	**0.42**	**0.47**	**0.48**	**0.08**	

C1, C2, C3, C4 and C5, C6 = Check 1, 2, 3, 4, 5 and 6 respectively.

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Table 3. 8 Mean squares and heritability estimates of 70 early maturing provitamin A quality protein maize inbred lines across drought, low-N, and optimal environments in Nigeria, 2016-2017

Source	DF	YIELD	DA	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP	DF	STGR
Env	7	82217326.1**	191.49**	1051.91**	404.54**	64747.46**	10398.83**	4.37**	5.45**	224.11**	98.33**	221.51**	405.59**	9.13**	4	126.47**
Rcond	2	119609298.8**	405.11**	2800.80**	1228.83**	164727.14**	10498.15**	12.96**	11.21**	494.23**	167.84**	577.05**	586.82**	20.05**	1	126.19**
Rep(Env)	8	263665.7**	2.16	2.96	3.26*	773.13**	529.22**	2.23**	0.32	4.76**	5.13**	5.41**	21.93**	0.08**	5	2.28**
Block(Env*Rep)	144	168729.5**	7.24**	9.33**	1.7	303.55**	112.11**	0.37	0.38	1.50**	1.58**	2.37**	5.6	0.05**	90	1.11**
Inbred	69	661182.5**	31.09**	37.33**	5.02**	1477.70**	432.19**	0.73**	0.99**	3.72**	1.95**	4.30**	16.18**	0.08**	69	1.50**
Rcond*Inbred	138	399198.2	4.94	7.50	3.64**	209.15	72.7	1.27	0.90**	3.33*	1.46	2.85*	8.98**	0.08	69	2.38**
Env*Inbred	483	346564.9**	3.54	5.66**	2.96**	195.14**	75.56*	0.42*	0.41	3.11**	1.12**	2.24**	6.98**	0.06**	276	1.28**
Error	408	89760.6	3.17	4.37	1.45	106.54	62.9	0.34	0.37	0.86	0.7	1.16	4.55	0.03	255	0.56
Heritability	**-**	**0.56**	**0.65**	**0.61**	**0.18**	**0.83**	**0.65**	**-**	**0.46**	**0.61**	**0.71**	**0.78**	**0.21**	**0.75**		**0.65**

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; Env = environment; Rcond= research condition; Rep=replication; YIELD = Grain yield; DA = days to 50% anthesis; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; RL = root lodging; SL = stalk lodging; HC = husk cover; PASP = plant aspect; EASP = ear plant aspect; EROT = ear rot; EPP =ears per plant; STGR= stay-green characteristic.

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Table 3. 9 Performance of 31 selected early maturing provitamin A quality protein maize inbred lines plus six checks across drought, low-N, and optimal environments in Nigeria, 2016-2017

Inbred	Grain yield (kg ha^{-1})	Days to Anthesis	Anthesis Silking Interval	Plant Height (cm)	Plant Aspect (1 – 9)	Ear aspect (1 – 9)	Husk cover (1-9)	Ears Per plant	STGR	Drought and Low N Base Index (MI)
TZEIORQ 12	1353	57	2	122.86	4	4	4	0.72	4	9.90
TZEIORQ 48	1385	57	2	119.35	4	4	4	0.68	4	8.26
TZEIORQ 6	935	56	1	110.13	4	5	4	0.71	4	8.26
TZEIORQ 8	1086	56	1	113.62	5	5	4	0.79	4	8.01
TZEIORQ 37	1280	56	2	115.08	6	4	4	0.67	3	7.97
TZEIORQ 25	927	55	1	106.13	5	4	4	0.64	4	7.89
TZEIORQ 7	992	56	1	113.74	4	4	4	0.78	4	7.79
TZEIORQ 27	1457	57	1	115.64	4	5	5	0.66	4	7.50
TZEIORQ 5	956	56	1	111.81	5	5	4	0.74	4	7.03
TZEIORQ 9	1212	56	2	113.45	4	4	5	0.75	4	6.20
TZEIORQ 28	1422	56	1	109.88	4	5	5	0.58	4	5.70
C6-TZEI 24	1393	55	2	92.98	4	5	4	0.67	4	5.17
TZEIORQ 17	1002	56	1	104.21	5	5	4	0.66	4	4.79
TZEIORQ 40	1068	54	1	103.47	4	5	3	0.68	4	4.69
TZEIORQ 26	958	55	1	110.10	6	5	3	0.68	4	4.65
C5-TZEI 129	1152	54	1	131.82	4	5	4	0.57	4	4.57
TZEIORQ 60	955	59	2	127.76	4	5	5	0.56	3	3.47
TZEIORQ 21	969	55	2	108.71	5	6	4	0.69	5	3.07
TZEIORQ 65	1249	58	1	123.46	5	5	4	0.63	4	1.87
TZEIORQ 24	454	57	1	113.61	4	5	3	0.56	4	1.55
TZEIORQ 29	727	60	2	85.98	5	5	4	0.65	4	-0.20
TZEIORQ 59	1065	59	1	119.65	5	5	4	0.57	4	-0.67
TZEIORQ 11	1082	56	2	112.84	5	4	3	0.67	5	-1.74
TZEIORQ 20	662	57	2	108.44	5	6	4	0.55	4	-3.59
TZEIORQ 13	1220	54	2	102.87	4	5	5	0.60	5	-3.85
TZEIORQ 72	799	59	3	104.69	5	6	5	0.50	4	-4.03
TZEIORQ 55	954	59	2	118.09	5	6	5	0.55	4	-4.45
TZEIORQ 62	720	58	1	114.11	5	6	5	0.52	4	-4.65
C4-TZEQI 82	858	59	3	98.19	5	6	4	0.51	4	-5.21
TZEIORQ 2	669	58	2	116.55	5	6	4	0.57	4	-5.52
TZEIORQ 66	599	57	2	118.41	5	6	5	0.58	4	-6.49
TZEIORQ 52	904	57	2	112.97	5	6	5	0.49	5	-7.26
C2 -TZEQI 91	1015	59	2	103.34	5	6	5	0.48	5	-7.49
TZEIORQ 46	747	55	2	86.05	5	6	4	0.58	6	-10.89
TZEIORQ 41	801	54	3	99.14	5	6	5	0.44	5	-11.82
C1-TZEQI 85	723	57	4	96.34	4	6	4	0.38	4	-13.22
C3-TZEQI 74	386	57	3	81.27	6	7	5	0.40	4	-13.26
Mean	**976.64**	**57**	**1.73**	**109.37**	**4.61**	**5.07**	**4.17**	**0.61**	**4.18**	
Sed	**84.88**	**0.50**	**0.34**	**2.92**	**0.24**	**0.31**	**0.26**	**0.05**	**0.27**	

C1, C2, C3, C4 and C5, C6 = Check 1, 2, 3, 4, 5 and 6 respectively.

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Table 3. 10 List of early maturing provitamin A quality protein maize inbred lines showing different levels of tolerance to drought and low-N environments

Drought (DT)	Low-N (LN)	DT & LN Simultaneously	Across DT and low-N (MI)
TZEIORQ 5	TZEIORQ 3	TZEIORQ-5	TZEIORQ 3
TZEIORQ 6	TZEIORQ 5	TZEIORQ-6	TZEIORQ 5
TZEIORQ 7	TZEIORQ 6	TZEIORQ-7	TZEIORQ 6
TZEIORQ 8	TZEIORQ 7	TZEIORQ-9	TZEIORQ 7
TZEIORQ 9	TZEIORQ 9	TZEIORQ-17	TZEIORQ 8
TZEIORQ 17	TZEIORQ 10	TZEIORQ-21	TZEIORQ 9
TZEIORQ 19	TZEIORQ 11	TZEIORQ-25	TZEIORQ 12
TZEIORQ 20	TZEIORQ 12	TZEIORQ-26	TZEIORQ 14
TZEIORQ 21	TZEIORQ 14	TZEIORQ-27	TZEIORQ 15
TZEIORQ 22	TZEIORQ 15	TZEIORQ-28	TZEIORQ 17
TZEIORQ 23	TZEIORQ 16	TZEIORQ-37	TZEIORQ 19
TZEIORQ 24	TZEIORQ 17	TZEIORQ-40	TZEIORQ 21
TZEIORQ 25	TZEIORQ 21	TZEIORQ-48	TZEIORQ 23
TZEIORQ 26	C6-TZEI 24	TZEIORQ-65	TZEIORQ 24
TZEIORQ 27	TZEIORQ 25	TZEIORQ-60	C6-TZEI 24
TZEIORQ 28	TZEIORQ 26	C5-TZEI-129	TZEIORQ 25
TZEIORQ 30	TZEIORQ 27		TZEIORQ 26
TZEIORQ 34	TZEIORQ 28		TZEIORQ 27
TZEIORQ 35	TZEIORQ 29		TZEIORQ 28
TZEIORQ 37	TZEIORQ 31		TZEIORQ 33
TZEIORQ 40	TZEIORQ 33		TZEIORQ 36
TZEIORQ 47	TZEIORQ 36		TZEIORQ 37
TZEIORQ 48	TZEIORQ 37		TZEIORQ 40
TZEIORQ 57	TZEIORQ 39		TZEIORQ 42
TZEIORQ 58	TZEIORQ 40		TZEIORQ 45
TZEIORQ 60	TZEIORQ 42		TZEIORQ 48
TZEIORQ 63	TZEIORQ 43		TZEIORQ 53
TZEIORQ 65	TZEIORQ 48		TZEIORQ 58
TZEIORQ 68	TZEIORQ 45		TZEIORQ 60
TZEIORQ 73	TZEIORQ 55		TZEIORQ 63
C3-TZEQI 74	TZEIORQ 54		TZEIORQ 65
C5-TZEI 129	TZEIORQ 60		TZEIORQ 68
	TZEIORQ 65		C5-TZEI 129
	TZEIORQ 69		
	TZEIORQ 70		
	TZEIORQ 72		
	C5-TZEI 129		

C3, C5 and C6= checks 3, 5 and 6 respectively; MI = multiple trait base index.

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Most of the measured traits exhibited significant ($P < 0.01$ or < 0.05) correlation coefficients with grain yield, and among each other (Table 3.11). Grain yield showed highly significant ($P < 0.01$) negative genetic correlations with plant and ear aspect under drought, low-N, optimal and across environments. Grain yield had high significant ($P< 0.01$) negative correlations with ASI, and stay-green characteristics under drought but not under low-N conditions. Under drought, ASI exhibited high significant positive genetic correlations with plant aspect (0.98), ear aspect (0.98) and stay green characteristic (0.86) but displayed high significant negative correlation with ears per plant (-0.98) as well as plant height (-0.98). Ear aspect consistently displayed significant ($P< 0.01$ or < 0.05) positive correlations with the flowering traits including days to 50% anthesis, days to 50% silking and ASI under each and across test environments.

The ANOVA for all carotenoids and tryptophan revealed significant ($P < 0.01$) variation among the 19 inbred lines assayed, except for α-carotene (Table 3.12). However, based on visual scores, no significant differences were detected among the inbred lines for kernel colour (Appendix 2). Estimated PVA varied from 3.47 μg g^{-1} for TZEIORQ 48 to 15.38 μg g^{-1} for TZEIORQ 55 with a mean of 6.47 μg g^{-1} (Table 3.13). Relative to the contents of the other carotenoids, the α-carotene levels of the selected inbred lines were very low. Ninety-five percent of the inbreds had > 0.075 % tryptophan per sample in whole grain. “Obatanpa”, the the standard QPM check, recorded the highest level of tryptophan and was 37 % higher than the second best inbred, TZEIORQ 42.

Ten out of the 19 selected inbred lines assayed for PVA carotenoids and tryptophan showed varying degrees of tolerance to drought while eleven inbreds were tolerant to low-N environments (Table 3.13). Furthermore, ten inbreds were tolerant across the two stresses with TZEIORQ 40, TZEIORQ 7, TZEIORQ 6, TZEIORQ 26, TZEIORQ 5 and TZEIORQ 48 identified as the most superior. The most drought and low-N tolerant inbreds

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Table 3. 11 Genetic correlation among grain yield and other agronomic traits of 70 early maturing provitamin A-quality protein maize inbred lines evaluated under drought, low-N, optimal and across environments in Nigeria, 2016-2017

	Genetic Correlation Co-efficients			
Traits	**Drought**	**Low N**	**Optimal**	**Across Environments**
YIELD x DA	-0.11	-0.24*	-0.09	-0.21
YIELD x DS	-0.33*	-0.21	-0.16	-0.25*
YIELD x ASI	-0.98**	-0.16	-0.43**	-0.34*
YIELD x PLHT	0.28*	0.11	0.19	0.22
YIELD x PASP	-0.82**	-0.72**	-0.68**	-0.76**
YIELD x EASP	-0.84**	-0.98**	-0.96**	-0.68**
YIELD x EPP	0.89**	0.09	0.37**	0.36**
YIELD x STGR	-0.67**	0.11	-	-
DA x DS	0.92**	0.98**	0.99**	0.99**
DA x ASI	0.48**	0.20	0.40**	0.36**
DA x PLHT	0.11	0.38**	-0.03	0.12
DA x PASP	0.17	0.12	0.13	0.26*
DA x EASP	0.37**	-0.01	0.28*	0.39**
DA x EPP	-0.42**	0.38**	-0.32*	-0.61*
DA x STGR	-0.21	-0.41**	-	-
DS x ASI	0.66**	0.38**	0.55**	0.47**
DS x PLHT	-0.25*	0.22	0.01	0.00
DS x PASP	0.51**	-0.14	0.19	0.28*
DS x EASP	0.64**	0.31*	0.35**	0.54**
DS x EPP	-0.70**	0.04	-0.28*	-0.82**
DS x STGR	0.13	-0.71**	-	-
ASI x PLHT	-0.98**	-0.43**	0.22	-0.47**
ASI x PASP	0.98**	-0.21	0.36**	0.20
ASI x EASP	0.98**	0.98**	0.59**	0.85**
ASI x EPP	-0.98**	-0.45**	0.05	-0.98**
ASI x STGR	0.86**	0.62**	-	-
PLHT x PASP	-0.73**	0.13	-0.25*	-0.40**
PLHT x EASP	-0.52**	-0.02	-0.29*	-0.39**
PLHT x EPP	0.31*	0.03	0.24*	0.2
PLHT x STGR	-0.65**	0.17	-	-
PASP x EASP	0.93**	-0.68**	0.68**	0.61**
PASP x EPP	-0.85**	0.98**	0.04	-0.12
PASP x STGR	0.87**	-0.10	-	-
EASP x EPP	-0.93**	-0.75**	-0.55**	-0.85**
EASP x STGR	0.75**	0.98**	-	-
EPP x STGR	-0.62**	0.98**	-	-

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; YIELD = Grain yield; DA= days to 50% anthesis; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height; PASP = plant aspect; EASP = ear plant aspect; EPP =ears per plant; STGR= stay-green characteristic.

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Table 3. 12 ANOVA for carotenoid contents of selected early maturing provitamin A quality protein maize inbred lines

		Mean squares of Carotenoids§ (μg g^{-1}dry weight)									
Source	**DF**	**Lut**	**Zeax**	**β-cryp**	**α-caro**	**β-caro**	**ŦPVA**	**Tcaro**	**Kern-col**	**DF**	**Tryp (%)**
Inbred	18	79.97**	31.94*	5.08**	0.36	9.26**	17.20**	149.68**	1.26	19	0.00156**
Rep	1	1.98	22.19	6.47*	0.11	0.75	0.06	55.72	1.93	1	0.00008
Error	18	9.57	11.91	0.58	0.37	0.14	0.39	21.7	2.38	19	0.00011

*, **, = significant at P < 0.05 and 0.01, respectively; DF= degrees of freedom; §Carotenoids are abbreviated as Lut= lutein; Zeax= Zeaxanthin; β-cryp= β-cryptoxanthin; α-caro= alpha-carotene; β-caro= β-carotene; ŦPVA= provitamin A; Tcaro= Total carotenoids; Kern-col= kernel colour; Tryp= tryptophan.

had low to moderate (3.47 – 8.34 μg g^{-1}) levels of PVA. TZEIORQ 55 and TZEIORQ 29 had PVA content of 15.38 μg g^{-1} and 12.10 μg g^{-1} respectivly and were only tolerant to low-N (Table 3.13).

Highly significant (P < 0.01) posistive phenotypic correlations were observed among PVA and its component carotenoids including β-cryptoxanthin (r = 0.75), α-carotene (r = 0.67) and β-carotene (r = 0.93) (Table 3.14). Significant (P<0.05) positive correlations were also observed between PVA and total carotenoids (r = 0.73) as well as kernel colour (r = 0.52). The correlations between grain yield and all carotenoids as well as tryptophan were not significant for the selected inbred lines except for β-cryptoxanthin and Zeaxanthin. The highest significant (P< 0.01) correlation coefficient was observed between PVA and β-carotene (r = 0.93), followed by the correlation between β-cryptoxanthin and α-carotene (r = 0.77).

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Table 3. 13 Carotenoids and tryptophan contents, kernel colour scores and reactions of 18 selected early maturing provitamin A-quality protein maize parental lines to drought and low-N environments in Nigeria, during 2016 and 2017

	Carotenoids§ (μg g^{-1}dry weight)								Reaction to drought and low-N			
Inbred lines	**Lut**	**Zeax**	**β-cryp**	**α-caro**	**β-caro**	**PVA**	**Tcaro**	**Kern-col**	**Tryp (%)**	**DTBI**	**Low-NBI**	**MI**
TZEIORQ 55	19.19	21.49	6.61	1.71	11.23	15.38	60.22	8.0	0.096	-1.00	-7.61	-6.49
TZEIORQ 29	27.07	6.31	6.11	2.10	7.69	12.10	49.28	9.0	0.119	-2.02	1.57	-0.2
TZEIORQ 20	15.04	21.70	3.84	1.31	5.78	8.36	47.67	8.0	0.087	1.93	-5.52	-3.59
TZEIORQ 42	10.63	23.19	4.99	1.27	5.21	8.34	45.30	8.0	0.124	-2.06	4.50	2.20
TZEIORQ 13	3.68	10.11	1.40	0.54	6.72	7.70	22.46	7.0	0.120	-5.25	1.18	-3.85
TZEIORQ 24	7.89	20.86	3.71	1.16	4.17	6.60	37.79	10.0	0.104	8.44	-2.85	1.55
TZEIORQ 59	25.79	15.17	2.11	1.00	4.64	6.19	48.71	8.0	0.121	-1.31	-0.04	-0.67
TZEIORQ 40	13.30	16.97	1.53	0.44	4.62	5.61	36.86	9.0	0.065	4.05	2.78	4.69
TZEIORQ 7	10.43	19.33	3.36	1.11	3.37	5.61	37.61	8.0	0.101	11.25	2.35	7.79
TZEIORQ 6	10.30	19.07	3.18	0.94	3.44	5.50	36.93	7.0	0.118	12.46	2.47	8.26
TZEIORQ 26	9.62	18.04	2.88	0.89	3.40	5.28	34.82	7.0	0.102	5.51	1.90	4.65
TZEIORQ 5	9.78	18.06	2.76	0.89	3.35	5.18	34.85	8.0	0.110	12.01	0.59	7.03
TZEIORQ 43	16.20	18.01	1.79	0.45	3.99	5.11	40.44	8.0	0.113	-3.66	0.99	-1.60
TZEIORQ 45	18.77	16.27	1.46	0.99	3.88	5.10	41.36	8.0	0.106	-4.52	5.62	0.25
TZEIORQ 23	9.52	18.58	2.82	0.91	3.20	5.06	35.03	7.0	0.091	6.97	-3.02	1.52
TZEIORQ 44	14.34	16.53	1.68	0.50	3.80	4.89	36.85	8.0	0.089	-2.35	-1.80	-2.81
TZEIORQ 2	4.89	16.58	1.83	0.50	2.65	3.82	26.44	7.0	0.102	-2.93	-5.79	-5.52
TZEIORQ 47	17.75	13.65	1.15	1.00	2.48	3.55	36.03	7.0	0.056	0.34	-2.33	-2.14
TZEIORQ 48	8.84	17.23	1.56	1.00	2.18	3.47	30.83	7.0	0.115	1.70	9.03	8.26
†OBATANPA	-	-	-	-	-	-	-	-	0.198	-	-	-
SED	2.19	2.44	0.54	0.43	0.27	0.44	3.29	1.12	0.008	-	-	-
Min.	3.68	6.31	1.15	0.45	2.18	3.47	22.46	7.0	0.056	-5.25	-7.61	-6.49
Max.	27.07	23.19	6.61	2.10	11.23	15.38	60.22	10.0	0.198	12.46	9.03	8.26
Mean	13.32	17.22	2.88	0.99	4.52	6.47	38.92	7.84	0.110	-	-	-

§Carotenoids are abbreviated as Lut= iutein; Zeax= Zeaxanthin; β-cryp= β-cryptoxanthin; α-caro= alpha-carotene; β-caor= β-carotene; ǂPVA= provitamin A; Tcaro= total carotenoids; Kern-col= kernel colour (scored on a scale of 1 to 12, 1= pale yellow, 12= darkest orange); Tryp= tryptophan; B.I= base index; MI= multiple trait base index, with positive value= Tolerance, and negative value= Susceptibility; †= QPM standard check.

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Table 3. 14 Phenotypic correlation co-efficients among traits of selected provitamin A quality protein maize inbred lines

Trait	ǂPVA	β-cryp	α-caro	β -caro	Tcaro	Kern-col	Lut	Zeax	Tryp
β-cryp	0.75***								
α-caro	0.67**	0.77***							
β-caro	0.93***	0.58*	0.54*						
Tcar	0.73**	0.69**	0.74**	0.75***					
Kern-col	0.52*	0.37	0.32	0.55*	0.63**				
Lut	0.19	0.07	0.24	0.32	0.62**	0.43*			
Zeax	0.23	0.55*	0.28	0.05	0.25	0.11	-0.24		
Tryp	0.20	0.27	0.20	0.14	0.04	-0.02	-0.04	-0.07	
Yield	-0.39	-0.73**	-0.41	-0.16	-0.32	-0.34	0.05	-0.64**	0.08

***, ** and *= Significant at P< 0.001, 0.01 and 0.05 respectively; Carotenoids are abbreviated as ǂPVA= provitamin A; β-cryp= β-cryptoxanthin; α-caro= alpha-carotene; β-caor= β-carotene; Tcaro= Total carotenoid; Kern-col= Kernel colour (scored on a scale of 1 to 12, 1= pale yellow, 12= darkest orange) Lut= Lutein; Zeax= Zeaxanthin; Tryp= Tryptophan; Yield= grain yield across drought, low-N and optimal environments.

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3.4 Discussion

The observed significant differences (P< 0.01) among inbred lines (G), environment (E), and inbred x environment interactions (GEI) for grain yield and most of the measured traits under drought, low-N, optimal and across test environments indicated the existence of genetic variability in the early maturing PVA-QPM inbred lines and also indicated that the research environments in Nigeria varied in terms of climatic and edaphic conditions. The result also implied that the research environments were discriminating enough for the identification of outstanding inbred lines (Badu-Apraku *et al*., 2011a; 2016a). The significant E and GEI observed for grain yield and most traits under each and across test environments suggested that the rankings of the traits measured for the inbred lines would not be consistent in the different environments and that inbred evaluations in more environments would be necessary to identify outstanding genotypes as reported by other authors under drought (Badu-Apraku *et al*., 2011a; Edmeades, 2013; Badu-Apraku and Fakorede, 2017) and under low-N (Meseka *et al*., 2006).

The high broad sense heritability recorded for plant aspect, ear aspect, ears per plant and the stay green characteristics compared with the relatively moderate H^2 estimate for grain yield under drought indicated that heritability of grain yield under drought could be low and that it is important to use these secondary traits (with high H^2) to complement grain yield in the identification of drought tolerant inbred lines (Bänziger *et al*., 2000; Badu-Apraku *et al*., 2011a). These results were correspondingly supported by the highly significant negative genetic correlations observed between grain yield and plant aspect, ear aspect and stay green characteristics, and also the highly significant positive correlation between grain yield and ears per plant. However, the very low H^2 estimate (< 0.30) of ASI among the inbreds under drought contradicts the findings of earlier workers (Bolaños and Edmeades, 1996; Bänziger *et al*., 2000; Badu-Apraku *et al*., 2011a; 2012a; 2015b; 2016b) who identified ASI as a major reliable secondary trait for selecting drought tolerant maize

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genotypes. Despite the low H^2 estimate of ASI under drought, it recorded a highly significant negative genetic correlation with grain yield indicating its significance under limited soil moisture conditions. These results suggested that heritabilities of the secondary adaptive traits including ASI, and their genetic correlations with grain yield are important genetic components which should be considered together but not separately in the selection of reliable secondary traits to complement yield under drought, and under stress in general. The inconsistent performance of the 20 inbred lines in the present study compared to the evaluation under drought in 2015 (Badu-Apraku *et al*. Unpublished) suggested significant variation in the severity of drought imposed in the different studies.

The moderate to high H^2 estimates recorded for grain yield, days to 50% anthesis, days to 50% silking, plant height, ear height and stay green characteristic under low-N indicated that initial selection for these traits under low-N conditions would be effective as widely reported (Bänziger and Lafitte, 1997b; Bänziger *et al*., 1999; Badu-Apraku *et al*., 2011a; 2013a). However, among these traits, days to 50% anthesis was the only trait which recorded significant negative genetic correlation with grain yield, indicating that it was the most reliable trait to complement grain yield under low-N conditions. Furthermore, the low to very low H^2 estimates recorded for ASI, plant aspect, ear aspect and ears per plant implied that early generation selection for these traits to improve low-N tolerance may be ineffective. However, the highly significant negative genetic correlations of plant and ear aspects with grain yield under low-N suggested the high possibility of these secondary traits being reliable under low-N. These results agreed with the report by Badu-Apraku *et al*. (2012a), who identified plant and ear aspects as reliable traits for the selection of improved grain yield under low-N using the extra-early maize cultivars. The unreliability of ears per plant to complement grain yield under low-N corroborates with the results of Lafitte and Edmeades (1994a) who found ears per plant not reliable for selection under low-N. The results also revealed that the stay green characteristic is relatively more important than ASI under low-

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N environments. These results were also consistent with the findings of Badu-Apraku *et al*. (2012a).

The high heritability estimates recorded for the six traits (except for ASI) employed in the combined drought and low-N multiple trait base index as well as their high genetic correlations with grain yield across drought, low-N and optimal environments confirmed the importance of those traits in the selection of drought and low-N tolerant maize inbred lines. Under optimal environments, the high to very high H^2 estimates recorded for most of the important secondary traits compared with grain yield underscored the need to select for those traits in early generations to improve grain yield. The inconsistent findings on the reliability of some of the secondary traits measured under drought or low-N environments could be due to the differences in the genetic materials (including the maturity periods and the level of inbreeding) and the severity of the stress imposed.

The 32 out of the 70 inbred lines identified as drought tolerant based on the drought base index would serve as an important source of genes for the development of superior drought tolerant hybrids (Betrán *et al*., 2003a), synthetics and for the improvement of the early maturing PVA-QPM population for drought tolerance. Similarly, the 37 low-N tolerant inbred lines identified would be crucial for the exploitation of low-N tolerant genes to develop superior hybrid and synthetic varieties under low-N conditions (Meseka *et al*., 2006; Ifie *et al*., 2015; Adofo-Boateng *et al*., 2015). Moreover, the 33 inbred lines (47% of 70) identified to possess drought and low-N tolerance according to the multiple trait base index underscores the similar adaptive mechanisms involved in the tolerance to the two stresses. These results implied that selection under drought could also improve low-N tolerance as widely reported by several workers (Kim and Adetimirin, 1997; Bänziger *et al*., 1999; Meseka *et al*., 2006; Badu-Apraku *et al*., 2011a; 2012a). At the commencement of the inbred evaluations under the contrasting environments, 24 inbreds were selected as parental lines based on data generated in 2015 for a combining ability study. From the previous data, 10

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out of the 24 inbreds were tolerant to both drought and low-N conditions while 14 were tolerant to either drought or low-N. Comparing the performance of the 24 parental lines with the result of the present study, 10 inbred lines were confirmed tolerant to both drought and low-N, 7 showed tolerance to one of the stresses, while 7 were susceptible to both stresses. The susceptibility of the 7 parental lines in the present study compared with the previous evaluations may be due to a more severe drought and low-N effects experienced during the evaluations in the present study.

The significant differences observed for PVA content among the 19 selected parental lines assayed and the range, 3.47 to 15.38 µg g^{-1}, obtained with a mean of 6.47µg g^{-1} indicated the existence of significant variation for the PVA carotenoids in the set of inbred lines used (Weber, 1987; Pfeiffer and McClafferty, 2007; Harjes *et al*., 2008; Mishra and Singh, 2010). This range of PVA values exceeded the 5.00 to 7.80 µg g^{-1} reported by Menkir *et al*. (2008) from 15 tropically adapted yellow maize inbred lines, but was similar to the 0.06 to 17.25 µg g^{-1} with a mean of 5.87 µg g^{-1} reported by Azmach *et al*. (2013) using 130 inbred lines. However, only TZEIORQ 55 recorded a PVA value beyond 15 µg g^{-1} which is the current breeding target set by HarvestPlus (HarvestPlus 2004; Ortiz-Monasterio *et al*. 2007; Harjes *et al*. 2008). Although the result signaled the potential of meeting the set target using this inbred set, there is the need to introgress the most favourable alleles of PVA from temperate germplasm sources into the tropical adapted inbred lines to facilitate the development of high PVA hybrids adapted to drought and low-N environments. The highest estimated mean of total carotenoids was 60.22 µg g^{-1} which was higher than the 42.71 µg g^{-1} obtained by Azmach *et al*. (2013), but was far below the 100 µg g^{-1} according to Burt *et al*. (2011). The significance of high total carotenoids is that inbred lines harbouring higher amounts of total carotenoids could be invaluable sources of the PVA carotenoids especially if the influx of assimilates to the carotenoid biosynthetic pathway favours the accumulation of the PVA carotenoids in the endosperm. Also, the results revealed relatively high level of lutein and

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Zeaxanthin (synthesized from the PVA carotenoids) at the expense of the PVA carotenoids for most of the inbreds. These results corroborated the report by Howitt and Pogson, (2006) who identified lutein and Zeaxanthin as the most predominant carotenoids in the maize endosperm. The results contradicted the findings of Babu *et al*. (2012) who found a large number of genotypes having high PVA contents (ranging from 15 to 20 µg g^{-1}) comparable to the non-PVA carotenoids when an improved PVA inbred lines and populations were assayed. Almost all the inbreds had tryptophan contents > 0.075 % per sample in whole grain substantiating the QPM trait in the inbred population as reported by Vivek *et al*. (2008) and Teklewold *et al*. (2015). However, new sources of PVA genes would be necessary to improve the existing early maturing PVA-QPM inbred population to speed up the development of the next generation of high PVA tropical maize hybrids for commercialization in SSA to combat "hidden hunger" due to vitamin A deficiency and protein energy malnutrition. The two inbred lines, TZEIORQ 55 and TZEIORQ 29 identified to possess high levels of PVA (15.38 and 12.10 µg g^{-1} respectively) and low-N tolerance could be invaluable sources of PVA genes for the improvement of the early PVA-QPM source population.

The lack of significant correlations among grain yield, PVA and tryptophan suggested that the traits could be improved independently and this contradicts an earlier report by Pixley *et al*. (2013) who found higher levels of tryptophan to be associated with the PVA biofortified maize varieties relative to the white types evaluated. These results might have accounted for why most of the inbreds that combined drought and low-N tolerance did not correspondingly have high levels of PVA. The results suggested that the inbreds are relatively adapted to topical environments in terms of drought and low-N with desirable tryptophan content but their PVA levels need further improvement. Also, the non-significant correlations observed between PVA and lutein as well as Zeaxanthin indicated that the levels of the PVA carotenoids (β-carotene, β-cryptoxanthin and α-carotene) could be

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improved without significant loss associated with the synthesis of lutein and Zeaxanthin as by products in the PVA biosynthetic pathway. Furthermore, the weak positive significant correlations observed between kernel colour and PVA, and β-carotene suggested that, to some extent, the degree of the orange colour of kernels could be a quick (but not the most reliable) strategy to identify inbred lines with high PVA levels. This observation contradicted the findings of Azmach *et al*. (2013) who found no significant correlations between kernel colour and PVA and β-carotene contents in the set of intermediate and late inbreds studied. The results of the correlation between kernel colour and PVA levels in the present study and that of Azmach *et al*. (2013) simply suggested that chemical analysis is the surest way to monitor and improve the levels of these carotenoids during the early breeding stages.

3.5 Conclusions

Days to 50% anthesis and silking, plant and ear heights, and plant and ear aspects complemented grain yield to identify 33 drought and low-N tolerant inbreds. Almost all the inbreds had desirable levels of tryptophan (> 0.075%) in sample in whole grain. However, low to moderate levels of PVA were recorded for most of the inbreds including those tolerant to drought and low-N and this indicated the need to introgress favourable alleles of PVA from temperate germplasm sources into the early PVA-QPM inbreds to accelerate progress of developing high PVA-QPM hybrids adapted to drought and low-N environments in SSA. No significant correlations were found among grain yield, PVA and tryptophan suggesting that these traits could be improved independently. Only TZEIORQ 55 and TZEIORQ 29 combined high PVA contents (15.38 and 12.10 µg g^{-1} respectively) with low-N tolerance while nine inbreds combined moderate PVA levels (5.06 – 8.34 µg g^{-1}) with drought and low-N tolerance. These two inbreds could be used for the development of a high PVA-QPM

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low-N tolerant hybrid if there is heterosis between them, and also for improving the PVA content of the early PVA-QPM inbred population. Moreover, the two inbreds together with the nine inbreds could be exploited for the development of superior PVA-QPM drought and low-N tolerant hybrids and synthetics.

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CHAPTER FOUR

4.0 Genetic diversity and population structure analyses of early maturing provitamin A quality protein maize inbreds using Diversity Array Technology markers

4.1 Introduction

In breeding programmes, information on the genetic variation and population structure of germplasm is important because it facilitates judicious utilisation of resources to achieve breeding objectives. Genetic diversity in maize has always been exploited to select diverse parents to maximise heterosis in developed hybrids. Maize as an open-pollinated species has a complex genome (Schnable *et al*., 2009) with a high degree of genetic diversity which is considered a major factor in heterosis (Gore *et al*., 2009). Semagn *et al*. (2012), demonstrated that high genetic variation in a source population could enhance the development of improved inbred lines and identification of best parental combinations for developing superior hybrids. Furthermore, studies have revealed tremendous genetic variability among tropical maize germplasm. Zhang *et al*. (2016) and Dao *et al*. (2014) estimated genetic diversity among tropical and temperate maize populations and found more diversity in the tropical than the temperate group. The early maturing PVA-QPM inbred lines are novel tropical maize lines developed from diverse germplasm sources (i.e. drought tolerance with an indirect selection for low-N, *Striga* resistance, quality protein and PVA) by the IITA-MIP. Assessment of the genetic variation within this set of inbred lines could therefore provide invaluable information to guide breeding strategies and facilitate progress in the development of drought and low-N tolerant hybrids and synthetics with elevated PVA and quality protein contents which are presently lacking in SSA.

In the present study, the Diversity Array Technology (DArT) which employs the Next Generation Sequencing (NGS) platform (DArTseq) (Semagn *et al*. 2012; Elshire *et al*., 2011;

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Govindaraj *et al*. 2015) was used to provide high-density and cost-effective whole genome genotyping. Although, the DArTseq technique involves several steps in its delivery, it was the method of choice because it is capable of providing genome profiles which are very useful for characterization of germplasm collections as well as reliable and precise phenotyping. The different genetic backgrounds of the set of new inbred lines coupled with the existence of natural genetic variability in tropical maize germplasm could facilitate genetic improvement and development of outstanding new products. This therefore calls for the assessment of the genetic diversity of the newly developed early maturing PVA-QPM inbred lines to increase the rate of genetic gain in improved varieties. Therefore, the objectives of the present study were to:

i. assess the genetic dissimilarities among the inbreds using high-density DArTseq markers, and
ii. study the genetic structure of the early PVA-QPM inbred lines to maximise heterosis in hybrid combinations.

4.2 Materials and methods

4.2.1 Plant materials

Seventy early maturing PVA-QPM inbred lines developed by the IITA-MIP were used in the present study. The background of the genetic materials is described in chapter three, section 3.2.1 while the summary of the pedigree information is presented in Tables 3.1a and b.

4.2.2 Leaf sample collection and DNA extraction

Maize leaf samples were collected from 10 plants (one leaf per plant) of each inbred line at 2 WAP. Genomic DNA samples were isolated from freeze dried leaf tissues of each inbred line following the DArT DNA extraction protocol as described online with the link:

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https://ordering.diversityarrays.com/files/DArT_DNA_isolation.pdf. The DNA concentration was obtained by spectrometry measurement using Nanodrop 8000 machine (Thermo Scientific, USA), and DNA quality was confirmed by running DNA samples on 0.8 % agarose gel. Short or degraded DNA was eliminated and DNA concentrations of 30 ng μl^{-1} were used.

4.2.3 Diversity Array Technology sequencing (DArTseq) genotyping

Genotyping by sequencing analysis of the inbred lines was performed using a high-density whole genome profiling of DArT services. DNA samples (100 µl of 50 ng μl^{-1}) were sent to the Integrated Genomic Service and Support (IGSS) platform of BecA-ILRI in Kenya for DArTseq analysis following the protocol described by Elshire *et al*. (2011) using 44391 DArTseq codominant markers.

4.2.4 Statistical analysis of DArTseq data

All the images from DArTseq platform were analysed using DArTsoft v.7.4.7 (DArT P/L, Canberra, Australia). The DArTseq markers were scored using DArTsoft as binary data zero (0), indicating the presence of the reference allele homozygote, one (1) representing the presence of the SNP allele homozygote or two (2) designating the presence of heterozygote in the genomic representation of each sample as described by DArT Pty Ltd, Australia (https://www.diversityarrays.com/).

4.2.5 Summary statistics and cluster analyses

DArTseq markers with >80% call rate were retained prior to statistical analysis. Thereafter, markers with <10% missing rate were filtered out using the TASSEL software version 5.2.12 (Bradbury *et al*., 2007). Minimum and maximum frequencies of 0.05 and 0.95 respectively, were also considered for the filtering of markers to finally retain 8171 markers for all subsequent analyses. Major allele frequency (i.e. 1 minus minor allele frequency), gene

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diversity, heterozygosity and polymorphic information content (PIC) were estimated using PowerMarker version 3.25 (Liu and Muse, 2005). PIC values which measure the allelic diversity at a locus were calculated using the formula:

$$PIC = 1 - \sum_{i=1}^{n} p_i^2$$

Where: i = the i^{th} allele of the j^{th} marker, n = the number of alleles at the j^{th} marker and p = allele frequency.

Gene frequency, and frequency based genetic distance matrix of the 70 inbred lines were estimated for the DArT-seq data using the Nei and Takezaki (1983) method implemented in PowerMarker version 3.25 (Liu and Muse, 2005). Using the frequency based genetic distance estimates, the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and 1000 nonparametric bootstrapping across different loci were used in PowerMarker version 3.25 to construct a phylogenetic tree to visualize patterns of genetic diversity in the panel of 70 inbred lines.

4.2.6 Population structure and principal component analyses

In order to assess the genetic structure of the inbred lines, the Bayesian model-based clustering algorithm implemented in the STRUCTURE software package version 2.3.4 (Pritchard *et al*., 2000a) was used for the 8171 DArTseq data. Admixture and shared allele frequencies model were used to determine the number of clusters (K). The number of K ranged from 1 to 12 and each K was run 10 times. For each run, the initial burn-in period was set to 10,000 with 100,000 Markov Chain Monte Carlo (MCMC) iterations. The LnP(D) in STRUCTURE output and the derived ΔK were used to determine the most suitable K value as implemented in the Structure Harvester (Evanno *et al*., 2005). The derived ΔK considers the rate of change of LnP(D) as K increases and the variance of LnP(D) among repeated runs and tends to be maximum at the true value of K. The formula used was:

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$\Delta K = M[|L(K - 1) - 2L(K) + L(K + 1)|]/S[L(K)]$ (Evanno *et al*., 2005).

where: L(K) is the K^{th} LnP(D), M is the mean of 10 runs, and S their standard deviation. The K with the highest maximum likelihood was used to assign individual genotypes to clusters. Individuals with membership probability greater than or equal to 0.70 were assigned to the same group while lines with membership probability less than 0.70 were assigned to a mixed group (Lu *et al*., 2009; Yang *et al*., 2011). Principal component analysis (PCoA) of the DArTseq markers was performed using GenALEx version 6.5 (Peakall and Smouse, 2006; 2012) to compare the clusters of the model-based structure with that of the PCoA.

4.3 Results

4.3.1 Summary statistics and phylogeny of inbred lines

The total SNPs used in the DArTseq genotyping were 44391. After filtering, DArTseq SNPs with <5% minor allele frequency, <0.8 call rate and >10% missing rate were excluded, and a sub-set of 8171 high-quality DArTseq markers that identified chromosome numbers and physical positions were used in the analysis of the data. In the subset of SNPs which constituted the DArTseq markers, changes in base pairs were A/C (892), A/G (2322), A/T (815), C/G (963), G/T (2278) and C/T (901). Among the polymorphic SNPs, the A/G and G/T transitions constituted the most informative which accounted for 28.4 and 27.9% respectively. The description of the summary statistics involving estimates of major allele frequency, gene diversity, heterozygosity, polymorphic information content and percent SNPs per chromosome are presented in Figure 4.1. Gene diversity varied from 0.042 to 0.500 with an average of 0.357. A similar trend was observed for the PIC values which varied from 0.041 to 0.3 75 with a mean of 0.287. Heterozygous individuals identified per marker varied from 0.000 to 0.929 with a mean of 0.056. About 73% of the informative SNPs identified over 95% homozygous individuals. Major allele frequency varied from 0.500 to 0.978 with

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a mean of 0.74. The genetic distance generated among the 70 inbred lines varied from 0.018 to 0.455 with a mean of 0.336.

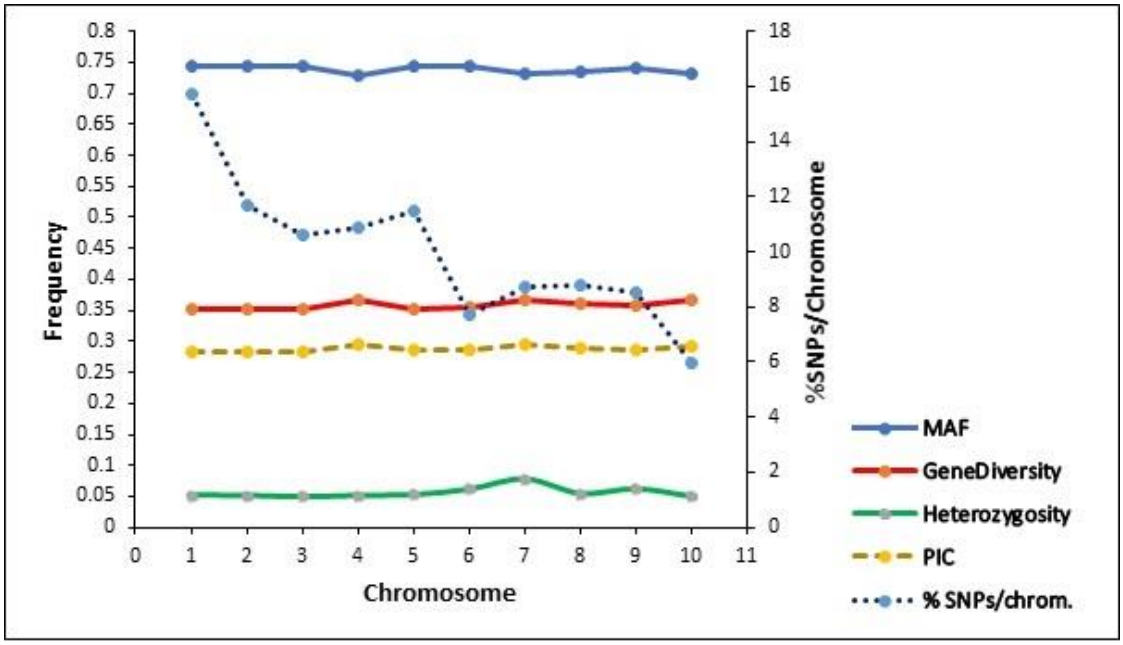

Figure 4. 1 Summary description of the DArTseq markers used in the genetic diversity study of 70 early provitamin A quality protein maize inbred lines

Based on the Nei's genetic distance, the UPGMA phylogenetic tree displayed five main groups for the 70 inbred lines (Fig 4. 2). Thirteen inbreds constituted group I, group II had 8, group III consisted of 18, group IV had 6 and group V had 25 inbred lines. Groups III and V were divided into two sub-groups (a and b) each. Except for the six checks, all the inbred lines had been improved for drought tolerance and *Striga* resistance, as well as increased PVA and quality protein levels. The six checks (2 normal yellow endosperm and 4 QPM yellow endosperm) constituted group IV. Available information on the inbred lines revealed that the different groups and sub-groups largely depended on pedigree information, the presence and the dosage of genes for drought tolerance, as well as PVA and quality protein alleles introgressed into the source population from which the inbreds were extracted.

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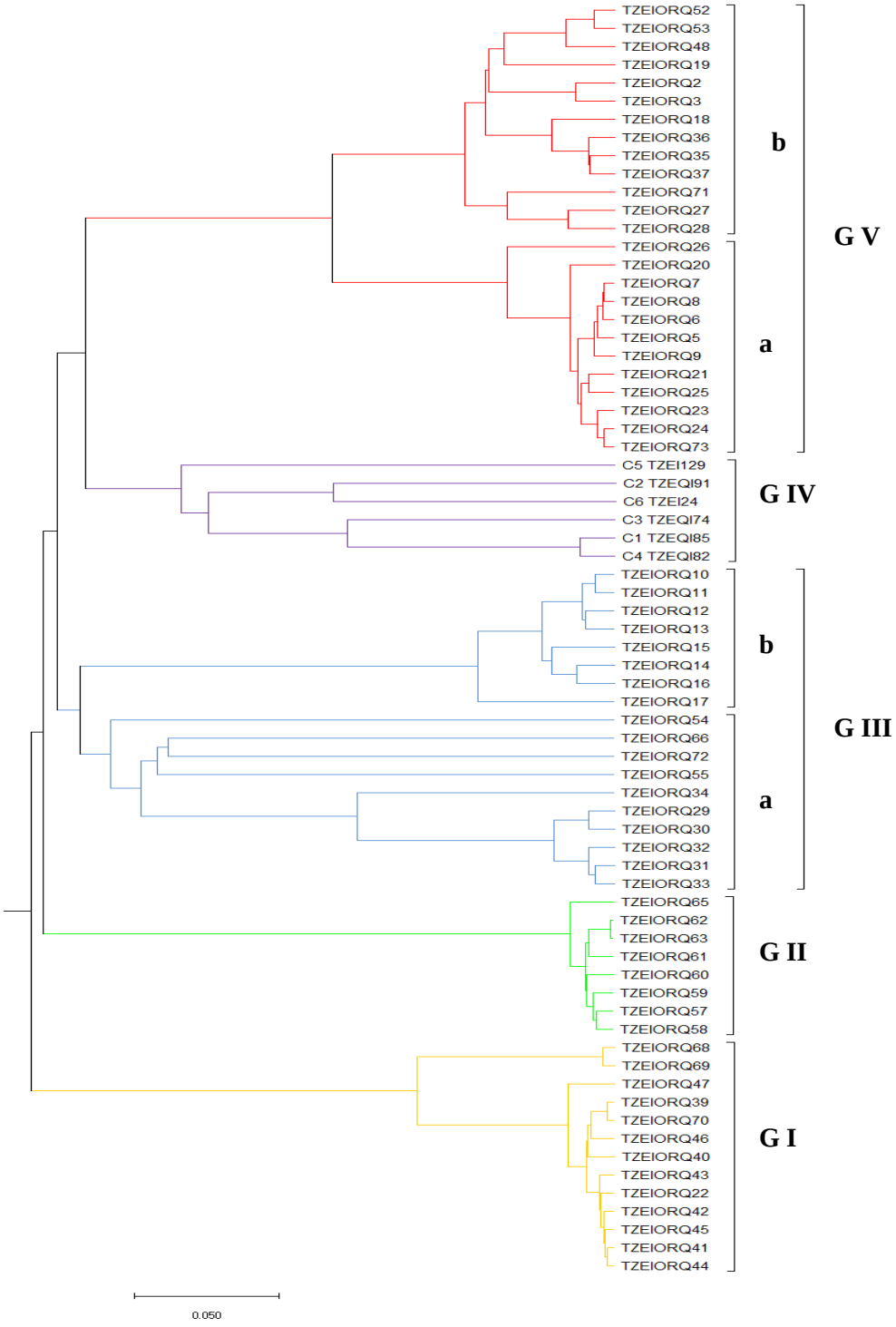

Figure 4. 2 Phylogenetic tree of 70 early provitamin A quality protein maize inbred lines using the UPGMA applied to Nei's 1983 genetic distance generated from the DArTseq markers

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4.3.2 Population structure and principal component analyses

The model-based clustering using an admixture implemented in the structure software was used to infer the number of clusters K within the 70 early PVA-QPM inbred population. In structure analysis, two criteria can be used to determine the best K in a population. These are the use of log likelihood for each K (Rosenberg *et al*., 2001; Evanno *et al*., 2005) and the use of an ad hoc quantity (ΔK) (Evanno *et al*., 2005). For the log likelihood criterion, increase in LnP(D) plateaued when K approached a true value (Fig. 4.3). On the other hand, the ΔK showed a clear peak at the true value of K (Fig. 4.4). The two plots consistently identified five clusters in the population. Sixty-six inbred lines which had probabilities ≥ 0.70 were assigned to a single group, while 4 inbreds (5.71% of the total) with probabilities less than < 0.70 (Appendix 3) and could not be distinctly assigned to any of the groups. The four inbreds were placed in a mixed group. The number and order of grouping of the inbred lines in the structure analysis were very similar to that of the UPGMA phylogeny. In the structure bar plot, the number of inbred lines classified into each cluster varied from 25 in group I, 8 in group II, 14 in group III, 13 in group IV, 6 in group V and 4 in mixed group (Fig. 4.5)

Principal Component Analysis (PCoA) of the DArTseq data was performed to alternatively study the structure of the inbred population (Patterson *et al*., 2006). The output of the PCoA was highly consistent with that of the structure analysis. As illustrated in Figure 4.6, the PCoA revealed a clear separation of the 5 groups identified in the structure analysis. These were; 25 inbreds in group I (red), 8 in group II (green), 14 in group III (blue), 13 in group IV (yellow), 6 in group V (purple) and 4 in mixed group (orange).

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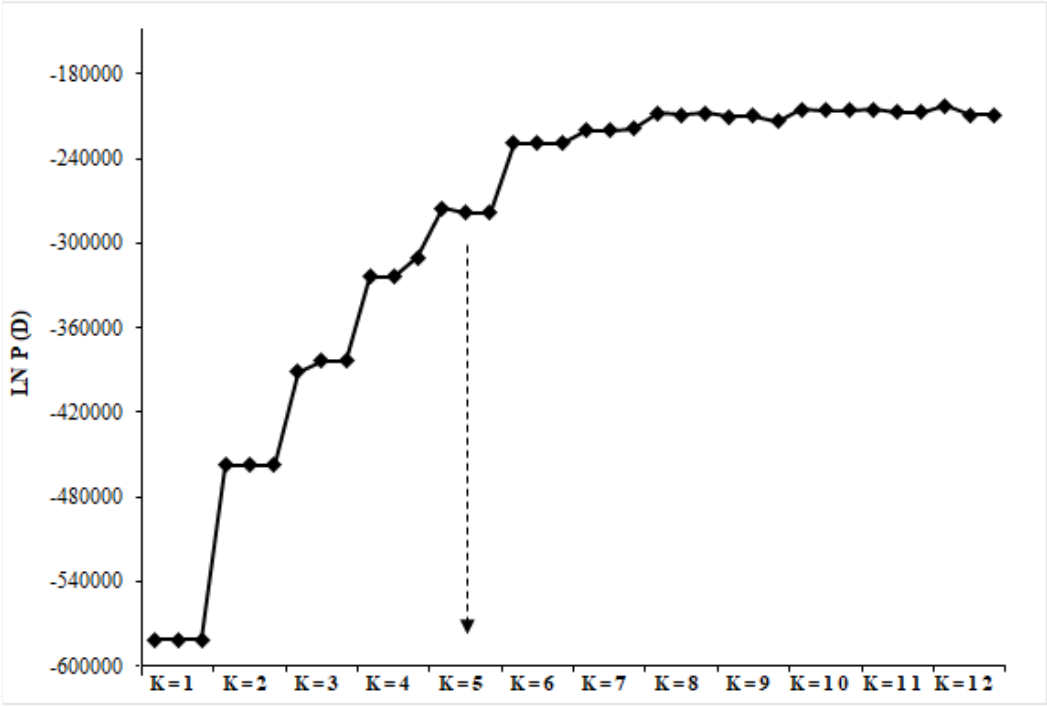

Figure 4. 3 Assessment of the best K in structure analysis using changing trends of estimated Ln probability of data LnP (D) over three repeats at each K value

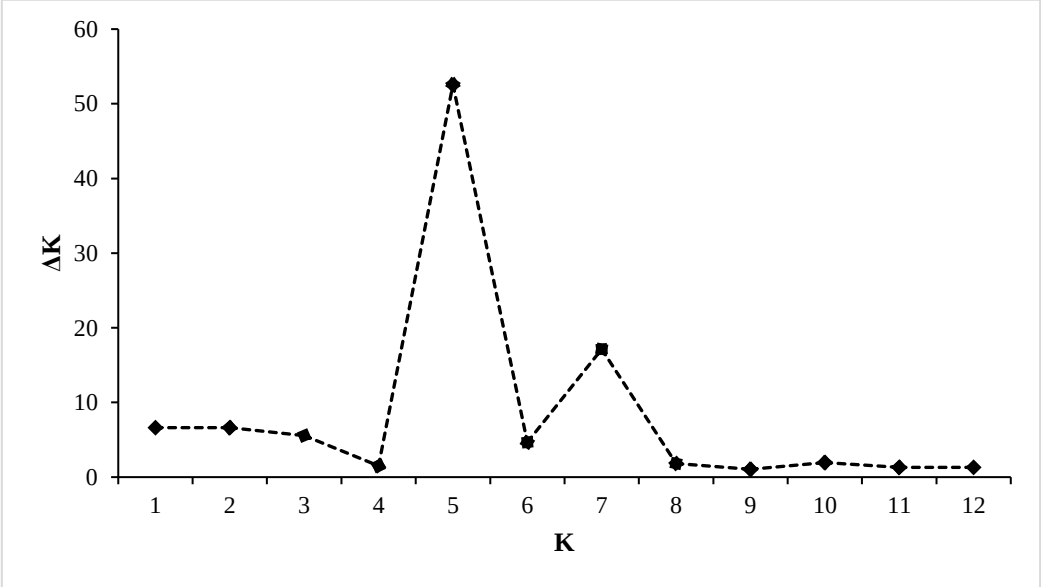

Figure 4. 4 Assessment of the best K in structure analysis using Pritchard's K (ΔK)

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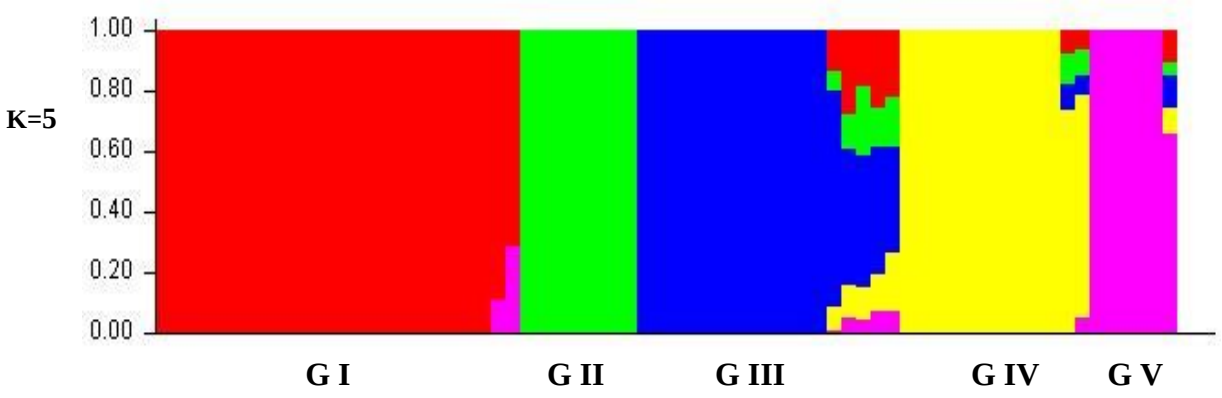

Figure 4. 5 Population structure bar plot of the 70 early PVA QPM inbred lines as membership coefficients (Q values)

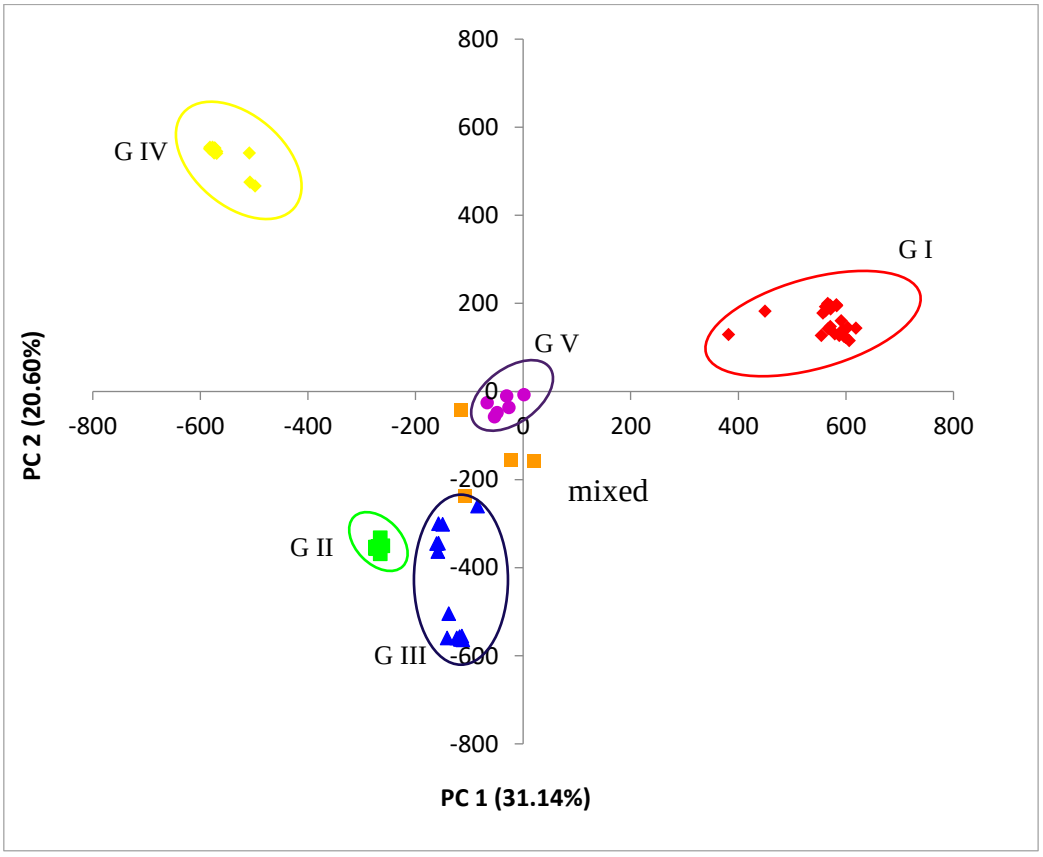

Figure 4. 6 Principal component analysis (PCoA) for the 70 early maturing provitamin A- quality protein maize inbred lines

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4.4 Discussion

The extent of genetic diversity, population structure, and patterns of relationship among the 70 early maturing PVA-QPM inbred lines was assessed using 8171 DArTseq markers. The summary statistics, UPGMA phylogeny, model-based population structure analysis, and the principal component analysis (PCoA) were used to investigate the inbred population to ascertain whether the population was homogeneous or harboured genetically distinct groups. From the summary statistics, the average gene diversity (GD) of 0.36 obtained was comparable to the 0.35 reported by Zhang *et al*. (2016) when a tropical group of maize was studied. The GD was also higher than that obtained in previous studies by Lu *et al*. (2009), and Van Inghelandt *el al*. (2010) which was around 0.32 but it was same as the value reported by Wu *et al*. (2015) which was 0.364. However, the GD observed in the present study was lower than that obtained by Yang *et al*. (2011) which was 0.39. The results agreed with previous findings that tropical maize germplasm is highly diverse with GD > 0.3 (Liu *et al*., 2003; Zhang *et al*., 2016). In addition, the observed average residual heterozygosity of 5.6% was higher compared with the 3.80% obtained by Dao *et al*. (2014), as well as the 3.34% found by Liu *et al*. (2018), but was lower than the 8.6% obtained by Jambrovic *et al*. (2008). The practically acceptable level of average heterozygosity indicated that the proportion of heterozygous individuals in the inbred population is reasonably low with most of the inbreds being about 94.4% homozygous with their loci fixed with minimal segregation. The average PIC obtained, 0.289 (ranging from 0.041 to 0.375) using 8171 SNPs for 70 inbred lines was higher than the 0.24 reported by Yu *et al*. (2009) using 1000 SNPs for a sample size of 274, as well as the 0.256 reported by Dao *et al*. (2014) using 1057 SNPs for sample size of 100. This indicated the existence of a high frequency of alleles and therefore high genetic diversity in the population as evident in the average major allele frequency of 0.74. The average PIC value was similar to that reported by Wu *et al*. (2015) which was around 0.29 using varying number of SNPs up to 43252 for a sample of 1857

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inbred lines. The differences in the summary statistics relative to other studies may be due to the differences in the genetic materials, the sample sizes and the number of SNPs used.

Based on the 8171 SNPs and the Nei's 1983 genetic distance method, the UPGMA phylogeny using 1000 non-parametric bootstrapping revealed five main clusters for the 70 inbred lines with groups III and V having two sub-groups (a and b) each. The clustering together of particular inbred lines illustrated that the DArTseq markers were identical in state at common loci for those inbred lines and that there is the tendency for such inbreds to be more related than those in different groups. As indicated in Section 3.2.1, the inbred lines were extracted from the source population 2009 TZE - OR2 DT STR QPM which is early, has PVA, drought tolerance (and indirect tolerance to low-N), *Striga* resistance and quality protein. The 13 inbred lines in group I followed pedigree information or were identical by descent, and were mostly drought and moderately low-N tolerant. The eight inbreds in group II were related by pedigree records and were mostly drought tolerant. Group III consisted of 18 inbred lines which had the PVA background coupled with low-N tolerance. Inbred lines in sub-group 'III-a' including TZEIORQ 10, TZEIORQ 12, TZEIORQ 13, TZEIORQ 14, TZEIORQ 15, TZEIORQ 16 and TZEIORQ 17 had the functional PVA gene (crtRB1) while inbreds in sub-group 'III-b', including TZEIORQ 55, TZEIORQ 29 and TZEIORQ 20, contain moderate to high PVA contents. It was striking to find all the six inbred checks used in the field evaluations classified into group IV. The checks had yellow kernels and most possessed the quality protein trait and hence their clustering might be influenced by common loci responsible for these traits. Group V was a relatively larger group including 25 inbreds which were related according to pedigree records. Sub-group 'V-a' was made up of 12 inbreds that combined drought and low-N tolerance, while sub-group 'V-b' consisted of 13 low-N tolerant inbred lines.

The log of likelihood method of determining the best K showed a steep increase in LnP(D) values from K= 1 to K= 5 after which the trend assumed a plateau. This observation

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indicated that the true K is 5 (Rosenberg *et al*., 2001; Evanno *et al*., 2005) and that five genetically distinct clusters exist in the entire population. Similarly, the Evanno criterion identified the peak level of ΔK at K= 5 (Evanno *et al*., 2005) inferring five genetically distinct clusters. The three multivariate methods illustrated the existence of genetically distinct groups in the population. Comparison of the different multivariate analyses revealed high consistency among the UPGMA clustering, the structure analysis, and the PCoA clustering in terms of the number of groups and number of individuals assigned to each group indicating that the identified groups are indeed genetically distinct. Individuals from the different groups are therefore, expected to harbour different favourable alleles for breeding for drought and/ low-N tolerant hybrids and synthetics with elevated PVA and quality protein contents. The results contradicted the findings of Dao *et al*. (2014) and Semagn *et al*. (2012) who reported a high consistency among the model-based structure analysis and the PCoA but a low concordance with the neighbour-joining phylogeny generated using the Roger's genetic distance method. This could be due to the difference in the inbred lines used in the different studies, the different methods of obtaining the genetic distances among the inbreds, as well as the different clustering algorithms. The clustering according to three multivariate analyses employed in the present study followed the available pedigree information, that is, expected related lines clustered together. These results substantiated other reports that the tropical maize populations are largely consistent with pedigree information (Warburton *et al*., 2005; Semagn *et al*., 2012; Dao *et al*., 2014). The results also demonstrated that the SNP-based DArT derived markers were informative in providing genome profiles which are very useful for the identification of unique characteristics among the maize germplasm (Huttner *et al*., 2005; Semagn *et al*., 2012).

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4.5 Conclusions

The UPGMA clustering, the model-based structure analysis and the PCoA consistently revealed clusters which largely followed the pedigree information indicating the existence of genetically distinct groups in the early PVA-QPM set of inbreds suggesting that inbreds that clustered together share common genetic backgrounds. The DArTseq markers were informative in providing very useful genome profiles for the identification of unique characteristics among the maize germplasm. Inbred lines from different clusters could be exploited to develop drought and/ low-N tolerant hybrids and synthetics with elevated PVA and quality protein contents for commercialization in SSA, and for the improvement of the early maturing PVA-QPM inbreds.

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CHAPTER FIVE

5.0 Assessment of combining ability and performance of provitamin A, quality protein maize inbreds under drought, low soil Nitrogen and optimal conditions

5.1 Introduction

Maize has the greatest potential for increased production and productivity in the savanna belt of sub-Saharan Africa (SSA). This is because the savanna belt of SSA is characterized by high solar radiation, low-night temperatures and low pests and diseases incidence. Despite this potentials, grain yield of maize in farmers' fields has been consistently low, averaging around 1.7 t ha^{-1}. This challenge has been ascribed to several factors including *Striga hermonthica infestation*, drought and low-N. The response of maize to drought and low-N has been found to be influenced by similar adaptive mechanisms (Badu-Apraku *et al*., 2012a) and the two stresses can together cause significant yield reduction in SSA (Kim and Adetimirin, 1997; Badu-Apraku *et al*., 2011a). Drought at flowering and grain filling (the most critical periods) may cause losses of 40-90% (Menkir and Akintunde, 2001). Yield reduction resulting from low-N varies between 10 and 50% (Wolfe *et al*., 1988). Improving maize for drought and low-N tolerance is crucial to the ongoing efforts to reduce the yield gap between optimal and stress conditions. Edmeades (2013) found that about 20-25% of the yield gap between well-watered potential yield and water-limited yield could be eliminated by genetic improvement for drought and low-N tolerance.

Through the concerted efforts of the IITA-MIP, PVA-QPM inbred lines with drought and low-N tolerance genetic backgrounds have been developed over the years for the production of hybrids and synthetics. Although several studies on the combining ability using different mating designs and heterotic grouping of maize inbred lines under contrasting environments have been conducted in SSA (Menkir *et al*., 2003; Badu-Apraku *et al*., 2013b;

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Badu-Apraku *et al*., 2015b, Annor and Badu-Apraku, 2016), it is important to carry out such studies for newly developed inbreds because there have been inconsistent reports on the mode of gene action conditioning heterosis in maize under drought and low-N conditions. The North Carolina Design II (NCD II) has the flexibility to investigate the relative importance of maternal (cytoplasmic) or paternal effects for the measured traits. Obtaining information on the combining ability of the newly developed inbred lines is necessary to guide breeding strategies under drought, low-N and optimal environments. Classifying the inbreds into different heterotic groups will help to reduce the development and evaluation of less productive crosses, while exploiting maximum heterosis by crossing inbred lines from opposing heterotic groups (Terron *et al*., 1997). The objectives of the study were to:

i. assess the combining ability of selected early maturing PVA-QPM inbreds under drought stress, low soil Nitrogen, and optimal conditions,
ii. classify the inbreds into heterotic groups and identify the best inbred and single-cross hybrid testers across environments, and
iii. assess the yield and stability of hybrids across research environments.

5.2 Materials and methods

5.2.1 Genetic materials and crosses

Twenty-four early maturing PVA-QPM inbred lines developed by the IITA-MIP were used in the present study. The inbred lines were selected based on their reactions to drought and low-N environments in previous studies. For the PVA and quality protein traits, kernel colour and endosperm modification, respectively, were used to select parental lines. This is because information on the levels of PVA and tryptophan of the inbreds was not available and the inbred evaluations and their nutritional analyses had to commence concurrently with the combining ability study without delay. The 24 inbreds were grouped

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into six sets and each set contained four inbred lines which were used to generate 96 single cross hybrids during the 2015/2016 dry season at IITA, Ibadan, Nigeria. The pedigree information of the parental lines, their reactions to drought and low-N, as well as the sets into which they were placed in the NCD II arrangement are presented in Table 5.1.

5.2.2 Field evaluation

The 96 PVA-QPM single cross hybrids plus four hybrid checks were evaluated under drought stress, low soil Nitrogen and optimal conditions in Nigeria from 2016 to 2017. Two of the four checks were single cross hybrids while the remaining two were top cross hybrids. Also, three of the checks were normal yellow endosperm hybrids commercialized in West Africa (Table 5.2) and the fourth check, TZEIOR 127 x TZEIOR 57 was an early maturing PVA single cross hybrid which has demonstrated outstanding performance in previous studies of the IITA-MIP under contrasting environments including drought and *Striga* infested conditions. These checks were used because early maturing PVA-QPM single cross hybrids were not available. The hybrids were evaluated under induced drought conditions at Ikenne (6° 53'N, 30° 42'E, 60 m altitude, 1200 mm annual rainfall) during the 2016/2017 dry season and at Kadawa (11°45′ N, 8°45′ E, 468.5 m above sea level, 884 mm annual rainfall) during the 2017/2018 dry season. The hybrids were evaluated under low-N (30 kg ha^{-1}) environments at Ile-Ife (7° 28' N, 4° 33' E, and 244 m above sea level, 1200 mm annual rainfall) and Mokwa (9°18'N, 5° 4'E, 457 m altitude, 1100 mm annual rainfall) during the 2016 and 2017 growing seasons. Hybrid evaluations were carried out under optimal growing conditions at Ile-Ife, Mokwa and Ikenne during the 2016 and 2017 growing seasons. A 10 × 10 alpha lattice design with two replications was used in all experiments conducted under the different environments.

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Table 5. 1 The 24 early maturing provitamin A quality protein maize inbred lines selected for the NCD II crosses at IITA Ibadan, Nigeria, from January to April 2016

S/N	Inbred	Pedigree	Reaction to drought	Reaction to low-N	Set
1	TZEIORQ 69	2009 TZE-OR2 DT STR QPM S_6 inb 57-2/2-2/2-1/1-1/2-1/1	Tolerant	Tolerant	A
2	TZEIORQ 29	2009 TZE-OR2 DT STR QPM S_6 inb 28-1/1-2/2-1/2-1/2-1/1	Tolerant	Susceptible	A
3	TZEIORQ 45	2009 TZE-OR2 DT STR QPM S_6 inb 35-2/3-3/3-4/4-3/4-1/1	Susceptible	Tolerant	A
4	TZEIORQ 48	2009 TZE-OR2 DT STR QPM S_6 inb 41-1/2-1/3-1/2-3/3-1/1	Tolerant	Susceptible	A
5	TZEIORQ 11	2009 TZE-OR2 DT STR QPM S_6 inb 7-1/3-1/2-1/2-4/4-1/1	Tolerant	Tolerant	B
6	TZEIORQ 20	2009 TZE-OR2 DT STR QPM S_6 inb 26-1/1-1/2-1/6-1/2-1/1	Tolerant	Tolerant	B
7	TZEIORQ 6	2009 TZE-OR2 DT STR QPM S_6 inb 2-2/3-2/3-2/4-1/5-1/1	Tolerant	Susceptible	B
8	TZEIORQ 44	2009 TZE-OR2 DT STR QPM S_6 inb 35-2/3-3/3-4/4-1/4-1/1	Tolerant	Susceptible	B
9	TZEIORQ 42	2009 TZE-OR2 DT STR QPM S_6 inb 35-2/3-3/3-2/4-2/2-1/1	Tolerant	Tolerant	C
10	TZEIORQ 59	2009 TZE-OR2 DT STR QPM S_6 inb 50-2/2-1/3-2/3-2/2-1/1	Tolerant	Tolerant	C
11	TZEIORQ 15	2009 TZE-OR2 DT STR QPM S_6 inb 7-2/3-1/2-3/4-1/3-1/1	Tolerant	Susceptible	C
12	TZEIORQ23	2009 TZE-OR2 DT STR QPM S_6 inb 26-1/1-1/2-4/6-1/3-1/1	Tolerant	Susceptible	C
13	TZEQI 82	TZE COMP5-Y C6S6 Inb 25 x Pool 18 SR QPM BC1S6 2-3-1-1-6-6	Tolerant	Tolerant	D
14	TZEIORQ 47	2009 TZE-OR2 DT STR QPM S_6 inb 35-3/3-3/3-1/3-2/2-1/1	Susceptible	Tolerant	D
15	TZEIORQ 7	2009 TZE-OR2 DT STR QPM S_6 inb 2-2/3-2/3-3/4-1/3-1/1	Tolerant	Susceptible	D
16	TZEIORQ 13	2009 TZE-OR2 DT STR QPM S_6 inb 7-1/3-1/2-2/2-3/3-1/1	Tolerant	Susceptible	D
17	TZEIORQ 2	2009 TZE-OR2 DT STR QPM S_6 inb 2-2/3-1/3-1/3-1/2-1/1	Tolerant	Tolerant	E
18	TZEIORQ 5	2009 TZE-OR2 DT STR QPM S_6 inb 2-2/3-2/3-1/4-3/3-1/1	Tolerant	Susceptible	E
19	TZEIORQ 26	2009 TZE-OR2 DT STR QPM S_6 inb 26-1/1-1/2-6/6-2/3-1/1	Tolerant	Tolerant	E
20	TZEIORQ 41	2009 TZE-OR2 DT STR QPM S_6 inb 35-2/3-3/3-4/4-1/4-1/1	Tolerant	Susceptible	E
21	TZEIORQ 24	2009 TZE-OR2 DT STR QPM S_6 inb 26-1/1-1/2-4/6-2/3-1/1	Tolerant	Tolerant	F
22	TZEIORQ 43	2009 TZE-OR2 DT STR QPM S_6 inb 35-2/3-3/3-3/4-1/2-1/1-	Tolerant	Susceptible	F
23	TZEIORQ 40	2009 TZE-OR2 DT STR QPM S_6 inb 35-2/3-2/3-1/2-2/2-1/1-	Susceptible	Tolerant	F
24	TZEIORQ 70	2009 TZE-OR2 DT STR QPM S_6 inb 60-2/2-1/2-1/3-1/4-1/1	Tolerant	Susceptible	F

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Each experimental unit was a single-row plot, 4 m long with row and hill spacing of 0.75 and 0.40 m, respectively. Three seeds were planted per hill and the seedlings later thinned to two at 2 WAP to obtain a final plant population density of about 66,666 ha^{-1}. The soil type at Ile-Ife, Ikenne and Mokwa and the details on the management of low-N, drought, and optimal experiments are described in Section 3.2.2 of this dissertation. The soil type at Kadawa is Eutric Gambisol (Soil Survey Staff, 1999). The managed drought at Kadawa was achieved by applying furrow irrigation water once per week up to 32 days after planting when the irrigation was withdrawn. Due to the intense heat at Kadawa during the period February – March when the experiment was conducted and the characteristic sandy and shallow top soil, the irrigation was resumed after grain filling (two weeks after flowering) to avoid total loss of the trial. Weed control was done as indicated for the low-N trials in section 3.2.2.1. The four sites (Ikenne, Ile-Ife, Mokwa and Kadawa) where the experiments were conducted as well as Ibadan where the breeding nurseries were planted are illustrated in Figure. 5.1.

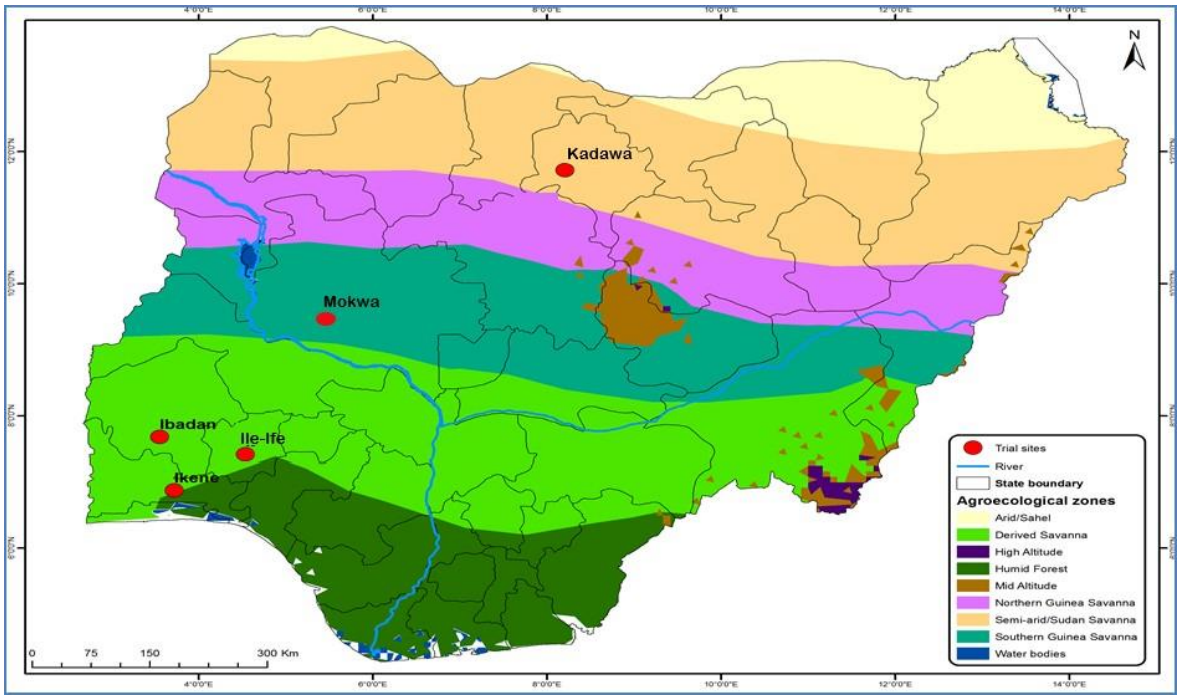

Figure 5. 1 Agro-ecological map of Nigeria indicating the experimental sites

Source of map image: International Institute of Tropical Agriculture (IITA) Geo-Spatial Service Laboratory

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Table 5. 2 Description of the commercial hybrid checks used in the study

Name	Year of Release	Country	Code/pedigree	Company	Type of Hybrid
Kunjor-wari	2015	Ghana	TZE-Y POP DT STR C4 x TZEI 17	SARI/CRI/IITA	Top cross
CSIR-Denbea	2017	Ghana	TZEI 124 x TZEI 25	SARI/CRI/IITA	Single cross
Tamalaka	2014	Mali	TZEI 124 x TZEI 25	IER/IITA	Single cross
Sammaz 41	2014	Nigeria	TZEI 124 x TZEI 25	IAR/IITA	Single cross
Sammaz 42	2014	Nigeria	TZE-Y Pop DT STR x TZEI 13	IAR/IITA	Top cross

SARI= Savanna Agricultural Research Institute; CRI= Crops Research Institute; IITA= International Instutute of Tropical Agriculture; IER= Institute of Rural Economy; IAR= Institute of Agricultural Research.

5.2.3 Data collection

Data collected from the drought, low-N and optimal experiments are as described in Section 3.2.4.

5.2.4 Statistical analysis

Data recorded for stalk and root lodging as well as ear rot, were converted to percentages and afterwards transformed using the square root transformation procedure. The location-year combination was considered as an environment whereas the environmental conditions (drought, low-N, and optimal growing season) were regarded as research conditions (treatments). Initially, separate Analysis of variance (ANOVA) was carried out on data collected under drought, low-N and optimal growing conditions with the general linear model procedure (PROC GLM) in Statistical Analysis System (SAS) using a random statement with test option (SAS Institute, 2012). Also, combined ANOVA was performed across the twelve test environments. In the ANOVA for each and across research conditions, environments, replicates within environment, and incomplete blocks within replicates × environment interaction were considered as random factors while the entries (hybrids) was regarded as a fixed factor. The statistical model corresponding to the experimental layout was:

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$$y_{klmi} = \mu_i + E_{ki} + R(E)_{kli} + G_{mi} + GE_{kmi} + \epsilon_{klmi}$$

Where y_{klmi} is the observed measurement of trait *i* with mean effect μ_i, E_{ki} is the effect of environment k on trait *i*, $R(E)_{kli}$ is the effect of replication *l* within environment *k* on trait *i*, G_{mi} is the effect of genotype *m* on trait *i*, GE_{kmi} is the effect of the interaction between genotype *m* and environment *k* on trait *i*, and $\epsilon_{klmi}$is the experimental error effect associated with genotype *m* and replication *l* within environment *k* on trait *i*. The entry means were adjusted for block effects, according to the lattice design (Cochran and Cox, 1960) and means were separated using standard error (S.E). The initial ANOVA was performed for each and across research conditions to obtain information on the variance due to hybrid (not partitioned), and hybrid × environment interaction.

Thereafter, NCD II ANOVA for each and across research conditions were performed on plot means for all data collected with PROC GLM in SAS (SAS Institute, 2012) using the random statement with test option. The general linear model used for the NCD II mating design was:

$$X_{ijklm} = \mu + S_l + g_i(S_l) + g_j(S_l) + h_{ij}(S_l) + E_m + r_k(SE)_{lm} + (SE)_{lm} + (E_g)_{im}(S_l) + (E_g)_{jm}(S_l) + (E_h)_{ijm}(S_l) + e_{ijklm}$$

Where:

X_{ijklm}= the observed value of the progeny of the ith female, jth male in the kth replication within set l and in the mth environment,

μ= population mean,

S_l= average effect of the lth set,

$g_i(S_l)$= GCA effect common to all hybrids of the ith female nested within lth set,

$g_j(S_l)$= GCA effect common to all hybrids of the jth male nested within lth set,

$h_{ij}(S_l)$= SCA effect of hybrid from the ith female and jth male nested within ^{lth} set,

E_m= average effect of the mth environment,

$rk(SE)_{lm}$ = effect of the kth replication nested within the lth set and mth environment,

$(SE)_{lm}$=Interaction between the set effect and the environment,

$(E_g)_{im}\ (S_l)\ and\ (E_g)_{jm}\ (S_l)$ = Interaction between environment and GCA nested within sets,

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$(E_h)_{ijm}(S_l)$= Interaction between environment and SCA nested within sets,

e_{ijklm}= the experimental error (Singh and Chaudhary, 1985).

The hybrids component of the variation was decomposed into variation due to male sets, female sets and female × male interactions sets in the NCD II ANOVA. The F-test for male, female and female × male mean squares were calculated using the mean squares for their respective interaction with the environment. The mean squares attributable to environment × female × male sets were tested using the pooled error mean squares. The main effects of the male sets and female sets is the GCA effect while the female × male sets interaction represents SCA effects (Hallauer and Miranda, 1988). The form of ANOVA used for the NCD II and repeated over environments is presented in Table 5.3 as follows;

Table 5. 3 Form of the analysis of variance of North Carolina Design II repeated over environments

source of variation	degrees of freedom(df)*	mean squares(ms)	expected mean squares
Environments (E)	e-1		
Sets (S)	s-1		
S x E	(e-1)(s-1)		
Replications/S/E	es(r-1)		
Males/S	s(m-1)	M_1	$\sigma_e^2 + r\sigma^2_{fme} + re\sigma^2_{mf} + r\sigma^2_{me} + ref\sigma^2_m$
Females/S	s(f-1)	M_2	$\sigma_e^2 + r\sigma^2_{fme} + re\sigma^2_{mf} + rm\sigma^2_{fe} + rem\sigma^2_f$
Males x Females/S	s(m-1)(f-1)	M_3	$\sigma_e^2 + r\sigma^2_{fme} + re\sigma^2_{mf}$
Males/S x E	s(m-1)(e-1)	M_4	$\sigma_e^2 + r\sigma^2_{fme} + rf\,\sigma^2_{me}$
Females/S x E	s(f-1)(e-1)	M_5	$\sigma_e^2 + r\sigma^2_{fme} + rm\sigma^2_{fe}$
Male x Females/S x E	s(m-1)(f-1)(e-1)	M_6	$\sigma_e^2 + r\sigma^2_{fme}$
Pooled error	se(r-1)(mf-1)	M_7	σ_e^2
Total	esrmf-1		

*e, s, r, m, and f refer to the number of environments, sets within an environment, replications, males and females, respectively.

The proportionate contribution of each trait was computed as percentage of the sum of squares for the crosses attributed to general combining ability (GCA) and specific combining ability (SCA) as follows:

Contribution of GCA-male (%) = [ssm / (ssm + ssf + ssmf) x 100]

Contribution of GCA-female (%) = [ssf / (ssm + ssf + ssmf) x 100]

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Contribution of SCA (%) = [ssmf / (ssm + ssf + ssmf) x 100]

where:

ssm = sum of squares due to males within sets,

ssf = sum of squares due to females within sets,

ssmf = sum of squares due to male x female within sets interaction.

The relative GCA-male, GCA-female and SCA effects for grain yield were computed from the adjusted means using line x tester approach (Singh and Chaudhary, 1985)

$$GCA_{males} = \overline{X}_j - \overline{Y}$$

$$GCA_{females} = \overline{X}_i - \overline{Y}$$

$$SCA = \overline{X}_{ij} - \overline{X}_i - \overline{X}_j + \overline{Y}$$

where:

$\overline{X}_j$ = the mean of hybrids with a given male averaged over replicates, environments and females,

$\overline{X}_i$ = the mean of hybrids with a given female averaged over replicates, environments and males,

$\overline{X}_{ij}$ = the mean of a given hybrid averaged over replicates, environments and females,

$\overline{Y}$ = the experimental mean.

Standard errors for GCAs effects were calculated as described by Cox and Frey (1984):

$$SE_{GCA} = [MS_{fe}(f-1) / mfer]^{½} \text{ or } [MS_{me}(m-1) / mfer]^{½}$$

$$SE_{SCA} = [MS_{fme}(m-1)(f-1) / mfer]^{½}$$

Where, MS_{fe}, MS_{me} and MS_{fme} are the respective female x environment, male x environment and female x male x environment interaction mean squares and are multiplied by the appropriate proportion of total number of observations (female x male x replicate x environment). The significance of the GCA-female, GCA-male, and SCA effects of the individual inbred lines were determined using the respective standard errors. In addition, the F test or variance ratio was used to compare the mean squares of the GCA male and female as suggested by Kearsey and Pooni (1996) to determine the relative significance of

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cytoplasmic effects. Restricted maximum likelihood (REML) estimates of the hybrids genetic and phenotypic variances were obtained with SAS PROC Varcomp and were used to compute broad-sense heritability for each trait as described in Section 3.2.4.

For the measured traits, mid-parent values for a cross was computed as the mean of the two parental lines averaged across research conditions separately, and across all research conditions. The relationship between per se performance of the parental lines and the grain yield of their respective hybrids under each and across research conditions was estimated using Spearman's rank correlation analysis implemented in SAS. Thus, the correlation co-efficients of traits of parental lines with grain yield of their hybrids were calculated using mean grain yields of hybrids and the corresponding mid-parent values. Additionally, the percent increase or decrease of F_1 hybrids over mid parent values was calculated to estimate possible heterotic effects for the measured traits (Fonseca and Patterson, 1968). Values for mid-parent heterosis (MPH) for a cross were computed for each trait as follows:

$$MPH = \left(\frac{F_1 - MP}{MP}\right) \times 100$$

Where;

F_1 = Mean of the hybrid,

MP = the mean of the parents that constituted the hybrids.

Mid-parent heterosis were averaged separately under drought, low-N, optimal and across all the twelve test environments. T-test was performed to determine whether F_1 hybrid means were statistically different from mid parent means as follows (Wynne *et al*., 1970):

$$tij = \frac{F_1 ij - MP}{\sqrt{\frac{3}{8} EMS}}$$

Where;

F_1ij = The mean of the ijth F_1 cross

MPij = The mid parent for the ijth cross

EMS = Error mean square

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Drought and low-N tolerant single cross hybrids were identified using the multiple trait base index (MI) proposed by Badu-Apraku *et al*. (2011a) as indicated in section 3.2.5.

Furthermore, stepwise multiple regression and sequential path diagrams were used to explain the causal relationships among traits under managed drought, low-N, optimal, and across environments using the procedure proposed by Mohammadi *et al*. (2003). The stepwise multiple regression analysis was performed using the Statistical Package for the Social Sciences, SPSS version 17.0 (SPSS Inc, 2007) to determine the first, second, and third order predictor traits on the basis of their contributions to the total variation in grain yield with minimized multicolinearity (Badu-Apraku *et al*., 2014; Talabi *et al*., 2017). Firstly, all other traits were regressed on grain yield to identify those with significant contributions to grain yield at $P \leq 0.05$ as first order traits. The rest of the traits were regressed on each of the first order traits and those with significant contributions to grain yield through the first-order trait were classified as second-order traits. The procedure was repeated and the remaining traits were categorized into subsequent orders. The standardized b values generated by the stepwise regression analysis were the path co-efficients (Mohammadi *et al*., 2003; Badu-Apraku *et al*., 2014; Talabi *et al*., 2017). The significance of the path coefficients was determined in the stepwise multiple regression analysis using t-test at 5% probability level and retained the traits with significant path co-efficients. Also, the relationships among traits within an order of traits were determined using the spearman correlation analysis implemented in SAS version 9.4 (SAS Institute, 2012).

Heterotic grouping based on GCA of multiple traits (HGCAMT) proposed by Badu-Apraku *et al.* (2013b), was used to group the inbred lines under each and across research environments. This was done by standardising the GCA effects of all traits that had significant mean squares under the respective test environments. Importance was however, attached to the six traits employed in the drought and low-N base index which included grain yield, ears per plant, anthesis silking interval, plant aspect, ear aspect, and the stay-green

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characteristic. The standardised GCA effects were subsequently subjected to Ward's minimum variance cluster analysis to group the inbred lines under drought, low-N, optimal and across environments using SAS software version 9.4 (SAS Institute, 2012). In addition, a similar cluster analysis procedure was followed to group the inbred lines using the frequency based genetic distance matrix obtained from 8171 DArTseq markers as described in section 4.2.5.

The HGCAMT method and the DArTseq based markers method of classifying inbreds into heterotic groups were compared to determine the more efficient method under drought, low-N, optimal, and across research conditions. This was done by arranging the 96 hybrids from the highest to the lowest based on grain yield under each and across environments. The procedure involved dividing the total number of hybrids for each method into two major groups. That is, inter-group and intra-group crosses. The hybrids were divided into high-yielding hybrids (yield group one with a mean grain yield ranking among the top 32 lines); intermediate hybrids (yield group 2 with a mean grain yield between the 33rd and 64th line) and low yielding hybrids (yield group 3 with a mean grain yield between 65th and 96th line). The better classification method was the one whose classified heterotic groups allowed inter-heterotic group crosses to produce more superior hybrids than the intra-group crosses (Fan *et al*., 2009). This implies that the more efficient grouping method should place more of the inter-group crosses in the higher yielding category and more of the intra-group crosses in the low yielding hybrid class. In addition, estimate of breeding efficiency was based on the method described by Badu-Apraku *et al*. (2016a). That is, the average of the proportion of total inter-heterotic group hybrids that is due to superior high yielding inter-heterotic group hybrids plus the proportion of total low-yielding intra-heterotic group hybrids that is due to the low yielding intra-heterotic group hybrids. Thus, breeding efficiency was estimated using the equation as follows:

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$$BE = \frac{\left[\frac{HYINTERGH}{TNINTERGH} X\ 100\right] + \left[\frac{LYINTRAGH}{TNINTRAGH} X\ 100\right]}{2}$$

where BE = Breeding efficiency

HYINTERGH = number of high yielding inter-heterotic group hybrids

TNINTERGH = total number of inter-heterotic group hybrids

LYINTRAGH = number of low yielding intra-heterotic group hybrids

TNINTRAGH = total number of intra-heterotic group hybrids.

The criteria proposed by Pswarayi and Vivek (2008) was adopted to identify inbred and single cross testers. An inbred line was considered a tester based on three main criteria. It must (i) belong to a known heterotic group (ii) have a high significant positive GCA across the test environments and (iii) have high yield *per se*. For the single cross tester identification, the parental lines of the hybrid must record significant positive GCA effects for grain yield, the lines must belong to the same heterotic group, and the hybrid must have a relatively good yielding ability under stress conditions to qualify its use as a seed parent in successful three-way and double-cross hybrids development to ensure its high seed production ability.

To identify the best performing hybrids under drought and low-N conditions, a multiple trait base index (MI) that combines superior grain yield under drought or low-N with good plant and ear aspects, delayed leaf senescence, short ASI and increased number of ears per plant was used as described in Section 3.2.5 according to Badu-Apraku *et al*. (2011a). Using the MI, a set of 25 hybrids (top 15 drought and low-N tolerant, and 10 most susceptible) plus the four checks were selected and for the genotype main effect plus geno-type × environment interaction (GGE) biplot analysis to break the G x E interactions into its component parts (Yan, 2001). The GGE biplot was used to obtain information on the most promising hybrids under drought, low-N and optimal environments and to investigate the stability of hybrids across these environments using GGE biplot software, a windows

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application that fully automates biplot analysis (Yan, 2001). The GGE biplot model equation used is as follows:

$\hat{Y}_{ij} - Y_j = \lambda_1\xi_{i1}\eta_{j1} + \lambda_2\xi_{i2}\eta_{j2} + \varepsilon_{ij}$

where $\hat{Y}_{ij}$ is the average yield of genotype i in environment j; Y_j is the average yield across all genotypes in environment j; λ_1 and λ_2 are the singular values for PC1 and PC2 respectively; ξ_{i1} and ξ_{i2} are the PC1 and PC2 scores, for genotype i; η_{j1} and η_{j2} are the PC1 and PC2 scores, for environment j; ε_{ij} is the error associated with the genotype i in environment j.

5.3 Results

5.3.1 Analysis of Variance of grain yield and other traits under drought, low-N, optimal and across environments

Across the two drought environments, the ANOVA showed significant ($P < 0.05$) differences among E, G and GEI mean squares for grain yield and all measured traits except the E mean squares for ASI, ear height, and stalk lodging as well as G and GEI effects for root lodging (Table 5.4). Significant set effects were observed for grain yield and most other traits except days to 50% silking, and root and stalk lodging. Decomposing the hybrid component of variation into male (set) (GCA-male), female (set) (GCA-female) and female x male interaction (set) (SCA) mean squares revealed significant variations for GCA-male, GCA-female and SCA for all measured traits except root lodging. Significant differences were observed for GCA-male x E, GCA-female x E, and SCA x E for grain yield and most of the measured traits except GCA-male x E effects for root and stalk lodging, husk cover and ear rot, GCA-female x E mean squares for root and stalk lodging and SCA x E effects for root lodging, husk cover and ear and plant aspects. Estimates of broad sense heritability varied from 25% for days 50% anthesis to 84% for ear aspect. Grain yield had H^2 estimate

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of 74%. Generally, very low to high H^2 estimates were observed for the measured traits, with no estimate for root lodging due to negative genotypic variance recorded for the trait.

Across four low-N environments, ANOVA indicated significant ($P < 0.05$) variations for E, G, GEI and mean square for grain yield and all measured traits except GEI forASI. Significant set x E effects were detected for grain yield and other traits with the exception of ASI, root lodging, plant aspect, ear rot, ears per plant and stay green characteristic (Table 5.5). The GCA-male, GCA-female and SCA effects showed significant differences for most of the measured traits under low-N. The exceptions included GCA-male effects for root lodging, SCA effect for ASI and root and stalk lodging. The result also revealed significant mean squares of GCA-male x E, GCA-female x E, and SCA x E for grain yield and all measured traits except the GCA-female x E effects for stalk lodging and SCA x E effect for root lodging. Broad sense heritability estimates ranged from 10% for stalk lodging to 88% for ear aspect. Grain yield recorded H^2 estimate of 75%. Overall, low to high H^2 estimates were observed for the measured traits.

Under optimal environments, significant ($P < 0.05$) differences were observed among E, G, and GEI mean squares for all measured traits but not for ASI (Table 5.6). Similarly, GCA-male, GCA-female, and SCA effects were significant ($P < 0.01$ or $P < 0.5$) for all measured traits except ASI. Furthermore, significant GCA-male x E, GCA-female x E and SCA x E were significant for all measured traits with the exception of ASI and root lodging. Estimates of broad sense heritability varied from 16% for ASI to 76% for days to 50% anthesis. Grain yield recorded H^2 estimate of 56%. Low to high H^2 estimates were observed for the measured traits, with no estimate for ear rot as a result of negative genotypic variance recorded for the trait.

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Table 5. 4 Mean squares and heritability estimates of grain yield and other agronomic traits of early maturing provitamin A - quality protein maize hybrids evaluated under drought at Ikenne in the 2016/2017 and 2017/2018 dry seasons

Source	DF	YIELD	DA	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP	STGR
Env	1	278571020.4**	22535.68**	21928.04**	4.15	3722.39**	26.32	38.80**	0.85	123.49**	38.34**	162.87**	1359.55**	3.54**	224.20**
Set	5	2553717.4*	5.37*	4.67	6.65**	345.38*	113.71*	0.56	1.93	1.97*	1.33*	1.85*	8.52*	0.07*	1.09*
Env*set	5	1028828.8	11.64**	13.26**	0.62	636.49**	159.19	2.12	1.7	2.78**	1.39*	0.91	5.36	0.08	0.87*
Rep(env*set)	10	706104.1	3.44	4.93	2.63	72.04	63.32	1.68	1.41	0.65	0.38	0.84	1.8	0.04	0.41
Block(env*rep)	36	1102824.7	5.18**	9.36**	3.28*	709.79**	244.60**	1.64	1.37*	2.19**	1.33**	1.10*	6.27**	0.03	0.73**
Hybrid	99	7678135.4**	15.63**	23.90**	5.05**	1100.55**	423.27**	1.17	2.45**	4.97**	2.89**	6.78**	6.24**	0.20**	1.43**
Male(set)	18	9683257.3**	17.48**	23.93**	3.41*	901.65**	316.07**	1.67	3.97**	2.74**	1.79**	6.27**	6.13**	0.15**	0.99**
Female(set)	18	6861305.6**	9.24**	12.48**	4.98**	1471.45**	538.24**	1.44	3.70**	4.69**	2.66**	5.95**	7.48**	0.19**	2.47**
Female*male(set)	54	8068468.2**	18.10**	28.89**	5.45**	1137.85**	463.40**	0.86	2.12**	6.27**	3.57**	7.88**	6.14**	0.24**	1.11**
Hybrid*env	99	1943928.1**	11.94**	11.62**	3.20**	264.96**	142.96*	1.18	1.31*	1.04*	0.70*	1.04*	4.48**	0.06**	0.91**
Env*male(set)	18	3080980.5**	16.35**	14.56**	5.57**	171.4*	215.58**	1.23	1.34	0.93	0.7*	1.60**	3.22	0.06*	1.57**
Env*female(set)	18	2343428.5**	10.30**	11.29**	4.70**	346.25**	91.27*	1.44	1.22	1.76**	0.96*	1.11*	5.23*	0.11**	1.31**
Env*female*male(set)	54	1571630.6*	11.45**	10.77**	2.21*	247.12*	139*	0.98	1.69**	0.66	0.54	0.75	4.31*	0.05*	0.54*
Error	144	973235	1.90	3.36	1.94	156	100.33	1.27	0.89	0.69	0.48	0.73	2.94	0.04	0.35
Heritability		0.74	0.25	0.51	0.35	0.78	0.70	-	0.53	0.81	0.78	0.84	0.28	0.70	0.38

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; Env = environment; Rep = replication; YIELD = Grain yield; DA= days to 50% anthesis ; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; RL = root lodging; SL = stalk lodging; HC = husk cover; PASP = plant aspect; EASP = ear aspect; EROT = ear rot; EPP =ears per plant; STGR= stay-green characteristic.

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Table 5. 5 Mean squares and heritability estimates of grain yield and other agronomic traits of early maturing provitamin A - quality protein maize hybrids evaluated under low-N environments at Ile-Ife and Mokwa during the 2016 and 2017 growing seasons

Source	DF	YIELD	DA	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP	STGR
Env	3	291853629.7**	111.51**	181.80**	25.46**	95457.86**	24241.01**	102.30**	7.12**	805.24**	35.80**	120.78**	550.25**	895.29**	3.77**
Set	5	1276289.4	7.13**	10.07**	0.73	1217.31**	266.04**	2.14	0.42	1.22*	3.29**	2.38**	2.94	6.06	0.03
Env*set	15	2048451.9**	5.09**	4.20**	0.39	297.40**	144.08**	1.45	0.46*	1.49**	0.29	1.52**	3.4	4.93	0.03
Rep(env*set)	20	914232.9	1.03	0.79	0.26	163.72	75.47	1.17	0.19	0.63	0.36	0.55	2.48	3.52	0.03
Block(env*rep)	72	1475216.4**	1.95**	2.71**	0.51**	351.11**	194.73**	2.13**	0.38*	0.93**	1.12**	1.19**	4.66**	5.31*	0.03
Hybrid	99	9948239**	11.74**	13.13**	0.51*	1316.85**	442.11**	2.38**	0.39**	2.84**	4.83**	6.55**	8.52**	0.09**	3.23**
Male(set)	18	8473940.5**	8.76**	11.13**	0.59*	1732.69**	632.07**	4.51	0.51*	1.68**	3.36**	5.72**	7.00**	10.50**	0.10**
Female(set)	18	8667775.8**	9.22**	11.85**	0.74**	1627.47**	534.09**	3.56**	0.52*	2.56**	4.63**	6.81**	12.43**	14.70**	0.06**
Female*male(set)	54	12215059.2**	12.98**	13.37**	0.42	1174.13**	408.57**	1.41	0.35	3.39**	5.80**	7.51**	4.65**	5.94**	0.11**
Hybrid*env	297	1607731.4**	2.30**	2.57**	0.39	253.47**	93.64**	1.81**	0.37**	1.88**	0.63**	0.89**	5.62**	0.04**	0.95**
Env*male(set)	54	1222434*	1.78*	2.09*	0.28*	302.00**	102.77**	3.03**	0.51**	1.82**	0.53*	0.8*	5.07**	4.99*	0.04**
Env*female(set)	54	1197533.8*	2.78**	3.23**	0.56*	325.76**	128.82**	2.45**	0.32	2.11**	0.57*	0.7*	6.54**	6.59*	0.03*
Env*female*male(set)	162	1805933.8**	2.01**	2.29**	0.38*	218.18**	75.22**	1.32	0.36*	1.80**	0.64*	0.92**	6.78**	5.46**	0.04**
Error	288	862481	1.08	1.41	0.34	113.28	54.42	1.13	0.26	0.49**	0.47	0.66	3.22	3.66	0.02
Heritability	-	0.75	0.81	0.81	0.27	0.83	0.81	0.31	0.10	0.38	0.78	0.88	0.18	0.55	0.72

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; Env = environment; Rep = replication; YIELD = Grain yield; DA= days to 50% anthesis ; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; RL = root lodging; SL = stalk lodging; HC = husk cover; PASP = plant aspect; EASP = ear aspect; EROT = ear rot; EPP =ears per plant; STGR= stay-green characteristic.

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Table 5. 6 Mean squares and heritability of grain yield and other agronomic traits of early maturing provitamin A - quality protein maize hybrids evaluated under optimal environments at Ikenne, Ile-Ife and Mokwa during the 2016 and 2017 growing seasons

Source	DF	YIELD	DA	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP
Env	5	187014729.5**	308.08**	307.66**	8.32	101981.11**	27999.72**	32.69**	170.28**	623.79**	24.88**	201.40**	547.09**	4.04**
Set	5	5812318.2**	6.82**	5.55*	17.45	1112.28**	255.4	0.35	5.17**	7.214**	2.02**	4.17*	10.45*	0.07*
Env*set	25	1423482.5	5.00**	4.37**	20.36	595.65**	189.05*	0.72	1.65**	2.65**	0.62	1.95	8.03**	0.04*
Rep(env*set)	30	1108645.8	0.99	1.74	6.16	177.94	96.26	1.03	0.7	1.02	0.6	1.63	4.3	0.02
Block(env*rep)	108	2280168.4**	3.51**	4.99**	22.27**	646.38**	285.58**	1.12	0.45	1.85**	1.48**	4.83**	4.28	0.04**
Hybrid	99	14416317**	14.44**	19.44**	14.64	1504.57**	650.92**	1.55**	1.53**	3.13**	4.06**	8.44**	10.62**	0.09**
Male(set)	18	15538832.5**	15.33**	17.34**	7.85	2676.02**	1217.68**	1.64*	1.56**	3.36**	6.57**	11.29**	12.10**	0.14**
Female(set)	18	10998389.3**	19.17**	21.33**	14.68	1612.83**	827.57**	3.31**	1.57**	3.65**	3.18**	6.39**	8.67**	0.06**
Female*male(set)	54	17538940.0**	12.70**	20.82**	17.32	1234.09**	486.41**	1.61*	1.16**	2.79**	4.45**	9.71**	11.90**	0.10**
Hybrid*env	495	3190671**	3.56**	4.23**	12.8	503.93**	202.85**	0.97*	1.26**	2.28**	0.91**	2.60**	5.87**	0.05**
Env*male(set)	90	2533736.8**	3.37**	4.68**	9.72	470.69**	195.37**	0.78	1.03**	2.20**	0.87*	2.20*	5.22*	0.04**
Env*female(set)	90	3065651.3**	3.98**	3.11*	12.64	655.73**	254.44**	1.05	1.17**	2.75**	0.81*	2.46**	5.68**	0.04**
Env*female*male(set)	270	3685993.8**	3.35*	4.54**	14.35	473.42**	196.47**	0.98	1.27**	2.09**	1.01**	2.93**	5.69*	0.05**
Error	432	1198307	1.74	2.18	13.89	235.14	115.76	0.93	0.53	0.98	0.56	1.65	3.91	0.02
Heritability	-	0.56	0.76	0.18	0.16	0.22	0.72	0.37	0.25	0.27	0.32	0.71	-	0.52

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; Env = environment; Rep = replication; YIELD = Grain yield; DA= days to 50% anthesis ; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; RL = root lodging; SL = stalk lodging; HC = husk cover; PASP = plant aspect; EASP = ear aspect; EROT = ear rot; EPP =ears per plant.

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Across environments, significant ($P < 0.01$ or $P < 0.05$) differences were observed among E, G, and GEI mean squares for all measured traits except ASI for GEI (Table 5.7). The mean squares for research conditions were significant ($P < 0.01$) for all measured traits. On the other hand, research condition x G interaction mean squares did not show significant differences among the measured traits. All measured traits revealed significant differences for GCA-male, GCA-female, and SCA effects except the GCA-male and GCA-female mean squares for ASI. Correspondingly, GCA-male x E, GCA-female x E, and SCA x E showed significant mean squares for all measured traits apart from GCA-male x E and GCA-female x E effects for ASI as well as SCA x E mean squares for fays to 50% anthesis, ASI and root lodging. Moderate to very high broad sense heritability estimates were recorded for the traits across environments varying from 31% for ASI to 93% for ear aspect. Grain yield had a moderately high H^2 estimate of 67%.

5.3.2 Proportionate contributions of combining ability effects under drought, low-N, optimal and across environments

The relative contributions of GCA and SCA effects were computed as the ratio of GCA component to the total genetic variation based on the sum of squares. As the ratio approaches unity, the greater the predictability of a specific hybrid's performance based on GCA alone (Baker, 1978). Under drought conditions, the total contributions of GCA (GCA-male + GCA-female) sum of squares relative to the total genetic variation among hybrids ranged from 54.19% for husk cover to 78.38% for root lodging while the contribution of SCA varied from 21.62% for root lodging to 45.81% for husk cover (Fig. 5.2). The contributions of GCA effect were greater than SCA effect for grain yield and all other measured agronomic traits. GCA accounted for 67.22% of the sum of squares for grain yield. Furthermore, the F test or variance ratio revealed no significant differences among the contributions of GCA-male and GCA-female for the measured traits with the exception of

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Table 5. 7 Mean squares and heritability of grain yield and other agronomic traits of early maturing provitamin A- quality protein maize hybrids across drought, low-N and optimal environments in 2016 and 2017

Source	DF	YIELD	DA	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP	DF	STGR
Env	11	335016271**	2656.27**	2992.07**	112.43**	80140.45**	24674.59**	57.40**	92.18**	573.61**	28.44**	149.51**	450.51**	3.66**	5	167.93**
Set	5	5437386**	12.94**	15.70**	8.6	2069.88**	232.12*	4.07	6.95**	4.40**	5.66**	6.78**	6.09*	0.12**	5	1.64**
Env*set	55	1696784**	6.15**	4.99*	10.60*	475.80**	176.07**	2.27	1.60**	2.70**	0.70*	1.69*	6.71**	0.04**	25	1.02**
Rep (env*set)	60	976756	3.48*	4.35	3.59	151.88	84.26	1.47	0.86	0.83	0.49	1.14	2.75	0.03	30	0.42
Block (env*rep)	216	1815631**	4.36**	6.07**	11.96**	558.37**	248.18**	2.78**	1.01	1.60**	1.34**	3.01	5.49**	0.03*	108	0.81**
Rcond	2	858934707**	2261.94**	4304.93**	577.42**	32333.57**	30394.48**	76.60**	79.84**	227.06**	5.48**	13.78**	25.14*	0.37**	1	84.67**
Hybrid	99	28365062**	29.52**	41.41**	9.59**	3290.63**	1208.08**	5.13**	2.26**	6.09**	10.54**	19.1**	8.56**	0.26**	99	3.51**
Male (set)	18	28550719**	25.13**	32.58**	4.75	4480.92**	1814.24**	6.74**	3.36**	4.24**	10.27**	18.73**	12.30**	0.27**	18	1.80**
Female (set)	18	22408812**	31.08**	37.04**	10.11	4053.69**	1633.79**	11.22**	4.36**	6.69**	9.05**	16.52**	10.96**	0.19**	18	3.28**
Female*male (set)	54	34936198**	30.61**	47.10**	11.23*	2993.21**	1071.98**	2.93**	0.93*	7.21**	12.72**	23.33**	7.08**	0.30**	54	4.19**
Rcond*hybrid	198	1884999	8.43	9.73	5.47	328.3	144.95	1.59	1.23	2.56	0.92	1.75	5.04	0.07	99	1.22
Hybrid*env	1089	2432847**	5.4**	5.98**	7.33	375.71**	157.51**	1.99**	1.22**	2.11**	0.82**	1.82**	6.12**	0.05**	972	0.98**
Env*male (set)	198	2278991**	6.32**	6.72**	5.89	368.66**	163.97**	2.76**	1.28**	1.80**	0.64*	1.72**	5.11**	0.04**	90	1.05**
Env*female (set)	198	2356833**	5.21**	5.64**	7.5	480.41**	180.35**	2.21**	1.31**	2.43**	0.81**	1.77**	6.93**	0.05**	90	1.26**
Env*female*male (set)	594	2620762**	5.16	6.00**	8.03	350.40**	150.02**	1.74	1.17**	2.02**	0.86**	1.90**	5.93**	0.05**	270	0.83**
Error	864	1048844	2.59	3.41	7.45	181.26	93.34	1.63503	0.73	0.76	0.51	1.16	3.23	0.03	432	0.44
Heritability		0.67	0.82	0.86	0.31	0.89	0.88	0.61	0.46	0.67	0.91	0.93	0.33	0.81		0.73

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; Env = environment; Rcond= research condition (treatments); Rep = replication; RCOND = Research condition; YIELD = Grain yield; DA= days to 50% anthesis ; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; RL = root lodging; SL = stalk lodging; HC = husk cover; PASP = plant aspect; EASP = ear aspect; EROT = ear rot; EPP =ears per plant; STGR= stay-green characteristic.

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stay green characteristic which showed significantly greater GCA-female effect relative to GCA-male effect.

Under low-N, the contributions of GCA effects were greater than SCA effects for all traits. The GCA effects compared to their respective genotypic sum of squares varied from 55.5% for husk cover to 85.07% for root lodging while SCA ranged from 14.92% for root lodging to 44.5% for husk cover (Fig 5.3). The GCA effects accounted for 58.4% for grain yield. The variation among GCA-male and GCA-female effects was not significant for measured traits under low-N conditions.

The contributions of GCA effect to the overall genotypic sum of squares among hybrids were larger than SCA effect for all measured traits under optimal environments. The GCA contributed 60.20% of the sum of squares for grain yield (Fig. 5.4). The F test revealed no significant differences among the contributions of GCA-male and GCA-female for grain yield and other measured traits except root lodging which had significantly greater GCA-female effect than GCA-male effect as well as ears per plant which recorded significantly larger GCA-male effect over GCA-female effect under optimal environments.

Across environments, the contributions of GCA effect were greater than SCA effects for the measured traits. The proportion of GCA sum of squares relative to the total genetic variation among hybrids varied from 54.76% for stay green characteristic to 89.28% for stalk lodging while the contribution of SCA ranged from 10.72% for stalk lodging to 45.23% for stay green characteristic (Fig 5.5). The GCA accounted for 59.33% of the sum of squares for grain yield. The proportionate contributions of GCA-male effect and GCA-female effect did not significantly vary among the hybrids for the measured traits across environments except for ASI and stay green characteristics which recorded significantly greater GCA-female effects than GCA-male effects.

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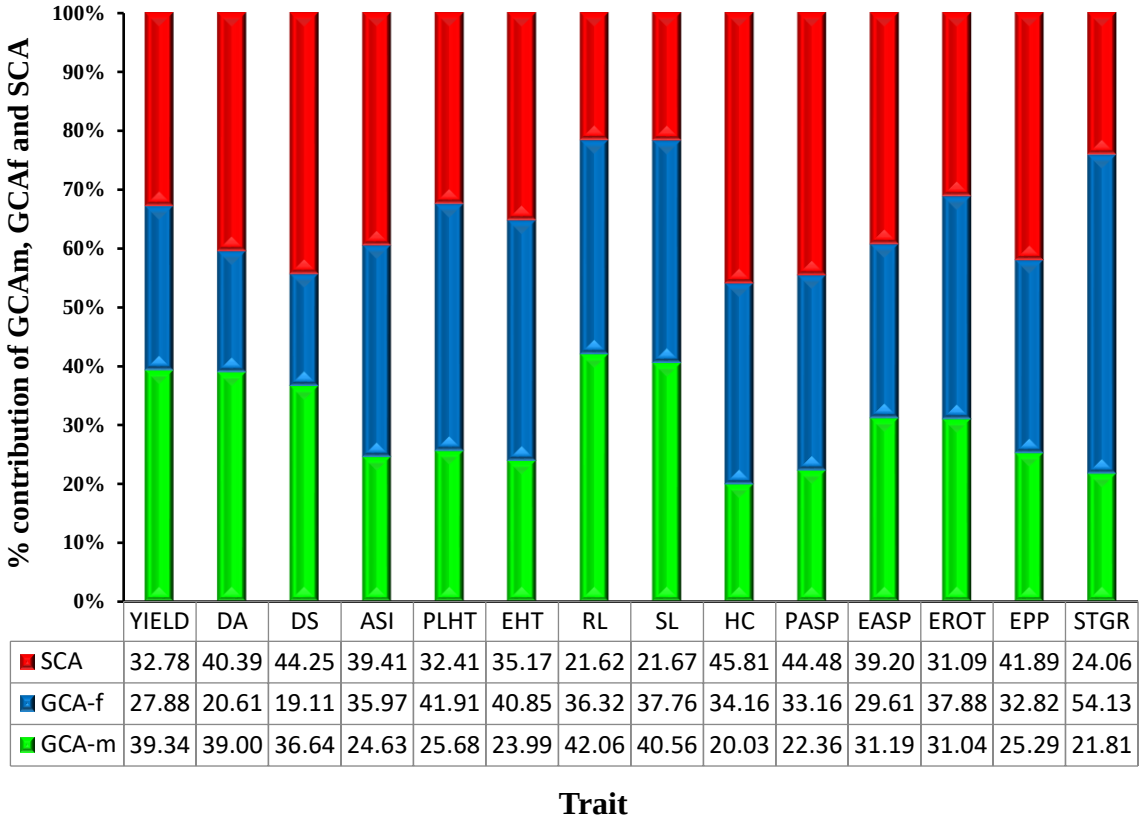

	YIELD	DA	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP	STGR
SCA	32.78	40.39	44.25	39.41	32.41	35.17	21.62	21.67	45.81	44.48	39.20	31.09	41.89	24.06
GCA-f	27.88	20.61	19.11	35.97	41.91	40.85	36.32	37.76	34.16	33.16	29.61	37.88	32.82	54.13
GCA-m	39.34	39.00	36.64	24.63	25.68	23.99	42.06	40.56	20.03	22.36	31.19	31.04	25.29	21.81

Figure 5. 2 Proportion of total genotypic sum of squares of grain yield and other agronomic traits of early maturing provitamin A-quality protein maize inbred lines attributable to general combining ability (GCA-male and GCA-female) and specific combining ability (SCA) effects under drought environments. YIELD = Grain yield; DA= days to 50% anthesis; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; RL = root lodging; SL = stalk lodging; HC = husk cover; PASP = plant aspect; EASP = ear aspect; EROT = ear rot; EPP =ears per plant; STGR= stay-green characteristic.

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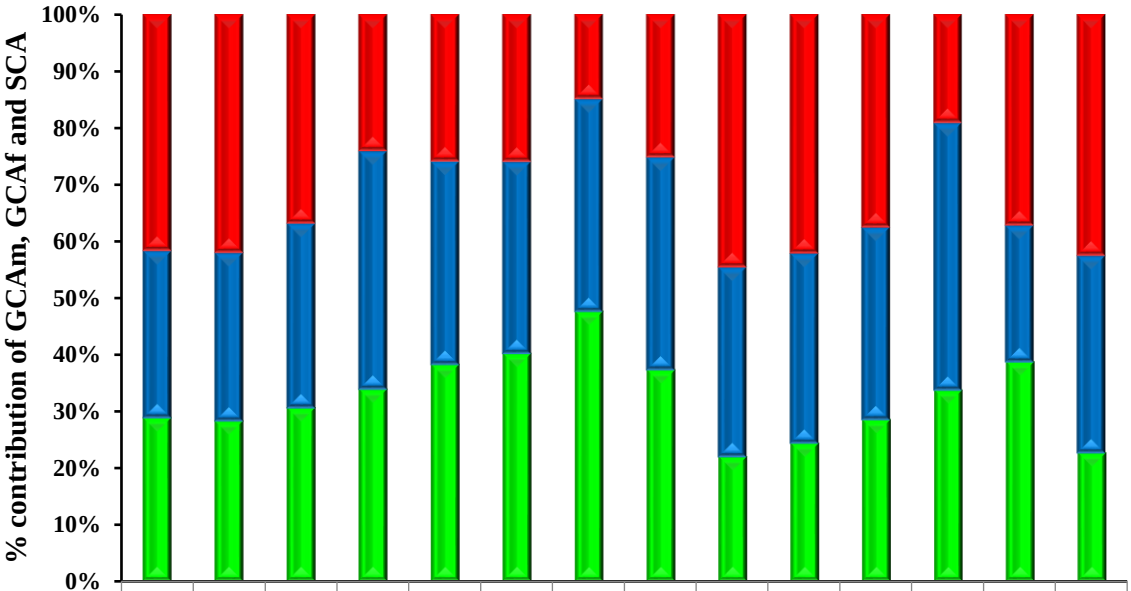

	YIELD	DA	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP	STGR
SCA	41.61	41.92	36.77	24.10	25.89	25.95	14.92	25.16	44.50	42.06	37.50	19.09	37.16	42.43
GCA-f	29.53	29.77	32.61	42.03	35.89	33.92	37.54	37.58	33.50	33.56	33.97	47.21	24.16	34.88
GCA-m	28.87	28.31	30.62	33.87	38.21	40.14	47.53	37.26	22.00	24.37	28.53	33.70	38.68	22.70

Trait

Figure 5. 3 Proportion of total genotypic sum of squares of grain yield and other agronomic traits of early maturing provitamin A-quality protein maize inbred lines attributable to general combining ability (GCA-male and GCA-female) and specific combining ability (SCA) effects under low-N environments. YIELD = Grain yield; DA= days to 50% anthesis; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; RL = root lodging; SL = stalk lodging; HC = husk cover; PASP = plant aspect; EASP = ear aspect; EROT = ear rot; EPP =ears per plant; STGR= stay-green characteristic.

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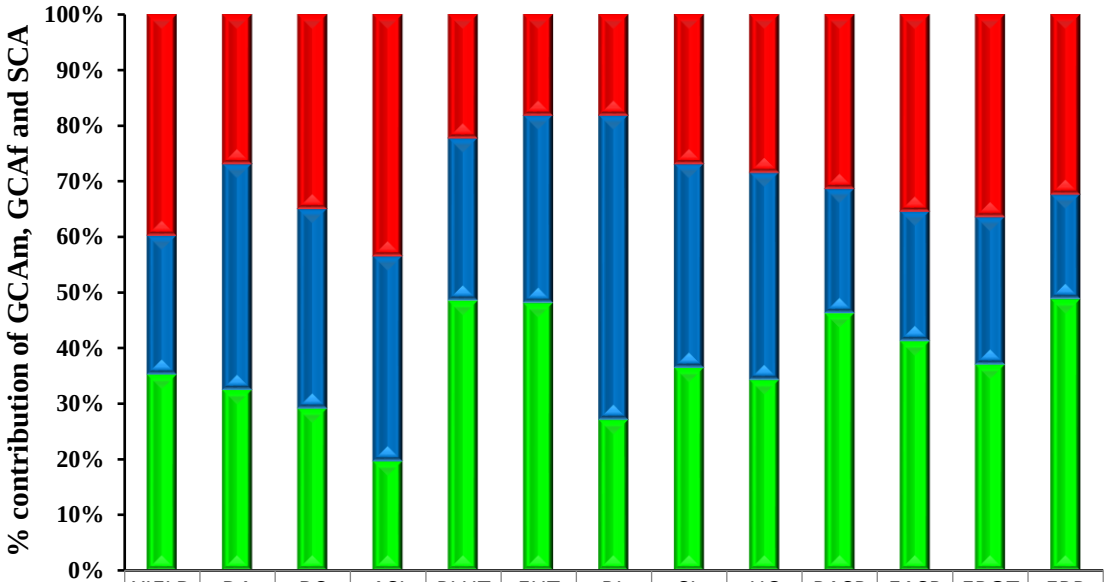

	YIELD	DA	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP
SCA	39.79	26.91	35.00	43.46	22.34	18.21	18.24	26.96	28.47	31.37	35.46	36.42	32.39
GCA-f	24.95	40.61	35.86	36.84	29.20	33.69	54.63	36.61	37.25	22.39	23.33	26.53	18.85
GCA-m	35.25	32.48	29.14	19.71	48.45	48.10	27.13	36.43	34.27	46.24	41.21	37.05	48.77

Trait

Figure 5. 4 Proportion of total genotypic sums-of squares of grain yield and other agronomic traits of earl maturing provitamin A-quality protein maize inbred lines attributable to general combining ability (GCA-male and GCA-female) and specific combining ability (SCA) effects under optimal environments. YIELD = Grain yield; DA= days to 50% anthesis; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; RL = root lodging; SL = stalk lodging; HC = husk cover; PASP = plant aspect; EASP = ear aspect; EROT = ear rot; EPP =ears per plant.

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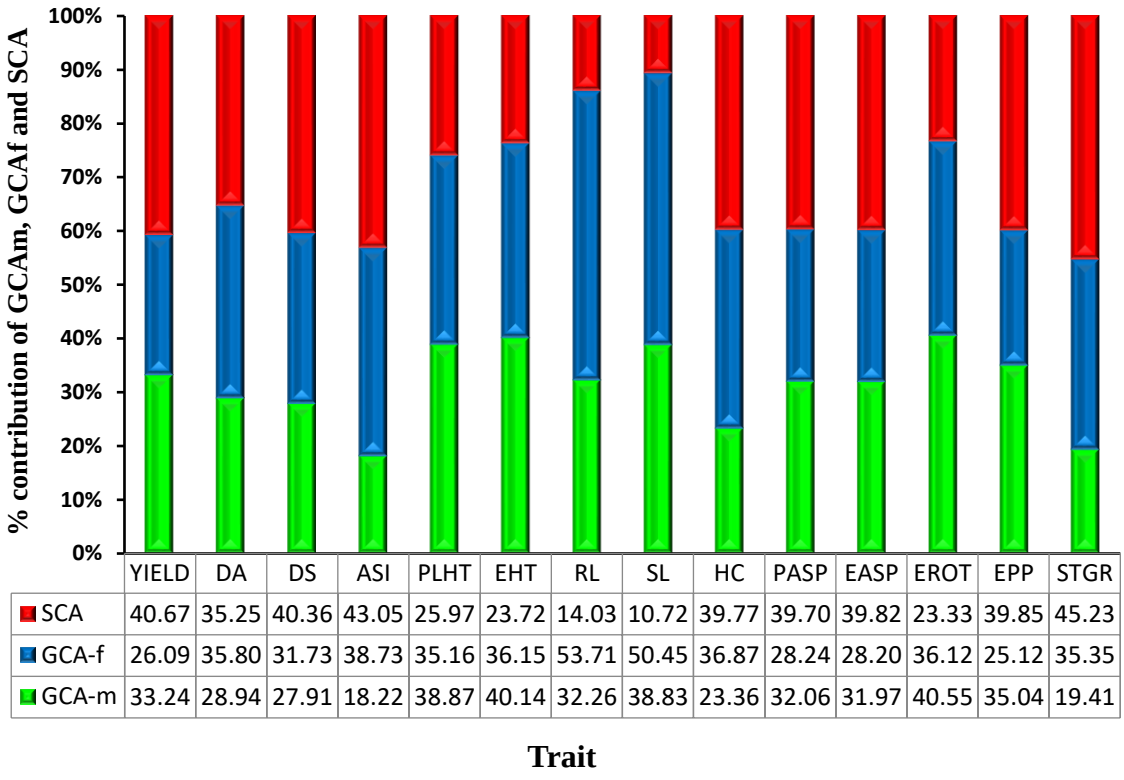

	YIELD	DA	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP	STGR
SCA	40.67	35.25	40.36	43.05	25.97	23.72	14.03	10.72	39.77	39.70	39.82	23.33	39.85	45.23
GCA-f	26.09	35.80	31.73	38.73	35.16	36.15	53.71	50.45	36.87	28.24	28.20	36.12	25.12	35.35
GCA-m	33.24	28.94	27.91	18.22	38.87	40.14	32.26	38.83	23.36	32.06	31.97	40.55	35.04	19.41

Figure 5. 5 Proportion of total genotypic sum of-squares of grain yield and other agronomic traits of early maturing provitamin A-quality protein maize inbred lines attributable to general combining ability (GCA-male and GCA-female) and specific combining ability (SCA) effects across drought, low-N and optimal environments.

YIELD = Grain yield; DA= days to 50% anthesis; DS = days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height; EHT = ear height; RL = root lodging; SL = stalk lodging; HC = husk cover; PASP = plant aspect; EASP = ear aspect; EROT = ear rot; EPP =ears per plant; STGR= stay-green characteristic.

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5.3.3 General combining ability effects

Under drought conditions, the GCA-male effects for grain yield ranged from -1733 for TZEIORQ 7 to 1574 for TZEIORQ 24 whereas GCA-female effects ranged from -1054 for TZEIORQ 45 to 1508 for TZEIORQ 29 (Table 5.8). Only TZEIORQ 29 had significant positive GCA-male and GCA-female effects for grain yield. TZEIORQ 13 and TZEIORQ 24 recorded significant positive GCA-male effects for grain yield, while TZEIORQ 6 had significant positive GCA-female effect. In addition, TZEIORQ 13 had significant negative GCA-male effect for ASI under drought.

Under low-N environments, GCA-male effects for grain yield varied from -1479 for TZEIORQ 7 to 840 for TZEIORQ 2 while GCA-female effects varied from -1148 for TZEIORQ 45 to 1041 for TZEIORQ 29 (Table 5.9). The inbreds TZEIORQ 11, TZEIORQ 59, TZEQI 82 and TZEIORQ 2. TZEIORQ 13 and TZEIORQ 24 had significant positive GCA-male effects for grain yield, while TZEIORQ 29, TZEIORQ 48 and TZEIORQ 43 recorded significant positive GCA-female effects for grain yield. It is striking that 5 out of the 9 inbreds which displayed significant positive GCA effects for grain yield under low-N were also tolerant to low-N environments. The inbred lines TZEIORQ 29 and TZEQI 82 had significant negative GCA-male and GCA-female effects, TZEIORQ 44 and TZEIORQ 24 had significant negative GCA-male effects, while TZEIORQ 70 recorded significant negative GCA-female effects.

Out of the 24 parental lines, only TZEIORQ 59 and TZEQI 82 had significant positive GCA-male and GCA-female effects for grain yield under optimal environments (Table 5.10). Other inbreds characterized by significant positive GCA effects for grain yield were TZEIORQ 11, TZEIORQ 13 and TZEIORQ 2 (GCA-male effects), and also TZEIORQ 29 (GCA-female effects). Furthermore, highly significant negative GCA-male and GCA-female effects for ear aspect were observed for TZEIORQ 29 and TZEIORQ 59.

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Table 5. 8 General combining ability effects of grain yield and other agronomic traits of early provitamin A quality protein maize inbred lines evaluated under drought environments in Nigeria, 2016-2017

	YIELD		ASI		PLHT		PASP		EASP		EPP		STGR	
INBRED	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**
TZEIORQ 69	-501.37	-891.01*	0.21	0.09	-5.36	-11.07*	0.31	0.47*	0.02	0.20	-0.12*	-0.1	-0.06	0.23
TZEIORQ 29	867.15*	1507.61**	-0.44	-0.74	1.36	11.33*	-0.46*	-0.56*	-0.87**	0.61*	0.12*	0.19*	-0.25	-0.47
TZEIORQ 45	647.39	-1053.54**	0.30	1.37*	-3.31	-19.52**	0.15	0.99**	0.17	-1.26**	-0.04	-0.20*	0.16	0.61*
TZEIORQ 48	-1013.17*	436.93	-0.07	-0.72	7.31*	19.25**	0.01	-0.90**	0.68*	1.20**	-0.12*	0.12	0.14	-0.37
TZEIORQ 11	268.04	58.63	0.23	-0.03	-5.44	-1.22	0.09	-0.13	0.07	-0.10	-0.04	0.06	0.24	0.22
TZEIORQ 20	77.00	67.82	0.00	-0.13	3.58	4.75	-0.22	-0.26	-0.25	-0.23	-0.02	0.02	-0.03	-0.09
TZEIORQ 6	-79.33	864.86*	-0.09	-0.38	4.38	6.18	-0.01	-0.22	0.01	-0.90**	0.02	0.12	-0.08	-0.38
TZEIORQ 44	-265.71	-991.31*	-0.14	0.54	-2.52	-9.72*	0.13	0.61*	0.18	1.23**	0.03	-0.2	-0.13	0.26
TZEIORQ 42	353.16	259.82	0.60	0.04	-7.75*	-4.31	-0.03	-0.38	-0.15	-0.33	0.03	0.09	0.20	-0.04
TZEIORQ 59	269.44	322.99	-0.50	0.09	14.69**	14.37**	-0.16	-0.18	-0.44	-0.35	0.09	0.11	-0.32	-0.47
TZEIORQ 15	-633.99	1.73	0.18	-0.41	-6.43*	-2.70	0.25	0.13	0.55*	0.11	-0.13*	-0.08	0.49	0.53*
TZEIORQ 23	11.39	-584.54	-0.28	0.29	-0.51	-7.36	-0.06	0.42	0.04	0.57*	0.02	-0.11	-0.38	-0.02
TZEQI 82	220.88	27.17	0.30	0.35	3.80	-3.34	-0.33	-0.1	-0.21	0.03	-0.06	0.02	-0.17	-0.02
TZEIORQ 47	429.83	-472.33	0.21	0.78	-2.60	-3.38	0.17	0.23	-0.31	0.30	0.01	-0.03	0.14	-0.07
TZEIORQ 7	-1732.88**	296.72	0.69	-0.81	-3.87	8.72*	0.44*	-0.26	1.26	-0.36	-0.15*	0.05	-0.23	-0.33
TZEIORQ 13	1082.17*	148.43	-1.20*	-0.31	2.66	-2.00	-0.29	0.14	-0.73*	0.03	0.20**	-0.01	0.27	0.42
TZEIORQ 2	383.28	47.61	-0.20	-0.81	10.41**	10.71*	-0.1	-0.3	-0.36	-0.07	0.16**	0.02	0.10	-0.1
TZEIORQ 5	447.71	46.87	-0.24	-0.04	5.42	2.89	-0.56**	-0.01	-0.39	0.01	0.02	-0.04	-0.21	-0.35
TZEIORQ 26	266.79	572.11	-0.26	0.08	2.22	-1.54	-0.09	-0.05	-0.37	-0.48*	0.01	0.01	-0.19	-0.39
TZEIORQ 41	-1097.78*	-666.59*	0.70	0.77	-18.05**	-12.06*	0.76**	0.36	1.12	0.55*	-0.18**	0.04	0.30	0.84**
TZEIORQ 24	1573.97**	-1045.41**	-0.41	0.13	11.92**	-8.86*	-0.63**	0.42	-1.31	0.64*	0.13*	-0.18*	-0.40	-0.34
TZEIORQ 43	-536.79	281.45	0.17	0.47	-1.56	-1.42	0.44*	0.04	0.39	-0.05	-0.01	0.08	-0.06	0.59*
TZEIORQ 40	-511.83	455.87	-0.13	-0.24	-6.73*	1.52	0.12	-0.23	0.34	-0.30	-0.07	0.06	0.19	-0.1
TZEIORQ 70	-525.35	308.09	0.37	-0.36	-3.64	8.76*	0.07	-0.24	0.58*	-0.30	-0.05	0.05	0.27	-0.15
SED	**380.03**	**331.43**	**0.51**	**0.47**	**2.83**	**4.03**	**0.18**	**0.21**	**0.27**	**0.23**	**0.05**	**0.07**	**0.27**	**0.25**

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; YIELD = Grain yield; DA= days to 50% anthesis; ASI = anthesis-silking interval; PLHT = plant height; PASP = plant aspect; EASP = ear aspect; EPP =ears per plant; STGR= stay-green characteristic; GCA-m = GCA effects of inbred as a male parent; GCA-f = GCA effects of inbred as a female parent.

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Table 5. 9 General combining ability effects of grain yield and other agronomic traits of early maturing provitamin A quality protein maize inbred lines evaluated under low-N environments in Nigeria, 2016-2017

	YIELD		ASI		PLHT		PASP		EASP		EPP		STGR	
INBRED	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**
TZEIORQ 69	24.99	-326.33	0.15	0.20	-1.77	-9.45**	-0.02	0.37**	0.08	0.20	-0.147**	0.012	0.26	-0.07
TZEIORQ 29	279.96	1041.31**	-0.17*	-0.05	-1.50	9.78**	-0.17	-0.70**	-0.46**	-0.89**	0.037	0.078*	-0.32*	-0.59**
TZEIORQ 45	-129.12	-1148.29**	0.09	0.02	-6.75*	-11.27**	0.26*	0.82**	-0.08	0.85**	-0.002	-0.149**	-0.05	0.72**
TZEIORQ 48	-175.83	433.31*	-0.07	-0.18	10.03**	10.94**	-0.06	-0.50**	0.46**	-0.16	-0.035	0.059*	0.11	-0.07
TZEIORQ 11	410.55*	559.12**	0.00	-0.16	2.44	8.15*	-0.21	-0.40**	-0.20	-0.25	0.005	0.051	0.03	-0.19
TZEIORQ 20	-25.92	34.82	-0.01	0.04	-3.66	0.12	-0.03	-0.02	-0.12	-0.14	-0.020	-0.023	0.11	0.16
TZEIORQ 6	-396.60*	-46.68	-0.05	0.09	2.90	1.01	0.18	-0.28*	0.20	-0.16	-0.007	0.013	0.20	-0.12
TZEIORQ 44	11.96	-547.25**	0.06	0.03	-1.68	-9.28**	0.06	0.69**	0.12	0.54**	0.022	-0.041	-0.33*	0.15
TZEIORQ 42	-98.24	147.60	-0.08	-0.03	-9.98**	-5.47	0.07	-0.05	0.16	-0.17	0.001	0.037	0.13	-0.11
TZEIORQ 59	339.73*	462.80*	0.03	0.00	9.21**	11.84**	-0.14	-0.44**	-0.32*	-0.53**	0.048	0.025	-0.16	-0.27
TZEIORQ 15	-138.51	-309.87	-0.01	0.07	2.30	1.54	0.04	-0.05	0.06	0.08	0.005	-0.013	-0.02	-0.15
TZEIORQ 23	-102.97	-300.53	0.06	-0.04	-1.53	-7.91*	0.02	0.54**	0.10	0.62**	-0.054	-0.049	0.05	0.52**
TZEQI 82	805.96**	488.28*	0.31**	0.28	2.31	6.09*	-0.36**	-0.40**	-0.72**	-0.75**	-0.005	0.005	-0.34*	-0.43*
TZEIORQ 47	38.47	-108.07	-0.02	-0.16	-8.84**	-3.24	0.30*	-0.04	0.10	0.32*	-0.015	-0.022	-0.14	0.02
TZEIORQ 7	-1478.53**	-179.97	-0.01	-0.02	-4.59	-3.01	0.50**	0.36*	0.97**	0.37*	-0.061	0.004	0.70**	0.35*
TZEIORQ 13	634.09**	-200.24	-0.28**	-0.10	11.11**	0.14	-0.44**	0.08	-0.35*	0.06	0.081*	0.014	-0.22	0.06
TZEIORQ 2	840.24**	583.59**	0.01	0.28	14.24**	11.63**	-0.49**	-0.26*	-0.52**	-0.25	0.060	0.013	-0.20	0.02
TZEIORQ 5	95.76	-235.08	-0.08	-0.08	-3.10	-3.87	-0.02	0.11	-0.18	-0.10	0.100**	0.005	-0.07	-0.09
TZEIORQ 26	125.14	-20.43	-0.17*	-0.17	1.72	-0.90	-0.26*	-0.13	-0.21	-0.09	-0.008	-0.009	0.02	0.09
TZEIORQ 41	-1061.14**	-328.08	0.24*	-0.04	-12.85**	-6.86*	0.77**	0.28*	0.92**	0.44**	-0.152**	-0.010	0.25	-0.01
TZEIORQ 24	580.46**	-880.57**	0.18*	0.11	9.35**	-0.69	-0.58**	0.24	-0.65**	0.72**	0.091*	0.012	-0.31*	0.49**
TZEIORQ 43	35.43	384.92*	-0.12	-0.32*	-6.72*	-2.33	0.39**	0.08	0.08	-0.38*	-0.057	-0.002	0.37*	-0.15
TZEIORQ 40	-402.88*	324.24	-0.10	0.13	-5.12	-3.84	0.20	-0.21	0.30*	-0.16	0.030	-0.025	-0.12	0.08
TZEIORQ 70	-213.01	171.41	0.04	0.09	2.48	6.87*	-0.01	-0.11	0.27	-0.18	-0.064*	0.015	0.07	-0.42*
SED	169.27	167.53	0.08	0.14	2.66	2.76	0.11	0.12	0.14	0.13	0.031	0.028	0.14	0.15

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; YIELD = Grain yield; DA= days to 50% anthesis; ASI = anthesis-silking interval; PLHT = plant height; PASP = plant aspect; EASP = ear aspect; EPP =ears per plant; STGR= stay-green characteristic; GCA-m = GCA effects of inbred as a male parent; GCA-f = GCA effects of inbred as a female parent.

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Table 5. 10 General combining ability effects of grain yield and other agronomic traits of early provitamin A quality protein maize inbred lines evaluated under optimal environments in Nigeria, 2016-2017

	YIELD		ASI		PLHT		PASP		EASP		EPP	
INBRED	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**
TZEIORQ 69	68.31	49.90	0.53	-0.02	-6.75*	-2.58	0.18	-0.01	0.31**	0.36	-0.140**	-0.001
TZEIORQ 29	198.64	647.86*	-0.22	-0.15	-1.32	1.64	-0.18	-0.18	-0.56**	-0.75**	0.025	0.024
TZEIORQ 45	-207.94	-1091.09**	0.23	0.77	-3.87	-10.33**	0.03	-0.05	0.31**	0.54*	0.033	-0.028
TZEIORQ 48	-59.01	393.30	-0.54	-0.59	11.94**	11.27**	-0.03	0.23*	-0.07	-0.16	-0.015	0.005
TZEIORQ 11	453.05*	269.85	-0.16	-0.02	3.54	2.57	-0.46*	-0.20	-0.33**	-0.33	0.001	0.026
TZEIORQ 20	-271.23	371.81	-0.63	-0.40	-0.38	4.20	0.01	0.03	-0.15	-0.24	-0.012	0.025
TZEIORQ 6	-204.71	-11.84	0.49	0.42	0.22	0.47	-0.03	0.22	-0.03	-0.02	-0.028	0.004
TZEIORQ 44	22.89	-629.82*	0.30	0.00	-3.39	-7.24*	0.49*	-0.05	0.51**	0.59	0.040	-0.055*
TZEIORQ 42	-144.19	-388.29	-0.45	-0.72	-4.44	-8.42*	0.03	0.11	0.32**	0.58	-0.007	-0.054*
TZEIOR 59	684.30**	1012.28**	0.30	0.35	6.81*	10.58**	-0.36	-0.30*	-0.35**	-0.74**	0.083**	0.062*
TZEIORQ 15	-311.66	35.55	0.62	-0.25	-3.00	-0.38	0.23	0.33*	-0.13	-0.02	-0.023	0.048
TZEIORQ 23	-228.45	-659.54**	-0.47	0.62	0.63*	-1.78	0.10	-0.14	0.16	0.18	-0.054*	-0.055*
TZEQI 82	825.06**	481.70*	-0.18	-0.31	1.32	0.51	-0.78**	-0.08	-0.23**	-0.39	0.008	-0.027
TZEIORQ 47	127.63	-149.33	0.12	0.51	-4.75	-4.22	-0.04	-0.12	0.21*	0.20	0.046	0.017
TZEIORQ 7	-1514.06**	-132.97	-0.19	0.23	-7.89*	4.91	1.17	0.26*	-0.05	0.05	-0.132**	0.019
TZEIORQ 13	561.38*	-199.40	0.25	-0.43	11.33**	-1.21	-0.35	-0.05	0.07	0.14	0.078*	-0.010
TZEIORQ 2	1060.60**	379.40	0.53	-0.25	15.81**	8.82*	-0.76**	-0.45**	-0.24*	-0.08	0.079**	-0.008
TZEIORQ 5	-245.94	-110.37	-0.82*	-0.75	-5.01	0.90	0.31	-0.09	0.00	-0.01	-0.028	-0.021
TZEIORQ 26	172.13	-0.06	0.12	0.93*	1.61	-2.15	-0.38	-0.17	0.14	0.07	0.036	-0.009
TZEIORQ 41	-986.79**	-268.97	0.17	0.07	-12.40**	-7.56*	0.82**	0.72**	0.09	0.01	-0.087**	0.038
TZEIORQ 24	302.61	-462.61*	0.02	0.07	0.45	2.82	-0.26	-0.07	0.12	0.32	-0.005	-0.005
TZEIORQ 43	-567.07*	250.79	0.28	0.34	-4.90	-1.67	0.61*	0.23*	-0.05	-0.17	-0.054*	0.046
TZEIORQ 40	-123.09	-95.38	-0.13	-1.14*	-6.41*	-3.12	0.05	0.19	0.01	-0.07	0.026	-0.009
TZEIORQ 70	387.56	307.21	-0.17	0.73	10.86**	1.98	-0.39*	-0.35**	-0.08	-0.08	0.033	-0.032
SED	198.97	218.86	0.39	0.44	2.71	3.20	0.19	0.11	0.10	0.20	0.026	0.025

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; YIELD = Grain yield; DA= days to 50% anthesis; ASI = anthesis-silking interval; PLHT = plant height; PASP = plant aspect; EASP = ear aspect; EPP =ears per plant; GCA-m = GCA effects of inbred as a male parent; GCA-f = GCA effects of inbred as a female parent.

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Across the 12 research environments, grain yield recorded GCA-male effects varying from -1541 for TZEIORQ 7 to 927 for TZEIORQ 2, and GCA-female effects from -1104 for TZEIORQ 45 to 920 for TZEIORQ 29 (Table 5.11). Five out of the 24 parental lines (TZEIORQ 29, TZEIORQ 11, TZEIORQ 59, TZEQI 82 and TZEIORQ 2) were good combiners both as males and females as evident in the significant positive GCA-male and GCA-female effects for grain yield. Furthermore, inbred lines TZEIORQ 13 and TZEIORQ 24 had highly significant positive GCA-male effects for grain yield and were good combiners as male parents whereas TZEIORQ 48, TZEIORQ 43 and TZEIORQ 70 recorded high significant positive GCA-female effects for grain yield and were good combiners as female parents. Moreover, TZEIORQ 29, TZEIORQ 59 and TZEQI 82 displayed superior significant negative GCA-male and GCA-female effects for stay green characteristic but not for ASI across drought, low-N and optimal environments.

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Table 5. 11 General combining ability effects of grain yield and other agronomic traits of early provitamin A quality protein maize inbred lines evaluated across drought, low-N and optimal environments in Nigeria, 2016-2017

	YIELD		ASI		PLHT		PASP		EASP		EPP		STGR	
INBRED	GCA-m	GCA-f	GCA-m	GCA-f	GCA-m	GCA-f	GCA-m	GCA-f	GCA-m	GCA-f	GCA-m	GCA-f	GCA-m	GCA-f
TZEIORQ 69	-42.21	231.00	0.33	0.14	-4.92*	-6.28**	0.09	0.36**	0.12	0.35*	-0.14	-0.01	0.16	0.03
TZEIORQ 9	337.50*	920.91**	-0.24	-0.23	-0.84**	5.92**	-0.28**	-0.60**	-0.39**	-0.88**	0.04	0.06	-0.29*	-0.55**
TZEIORQ 45	-38.64	-1103.86**	0.23	0.57*	-4.73*	-12.13**	0.19*	0.6**	0.02	0.75**	0.01	-0.10	0.02	0.69**
TZEIORQ 48	-256.65	413.95**	-0.32	-0.48	10.50**	12.48**	0.00	-0.35**	0.25*	-0.23	-0.04	0.04	0.12	-0.17
TZEIORQ 11	406.63**	331.39*	0.06	-0.07	2.04	3.89*	-0.26**	-0.32**	-0.31*	-0.26*	0.00	0.04	0.10	-0.05
TZEIORQ 20	-130.08	209.29	-0.30	-0.25	-0.36*	2.98	0.01	-0.13	-0.11	-0.21	0.00	0.01	0.06	0.08
TZEIORQ 6	-247.74	121.68	0.21	0.19	1.41	1.49	0.05	-0.14	0.08	-0.21	-0.02	0.03	0.11	-0.2*
TZEIORQ 44	-28.81	-662.37**	0.04	0.12	-3.09	-8.36**	0.20*	0.59**	0.34*	0.68**	0.02	-0.08	-0.27*	0.18
TZEIORQ 42	-45.33	-103.05	-0.14	-0.30	-6.82*	-7.24**	0.08	0.10	0.04	0.21	0.00	-0.01	0.16	-0.08
TZEIORQ 59	501.10**	714.28**	0.11	0.19	8.95**	12.07**	-0.21**	-0.38**	-0.36**	-0.64**	0.07	0.06	-0.21*	-0.33**
TZEIORQ 15	-308.32*	-83.89	0.29	-0.06	-1.83	0.37	0.12	-0.08	0.23	0.01	-0.03	0.01	0.15	0.08
TZEIORQ 23	-147.45	-527.34**	-0.26	0.25	-0.30	-5.20*	0.01	0.36**	0.09	0.42**	-0.04	-0.07	-0.09	0.34**
TZEQI 82	718.97**	410.09**	0.06	0.04	2.13	1.78	-0.40**	-0.27**	-0.68**	-0.44**	0.00	-0.01	-0.28*	-0.3**
TZEIORQ 47	149.09	-188.76	0.08	0.31	-5.70**	-3.70	0.13	0.13	-0.05	0.26*	0.02	0.00	-0.05	-0.01
TZEIORQ 7	-1541.28**	-79.12	-0.04	-0.01	-6.38**	2.83	0.71**	0.05	1.15**	0.09	-0.12	0.02	0.39**	0.12
TZEIORQ 13	673.23**	-142.21	-0.10	-0.34	9.96**	-0.90	-0.44**	0.09	-0.42**	0.10	0.10	0.00	-0.06	0.18
TZEIORQ 2	927.14**	395.88*	0.26	-0.15	13.58**	10.26**	-0.48**	-0.26**	-0.56**	-0.14	0.08	0.00	-0.02	-0.01
TZEIORQ 5	-35.51	-124.92	-0.45*	-0.44	-2.26	-0.78	-0.13	0.06	0.04	-0.01	0.01	-0.02	-0.18	-0.18
TZEIORQ 26	154.62	83.32	-0.05	0.43	1.73	-1.46	-0.22**	0.01	-0.32*	-0.09	0.02	0.00	-0.07	-0.07
TZEIORQ 41	-1046.25**	-354.28*	0.24	0.16	- 13.5**	-8.02**	0.83**	0.19*	0.84**	0.23	-0.11	0.02	0.27*	0.27*
TZEIORQ 24	607.16**	-717.99**	-0.05	0.11	5.30**	-0.38	-0.47**	0.26**	-0.57**	0.57**	0.05	-0.05	-0.34**	0.21*
TZEIORQ 43	-361.16*	282.97*	0.03	0.12	-4.98*	-1.48	0.42	0.01	0.40**	-0.27*	-0.05	0.05	0.23*	0.10
TZEIORQ 40	-281.11*	118.59	-0.09	-0.59	-6.03**	-2.17	0.17*	-0.17*	0.18	-0.20	0.01	0.01	-0.02	0.02
TZEIORQ 70	35.11	316.42*	0.11	0.36	5.70**	4.03*	-0.12	-0.10	-0.01	-0.10	-0.01	-0.01	0 13	-0.33**
SED	133.43	135.69	0.22	0.24	1.70	1.94	0.07	0.08	0.12	0.12	0.20	0.23	0.10	0.10

*, ** = Significant at 0.05 and 0.01 probability levels, respectively; YIELD = Grain yield; DA= days to 50% anthesis; ASI = anthesis-silking interval; PLHT = plant height; PASP = plant aspect; EASP = ear aspect; EPP =ears per plant; STGR= stay-green characteristic; GCA-m = GCA effects of inbred as a male parent; GCA-f = GCA effects of inbred as a female parent.

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5.3.4 Heterotic grouping of early maturing provitamin A- quality protein maize inbreds under drought, low-N, optimal and across environments and comparison of efficiencies of the grouping methods

5.3.4.1 Heterotic grouping based on GCA of multiple traits (HGCAMT) method

Under drought, the dendrogram constructed using the HGCAMT method displayed 5 groups when 50% (r^2 = 0.5) of the variation among inbreds was explained (Fig. 5.6). Assessing the performance of the inbreds within the heterotic groups under drought environments, it was found that ten out of the 24 parents exhibited tolerance to drought. Out of the ten drought tolerant inbreds, six (TZEIORQ 20, TZEIORQ 26, TZEIORQ 47, TZEIORQ 5, TZEIORQ 23 and TZEIORQ 24) were placed in group V. Groups III and IV had two each of the remaining four drought tolerant parents (TZEIORQ 40 and TZEIORQ 7, and TZEIORQ 48 and TZEIORQ 6 respectively).

The 24 parental lines were classified into four heterotic groups under low-N using the HGCAMT method with 50% co-efficient of determination (r^2) (Fig. 5.7). Thirteen low-N tolerant parental lines were identified and these were generally spread across the 4 heterotic groups under low-N environments. Under optimal environments, the HGCAMT method placed the 24 parents into 5 heterotic groups (r^2 = 50%) (Fig. 5.8).

Across research environments, the dendrogram contrasted using the HGCAMT method placed the inbred lines into 4 heterotic groups (Fig. 5.9). It is striking that the HGCAMT method consistently placed inbred lines TZEIORQ 69 into heterotic group I under drought, low-N, optimal and across environments. Furthermore, TZEIORQ 29 and TZEIORQ 59 as well as TZEIORQ 44 and TZEIORQ 45 were placed into the same heterotic group under each and across the test environments. In addition, TZEIORQ 24 and TZEIORQ 26 were classified into the same group under each and across environments except for drought conditions.

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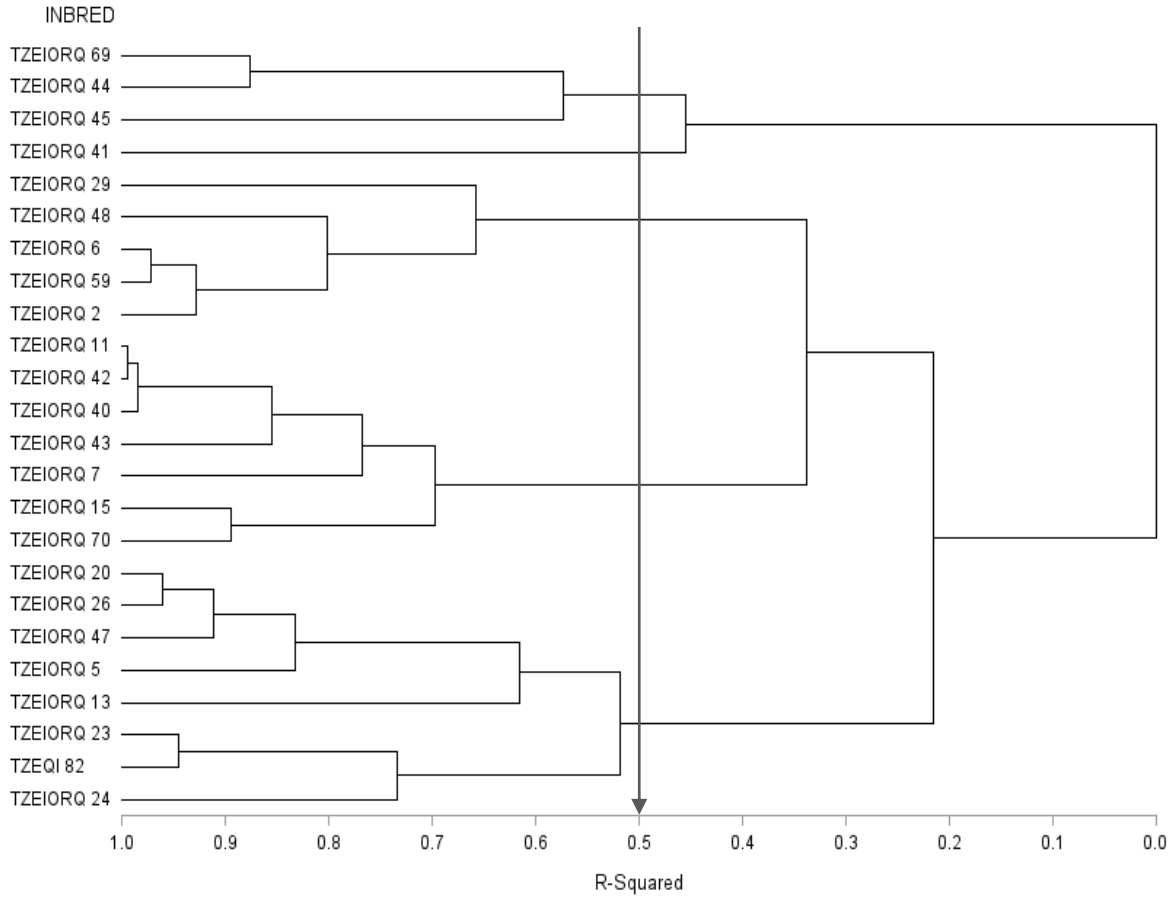

Figure 5. 6 Dendrogram of 24 early maturing provitamin A-quality protein maize parental lines constructed from GCA effects of grain yield and other traits (HGCAMT) using Ward's minimum variance cluster analysis method under drought environments in Nigeria, 2016-2017.

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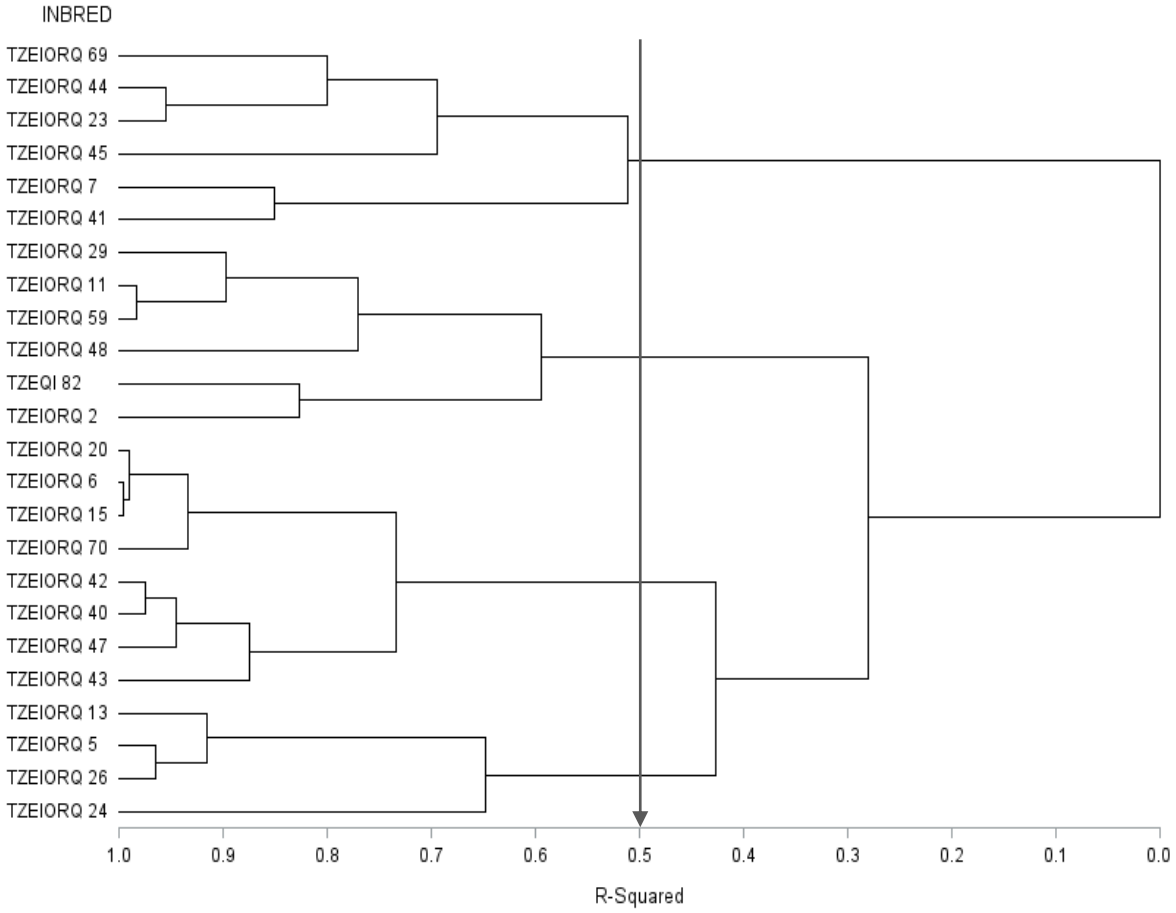

Figure 5. 7 Dendrogram of 24 early maturing provitamin A-quality protein maize parental lines constructed from GCA effects of grain yield and other traits (HGCAMT) using Ward's minimum variance cluster analysis method under low-N environments in Nigeria, 2016-2017.

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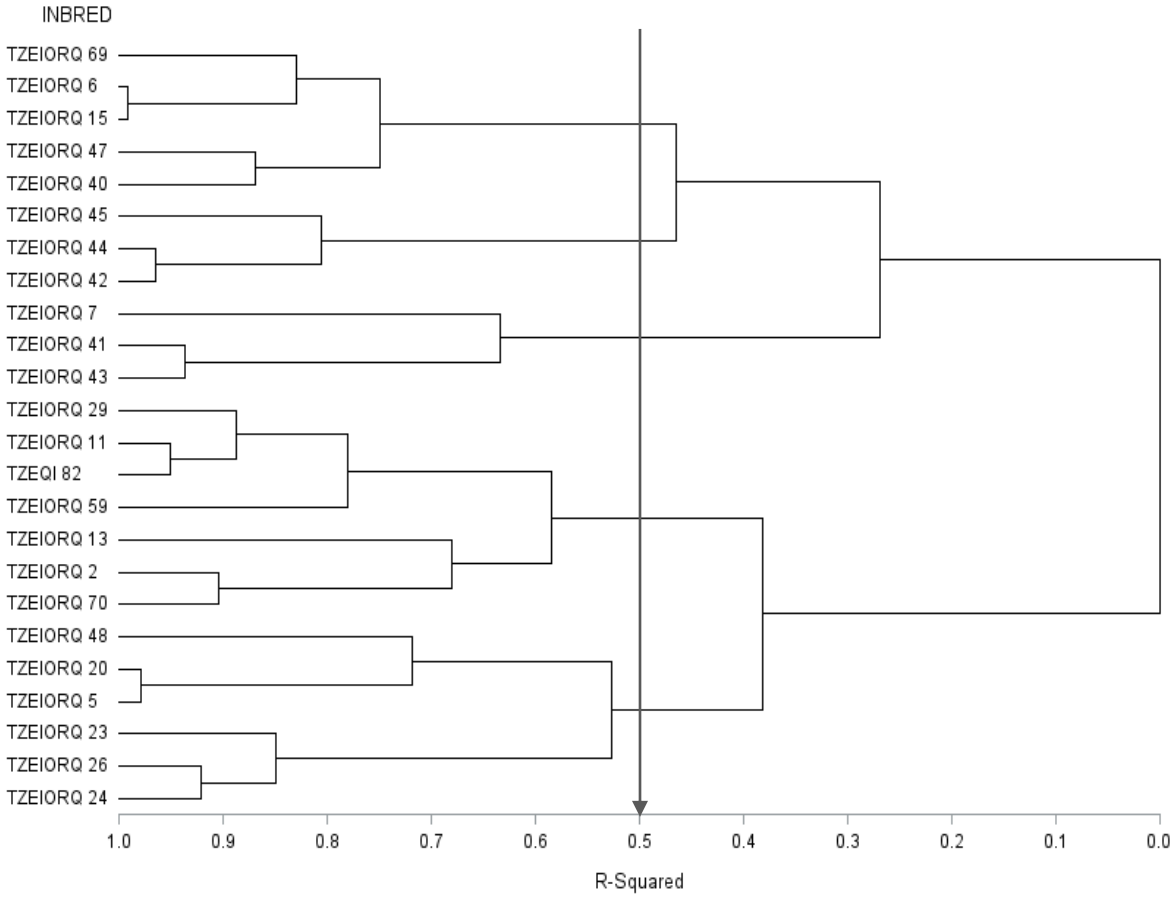

Figure 5. 8 Dendrogram of 24 early maturing provitamin A-quality protein maize parental lines constructed from GCA effects of grain yield and other traits (HGCAMT) using Ward's minimum variance cluster analysis method under optimal environments in Nigeria, 2016-2017.

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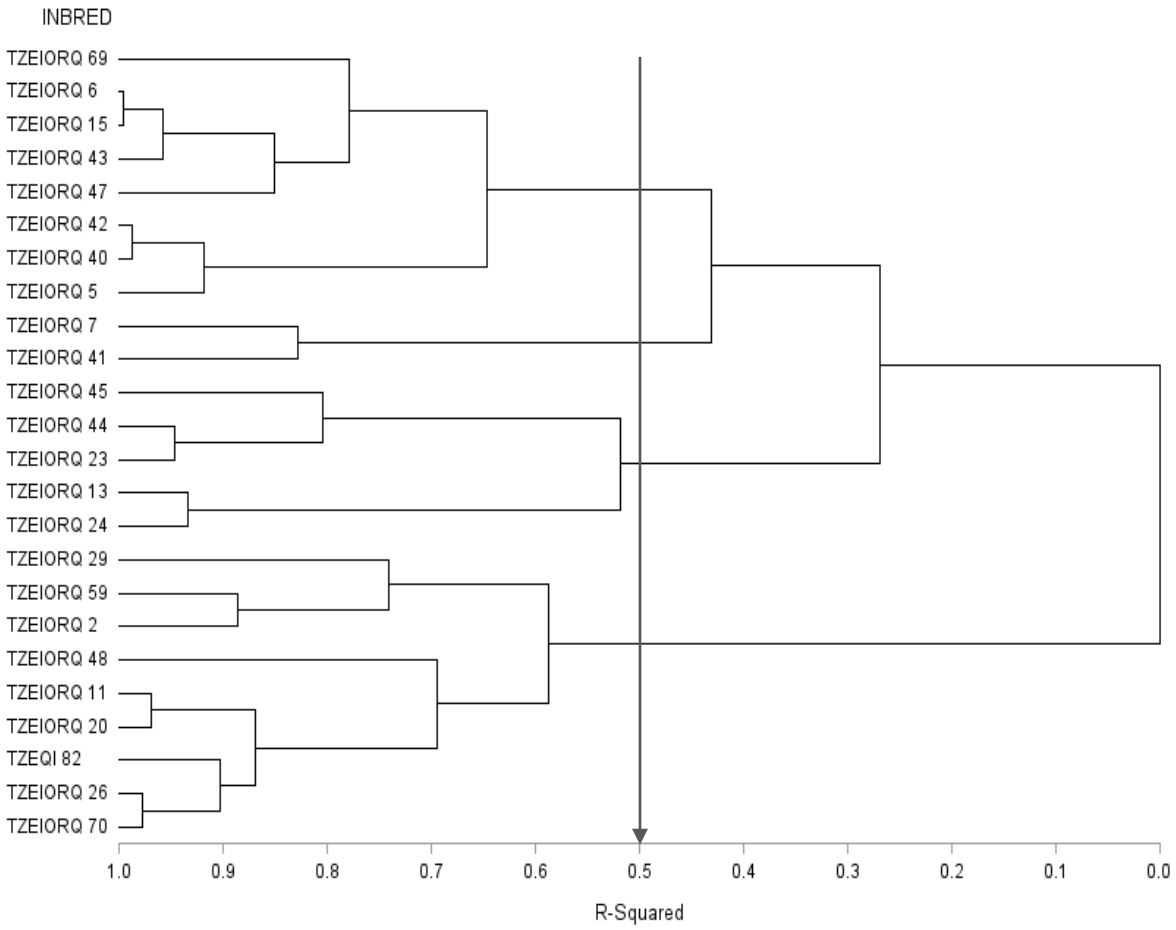

Figure 5. 9 Dendrogram of 24 early maturing provitamin A-quality protein maize parental lines constructed from GCA effects of grain yield and other traits (HGCAMT) using Ward's minimum variance cluster analysis method across drought, low-N and optimal environments in Nigeria, 2016-2017.

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5.3.4.2 Heterotic grouping using Diversity Array Technology sequencing (DArTseq) markers

The molecular markers (DArTseq) grouping method classified the inbred lines into 3 groups and explained 80% of the variation among the inbred lines (Fig. 5.10). The SNP-based DArT marker method classified the inbred lines that were more likely to be related based on available pedigree information into the same groups. For instance, inbred lines TZEIORQ 11, TZEIORQ 13 and TZEIORQ 15 with high probability of being related were classified into group I. Similarly, TZEIORQ 5, TZEIORQ 6 and TZEIORQ 7 were placed in group II, while inbred lines TZEIORQ 40, TZEIORQ 41, TZEIORQ 42, TZEIORQ 43, TZEIORQ 44, TZEIORQ 45 and TZEIORQ 47 were placed in group III (Fig. 5.10). Almost all the inbred lines placed in group II exhibited tolerance to combined drought and low-N.

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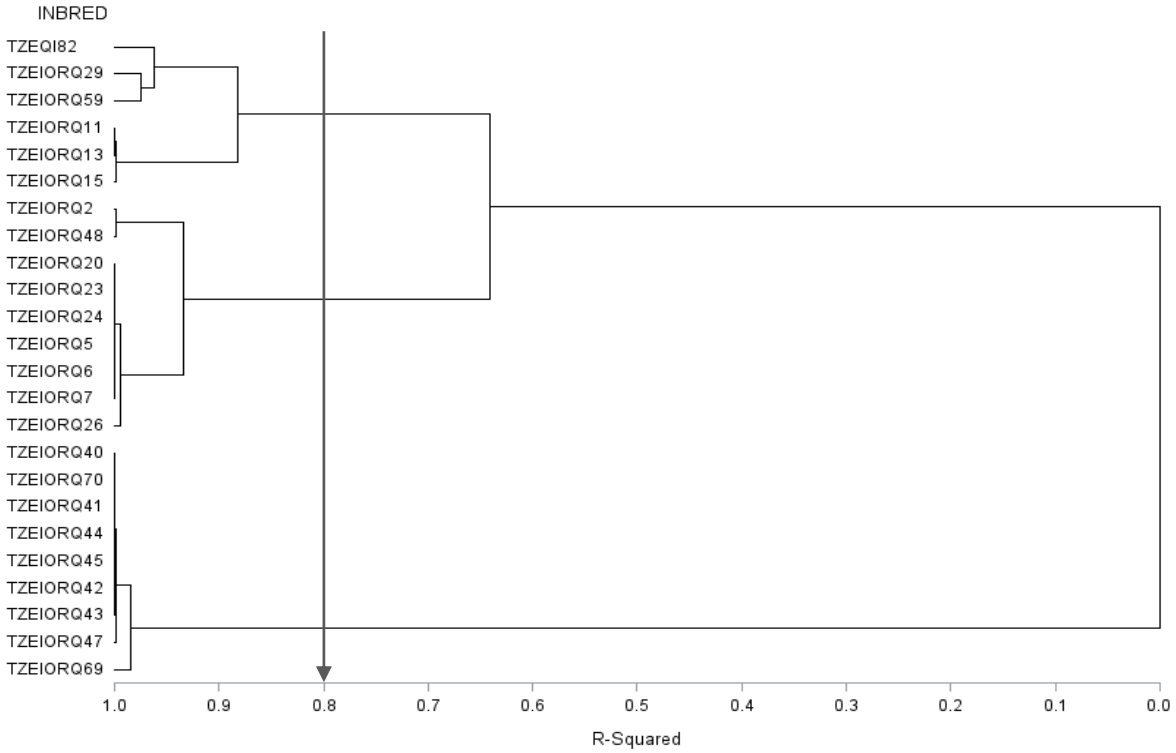

Figure 5. 10 Dendrogram of 24 early maturing provitamin A quality protein maize parental lines constructed based on the Nei's 1983 genetic distance generated from the Diversity Array Technology sequencing (DArTseq) markers using Ward's minimum variance cluster analysis method.

5.3.4.3 Comparison of the efficiency of the HGCAMT and DArTseq markers heterotic grouping methods under drought, low-N, optimal and across environments

Of the 96 hybrids studied, the HGCAMT method identified 22, 23, 28 and 25 as the number of high-yielding inter group hybrids while 7, 6, 9 and 7 were the number of low-yielding intra group hybrids under drought, low-N, optimal and across environments, respectively (Table 5.12). In contrast, the SNP-based DArT markers method identified 32, 29, 30 and 32 as the number of high-yielding inter group hybrids whereas 27, 28, 29 and 29 were the number of low-yielding intra group hybrids under drought, low-N, optimal and across environments, respectively. The breeding efficiency (BE) of the DArTseq markers method under each and across environments was higher than those of the HGCAMT method.

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5.3.5 Identification of inbred testers

The choice of lines as potential testers for classifying other lines into heterotic groups should be based on significant high and positive GCA effects of grain yield, classification into heterotic groups, and high grain yield of inbred per se (Pswarayi and Vivek, 2008). Based on these criteria, five out of the 24 parental lines namely TZEIORQ 29, TZEIORQ 11, TZEIORQ 59, TZEQI 82 and TZEIORQ 2 recorded significant positive GCA-male and GCA-female effects of grain yield across drought, low-N and optimal environments as indicated in Table 5.11. Moreover, the five inbred lines had moderately high to high grain yield across drought, low-N and optimal environments, as presented in chapter three (Table 3.9). The DArTseq markers method classified four (TZEIORQ 29, TZEIORQ 11, TZEIORQ 59 and TZEQI 82) out of the five inbred lines identified into heterotic group I and placed TZEIORQ 2 into heterotic group II (Fig 5.11). TZEIORQ 29 was identified as the best male and female early PVA-QPM inbred tester for heterotic group I. Furthermore, TZEIORQ 24 recorded high significant positive GCA-male effect for grain yield, was placed in heterotic group II according to the DArTseq markers method and had an average grain yield of 454 kg ha^{-1} across drought, low-N and optimal environments. TZEIORQ 24 was identified as the best male tester for heterotic group II. None of the inbred lines in heterotic group III adequately met the set criteria for inbred tester identification.

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Table 5. 12 Breeding efficiencies of the heterotic group's general combining ability of multiple traits and the DArTseq markers methods under drought, low- N, optimal and across environments.

Yield Group	Cross type	HGCAMT	DArTseq Markers
Drought environments			
1	Inter	22	32
1	Intra	10	0
2	Inter	28	26
2	Intra	4	6
3	Inter	25	5
3	Intra	7	27
BE (%)		**31.33**	**66.31**
Low N environments			
1	Inter	23	29
1	Intra	9	3
2	Inter	27	30
2	Intra	5	2
3	Inter	26	4
3	Intra	6	28
BE (%)		**30.13**	**65.44**
Optimal environments			
1	Inter	28	30
1	Intra	4	2
2	Inter	24	30
2	Intra	8	2
3	Inter	23	3
3	Intra	9	29
BE (%)		**40.11**	**67.75**
Across environments			
1	Inter	25	32
1	Intra	7	0
2	Inter	18	29
2	Intra	14	3
3	Inter	25	3
3	Intra	7	29
BE (%)		**30.88**	**70.31**

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5. 3.6 Performance of early maturing provitamin A-quality protein maize hybrids under drought, Low-N, optimal and across environments

Under drought environments, there were no significant differences in grain yield among the nine top-yielding hybrids (Table 5.13). However, the highest yielding and the best performing hybrid, TZEIORQ 29 x TZEIORQ 24 and the five other high yielding hybrids, TZEIORQ 40 x TZEIORQ 26, TZEIORQ 20 x TZEIORQ 45, TZEIORQ 6 x TZEIORQ 29, TZEIORQ 26 x TZEIORQ 47 and TZEIORQ 7 x TZEIORQ 42 were significantly different from the highest yielding drought tolerant commercial hybrid check, TZEI-124 x TZEI-25. Grain yield of the highest yielding hybrid exceeded the best check by 34%. Comparison of the grain yield of hybrids under drought to that of optimal environments, revealed wider range of yield reductions (2 to 80%) with a mean of 35%. Higher percentage yield reductions were observed among the susceptible hybrids than among the tolerant hybrids. In general, the drought tolerant single cross hybrids had one or both parents characterized as tolerant to drought while the susceptible hybrids had one or both parents characterized as susceptible to drought. However, the differences in grain yield between hybrids involving T x T, T x S, and S x T were generally, not significant (Table 5.13). Anthesis-silking interval varied from 1 to 7 days with a mean of 3 days. The 15 top performing hybrids had shorter ASI of 1 and 2 days while the susceptible hybrids had intervals ranging from 3 to 7 days. Furthermore, the drought tolerant hybrids were characterized by increased ears per plant, desirable plant and ear aspects as well as reduced stay green characteristic (Appendix 4).

Under low-N conditions, grain yield of the 96 single cross hybrids ranged from 669 kg ha^{-1} for TZEIORQ 44 x TZEIORQ 45 to 5055 kg ha^{-1} for TZEIORQ 29 x TZEIORQ 43 with a mean of 3163 kg ha^{-1} (Table 5.14). The two top performing hybrids were not significantly different from each other. However, the highest yielding hybrid, TZEIORQ 29 x TZEIORQ 43 significantly out-yielded the best check, TZEIOR 127 x TZEIOR 57 by 25%.

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Table 5. 13 Grain yield and other agronomic traits of 25 early provitamin A quality protein maize hybrids (best 15 and worst 10) and four checks evaluated under drought environments in 2016/2017 and 2018 dry seasons at Ikenne and Kadawa, respectively

HYBRID	YIELD	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP	STGR	YRD	INBcom	DTBI
TZEIORQ 29 x TZEIORQ 24	5500	57	1	178.72	97.61	0.13	0.75	4	4	3	0.48	1.01	4	1.62	S x T	9.16
TZEIORQ 24 x TZEIORQ 41	4499	57	1	159.56	83.97	0.28	1.23	4	4	3	0.95	0.97	3	22.10	T x S	8.98
TZEIORQ 40 x TZEIORQ 26	4853	55	2	165.13	80.29	0.18	0.25	4	4	3	2.15	1.04	3	13.97	T x T	8.70
TZEIORQ 20 x TZEIORQ 45	5012	55	1	179.46	97.80	0.18	0.40	4	4	4	4.53	1.02	4	7.13	T x S	8.17
TZEIORQ 6 x TZEIORQ 29	4728	57	1	167.15	87.36	0.08	0.13	4	4	4	0.00	1.02	4	6.22	T x S	8.06
TZEIORQ 26 x TZEIORQ 47	5085	57	2	172.69	88.76	0.18	0.05	4	4	3	2.48	1.01	4	12.83	T x T	7.87
TZEIORQ 47 x TZEIORQ 23	4166	56	2	164.79	87.60	0.28	0.20	4	4	4	1.90	0.99	3	17.57	T x T	7.13
TZEIORQ 7 x TZEIORQ 42	4781	55	2	175.05	88.62	0.33	0.00	4	4	4	1.13	1.03	4	11.01	T x S	6.99
TZEIORQ 40 x TZEIORQ 5	4624	54	2	171.96	87.41	0.28	0.23	4	4	3	1.90	0.83	4	22.99	T x T	6.97
TZEI 124 x TZEI 25 (check 2)	3607	57	1	164.94	74.98	0.23	0.20	4	4	4	1.73	0.86	3	38.82	-	6.36
TZEIORQ 5 x TZEIORQ 47	4141	56	2	164.41	78.71	0.10	0.18	4	4	4	0.85	1.01	3	14.68	T x T	6.16
TZEIORQ 23 x TZEIORQ 44	3854	55	1	171.32	92.32	0.23	0.15	4	4	4	2.13	1.00	4	31.03	T x S	6.03
TZEIORQ 42 x TZEIORQ 20	3908	56	2	178.22	98.42	0.03	1.20	4	4	4	0.53	0.97	4	26.08	S x T	6.02
TZEIORQ 6 x TZEIORQ 45	4773	57	3	170.13	86.70	0.13	0.53	5	5	4	2.55	1.01	6	11.15	T x S	6.00
TZEIORQ 70 x TZEIORQ 2	4485	58	2	170.13	82.91	0.08	0.13	4	5	4	1.45	1.06	4	25.76	S x T	5.92
TZEIORQ 6 x TZEIORQ 69	4460	56	2	163.05	78.72	0.20	0.05	5	4	4	1.18	0.92	4	11.60	T x S	5.70
TZE Pop DT STR x TZEI 17 (check 4)	3147	58	2	165.90	76.23	0.38	0.25	5	5	5	1.93	0.83	3	38.96	-	3.59
TZEIOR 127 x TZEIOR 57 (check 1)	2749	57	2	173.95	83.44	0.80	0.05	4	5	4	1.40	0.80	4	39.92	-	2.77
TZE Pop DT STR x TZEI 13 (check 3)	2265	62	4	167.11	91.16	0.20	0.28	5	5	5	2.05	0.68	4	44.10	-	-1.72
TZEIORQ 42 x TZEIORQ 44	1769	62	3	119.76	58.69	0.78	0.55	7	6	7	1.48	0.49	5	33.70	S x S	-8.37
TZEIORQ 15 x TZEIORQ 11	1635	62	3	132.70	66.16	0.38	1.28	7	6	8	0.28	0.40	6	46.24	S x S	-9.39
TZEIORQ 44 x TZEIORQ 45	1870	63	3	117.66	59.56	0.83	3.05	8	7	8	0.48	0.12	5	19.04	S x S	-11.15
TZEIORQ 45 x TZEIORQ 40	806	62	3	121.71	63.26	0.18	2.85	7	7	8	2.98	0.39	5	65.47	S x T	-11.20
TZEIORQ 45 x TZEIORQ 43	697	63	5	126.64	63.53	0.78	2.58	8	7	7	3.13	0.49	5	70.55	S x S	-11.74
TZEIORQ 45 x TZEIORQ 70	831	53	4	126.32	66.06	0.10	0.58	7	7	8	4.73	0.35	6	79.88	S x S	-12.16
TZEIORQ 43 x TZEIORQ 41	632	62	4	120.13	62.52	0.80	1.88	7	7	8	2.73	0.34	6	74.43	S x S	-12.67
TZEIORQ 13 x TZEIORQ 15	848	58	4	129.53	65.12	0.25	2.38	7	7	8	1.28	0.24	6	69.61	S x S	-12.87
TZEIORQ 47 x TZEIORQ 42	617	65	7	119.61	61.23	1.50	0.00	7	7	8	2.93	0.37	5	78.42	T x S	-14.56
TZEIORQ 41 x TZEIORQ 47	720	58	7	115.19	64.00	0.18	2.38	8	7	7	1.95	0.36	7	77.37	S x T	-17.23
MEAN	**3140**	**58**	**3**	**153.55**	**78.38**	**0.34**	**0.82**	**5**	**5**	**5**	**1.83**	**0.75**	**4**	**34.90**		
SED	**521.72**	**1.02**	**0.75**	**6.61**	**5.35**	**0.57**	**0.47**	**0.46**	**0.38**	**0.46**	**0.98**	**0.11**	**0.33**			

YIELD = Grain yield (kg ha^{-1}); DA= days to 50% anthesis ; DS= days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height (cm); EHT = ear height (cm); RL = root lodging; SL = stalk lodging; HC = husk cover (rated on a scale of 1-9); PASP = plant aspect (1-9); EASP = ear aspect (1-9); EROT = ear rot; EPP =ears per plant; STGR= stay green characteristic; YRD= percentage yield reduction; INBcom= parental inbred line combinations; tolerant (T) or susceptible (S) to drought; DTBI= drought base index.

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Comparison of the yield under low-N and optimal environments revealed a yield reduction varying from 4% for TZEIORQ 29 x TZEIORQ 43 to 75% for TZEIORQ 24 x TZEIORQ 26 with a mean of 36%. The low-N tolerant hybrids recorded lower yield reductions than the susceptible hybrids. For most of the high yielding hybrids, it was observed that the cross combinations involved at least one low-N tolerant inbred line while the susceptible hybrids had one or both parents susceptible (Table 5.14). Also, most of the susceptible hybrids were associated with more days to anthesis and silking, increased ASI, poor plant and ear aspects, fewer ears per plants and early leaf senescence.

Grain yield of the early maturing PVA-QPM hybrids under optimal environments ranged from 1891 kg ha^{-1} for TZEIORQ 40 x TZEIORQ 41 to 5895 kg ha^{-1} for TZEI 124 x TZEI 25 (commercial hybrid check) with a mean of 4382 kg ha^{-1} (Table 5.15). The best commercial check (the highest yielding hybrid) was not significantly different from the top 2 hybrids TZEIORQ 40 x TZEIORQ 26 and TZEIORQ 23 x TZEIORQ 44. Days to silking and anthesis, and ASI of the hybrids were generally fewer compared to those under drought, but were relatively closer to the values under low-N environments.

Across drought, low-N and optimal environments, grain yield of the hybrids ranged from 1316 kg ha^{-1} for TZEIORQ 42 x TZEIORQ 44 to 5151 kg ha^{-1} for TZEIORQ 26 x TZEIORQ 47 with a mean of 3951 kg ha^{-1} (Table 5.16). Grain yield of the best performing hybrid (TZEIORQ 40 x TZEIORQ 26) was not significantly different from the best check (TZEI 124 x TZEI 25). Poor plant and ear aspects, fewer numbers of ears per plant and increased stay green characteristic were observed among the susceptible hybrids across drought, low-N and optimal environments.

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Table 5. 14 Grain yield and other agronomic traits of 25 early provitamin A quality protein maize hybrids (best 15 and worst 10) and four checks evaluated under low-N environments in 2016 and 2017 growing seasons at Ile-Ife and Mokwa

HYBRID	YIELD	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP	STGR	YRD	INBcom	LNBI
TZEIORQ 29 x TZEIORQ 43	5055	54	1	163.28	74.73	0.41	0.90	2	5	4	0.60	0.96	3	4.23	T x T	8.08
TZEIORQ 43 x TZEIORQ 5	4359	51	1	147.38	66.76	0.86	0.11	3	5	4	2.34	0.96	3	25.63	T x T	6.86
TZEIORQ 40 x TZEIORQ 26	4155	52	1	148.17	66.50	0.40	0.01	4	4	4	2.34	0.95	3	26.33	T x T	6.78
TZEIORQ 42 x TZEIORQ 20	4584	52	1	150.53	71.64	0.43	0.08	3	4	3	1.88	0.93	3	13.28	T x S	6.44
TZEIORQ 70 x TZEIORQ 2	4069	54	1	166.36	75.83	1.01	0.25	3	5	4	1.90	0.99	3	32.64	T x S	5.99
TZEIORQ 43 x TZEIORQ 2	4440	52	1	160.75	74.90	1.58	0.44	4	5	4	2.46	0.98	4	21.87	T x S	5.66
TZEIORQ 42 x TZEIORQ 11	4432	52	1	154.27	70.47	1.71	0.38	3	5	4	1.95	0.96	3	18.76	T x T	5.41
TZEIORQ 43 x TZEIORQ 26	4030	52	1	152.36	71.33	1.00	0.18	3	5	4	2.00	0.87	3	25.09	T x T	5.39
TZEIORQ 48 x TZEIORQ 43	4694	53	1	165.07	76.38	2.90	0.14	4	5	4	3.45	0.97	3	11.81	T x T	5.34
TZEIORQ 26 x TZEIORQ 47	4160	52	1	144.39	64.81	0.40	0.38	4	4	4	2.54	0.85	3	28.70	T x S	5.24
TZEIORQ 26 x TZEIORQ 13	4384	53	1	161.84	75.53	0.83	0.16	4	4	4	1.68	0.95	4	24.36	T x S	5.02
TZEIORQ 7 x TZEIORQ 42	4016	52	1	147.72	69.31	0.15	0.00	3	5	4	2.60	0.97	3	25.24	T x T	4.93
TZEIORQ 47 x TZEIORQ 15	4004	52	1	157.63	68.66	1.11	0.34	3	4	4	3.14	0.90	3	21.93	S x T	4.92
TZEIORQ 23 x TZEIORQ 11	4623	53	1	164.84	76.01	0.04	0.23	3	4	4	2.48	0.92	3	10.42	S x T	4.88
TZEIORQ 40 x TZEIORQ 2	4556	53	1	155.78	71.00	3.69	0.36	3	4	4	1.75	0.97	4	20.23	T x S	4.06
TZEIOR 127 x TZEIOR 57 (check 1)	3805	53	1	163.12	69.42	0.09	0.03	3	5	4	2.70	0.92	3	25.14	-	3.99
TZEI 124 x TZEI 25 (check 2)	3690	55	1	161.72	68.98	1.13	0.16	4	4	4	1.28	0.78	3	37.40	-	2.73
TZE Pop DT STR x TZEI 17 (check 4)	2962	54	1	147.86	68.49	0.41	0.40	4	5	5	3.23	0.83	3	42.54	-	0.58
TZEIORQ 41 x TZEQI 82	3576	55	1	149.19	66.16	0.36	0.18	4	5	5	1.33	0.84	4	35.44	S x S	-0.62
TZE Pop DT STR x TZEI 13 (check 3)	2430	56	1	158.34	69.25	0.20	0.11	4	5	5	2.89	0.73	4	40.02	-	-2.83
TZEIORQ 24 x TZEIORQ 2	2331	56	1	154.85	72.63	1.56	0.03	5	5	5	2.38	0.73	4	55.16	S x S	-4.01
TZEIORQ 24 x TZEIORQ 26	877	56	1	133.94	58.33	0.40	0.09	4	6	6	2.89	0.75	5	74.59	S x T	-9.43
TZEIORQ 43 x TZEIORQ 41	747	57	3	110.96	44.54	0.44	0.75	5	7	7	0.54	0.62	5	69.79	T x S	-10.35
TZEIORQ 41 x TZEIORQ 47	933	54	2	111.25	41.62	0.83	0.36	5	7	7	1.75	0.67	5	70.68	S x S	-10.38
TZEIORQ 23 x TZEIORQ 20	935	56	2	116.18	56.56	0.10	0.33	5	7	7	1.71	0.64	5	55.25	S x S	-10.77
TZEIORQ 7 x TZEIORQ 23	1444	57	1	130.61	61.32	0.84	0.10	5	7	7	1.63	0.59	5	47.99	T x S	-11.15
TZEIORQ 7 x TZEIORQ 42	840	55	1	126.13	52.71	0.88	0.49	4	6	7	2.46	0.64	5	70.62	S x T	-11.61
TZEIORQ 44 x TZEIORQ 45	669	56	1	117.38	47.97	0.80	1.26	4	7	6	0.83	0.62	4	71.04	S x T	-11.74
TZEIORQ 40 x TZEIORQ 41	932	55	2	118.84	51.14	0.61	0.64	5	7	7	1.11	0.55	5	50.71	T x S	-15.24
MEAN	**3163**	**54**	**0.97**	**146.23**	**65.62**	**0.87**	**0.31**	**4**	**5**	**5**	**2.06**	**0.83**	**4**	**36.44**		
SED	**355.52**	**0.45**	**0.23**	**4.2**	**2.85**	**0.62**	**0.24**	**0.28**	**0.26**	**0.31**	**0.68**	**0.06**	**0.27**			

YIELD = Grain yield (kg ha^{-1}); DA= days to 50% anthesis ; DS= days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height (cm); EHT = ear height (cm); RL = root lodging; SL = stalk lodging; HC = husk cover (rated on a scale of 1-9); PASP = plant aspect (1-9); EASP = ear aspect (1-9); EROT = ear rot; EPP =ears per plant; STGR= stay green characteristic; YRD= percentage yield reduction; INBcom= parental inbred line combinations; tolerant (T) or susceptible (S) to low N; LNBI= low N base index.

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Table 5. 15 Grain yield and other agronomic traits of 25 early provitamin A quality protein maize hybrids (best 15 and worst 10) and four checks evaluated under optimal environments in 2016 and 2017 growing seasons at Ikene, Ile-Ife and Mokwa

HYBRID	YIELD	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP	MI
TZEIORQ 40 x TZEIORQ 26	5641	51	0	160.49	76.78	0.74	0.87	3	5	3	1.98	0.91	8.09
TZEIORQ 23 x TZEIORQ 44	5589	52	0	170.38	77.75	0.03	0.93	4	5	5	2.06	0.90	7.23
TZEIORQ 47 x TZEIORQ 23	5054	52	1	165.53	81.15	0.57	1.03	4	5	5	1.51	0.82	7.14
TZEIORQ 26 x TZEIORQ 47	5834	52	1	153.81	72.74	0.07	0.90	4	5	4	1.89	0.90	6.71
TZEIORQ 42 x TZEIORQ 20	5286	52	0	167.92	81.80	0.02	1.10	4	5	5	2.23	0.82	6.67
TZEIORQ 24 x TZEIORQ 41	5776	52	0	167.70	78.17	0.53	0.57	5	5	4	1.80	0.82	6.66
TZEIORQ 20 x TZEIORQ 45	5396	52	1	170.44	78.22	0.02	0.98	4	5	4	2.98	0.89	6.57
TZEIORQ 29 x TZEIORQ 43	4623	53	1	148.25	69.74	0.51	2.05	5	5	5	2.81	0.72	6.29
TZEIORQ 48 x TZEIORQ 43	5322	54	1	161.60	78.26	1.70	1.17	4	5	5	1.43	0.77	6.05
TZEIORQ 7 x TZEIORQ 42	5372	51	0	168.17	82.09	0.01	0.91	4	5	4	2.69	0.94	6.01
TZEIORQ 43 x TZEIORQ 5	5862	51	1	159.52	76.98	0.12	0.96	4	5	5	1.90	0.92	5.87
TZEIORQ 6 x TZEIORQ 29	5042	53	1	160.25	76.17	0.04	0.74	4	5	5	1.99	0.86	5.45
TZEIORQ 29 x TZEIORQ 24	4923	53	1	158.65	79.47	0.03	0.64	3	5	4	1.88	0.84	5.29
TZEIORQ 23 x TZEIORQ 11	5161	52	1	166.64	79.98	0.12	0.70	4	5	4	2.08	0.81	5.27
TZEI 124 x TZEI 25 (check 2)	5895	53	1	174.95	77.59	0.20	0.73	4	4	4	2.14	0.90	5.19
TZEIORQ 26 x TZEIORQ 13	5795	52	1	174.77	83.29	0.28	0.35	4	5	4	1.56	0.92	5.02
TZEIOR 127 x TZEIOR 57 (check 1)	5083	52	0	175.68	71.34	0.30	0.62	5	5	4	3.43	0.90	4.13
TZE Pop DT STR x TZEI 17 (check 4)	5156	52	1	164.03	79.02	0.10	0.91	4	5	5	2.14	0.80	1.96
TZE Pop DT STR x TZEI 13 (check 3)	4052	54	1	166.33	77.16	0.05	1.54	4	5	5	1.93	0.73	-2.74
TZEIORQ 5 x TZEIORQ 7	3419	55	1	153.37	74.59	0.38	0.10	3	6	6	3.96	0.68	-11.33
TZEIORQ 24 x TZEIORQ 26	3451	55	1	155.53	72.11	0.03	0.05	4	6	6	4.78	0.81	-11.78
TZEIORQ 43 x TZEIORQ 41	2472	54	1	139.07	59.59	0.46	1.23	5	7	6	3.80	0.68	-12.09
TZEIORQ 23 x TZEIORQ 20	2090	55	2	146.64	68.97	0.18	0.73	4	6	6	3.39	0.62	-12.39
TZEIORQ 40 x TZEIORQ 41	1891	55	0	129.75	56.28	0.78	0.32	5	7	7	3.42	0.62	-12.63
TZEIORQ 44 x TZEIORQ 45	2310	54	1	135.85	64.22	0.63	0.38	4	7	7	2.76	0.75	-12.83
TZEIORQ 26 x TZEIORQ 7	2161	57	2	142.18	72.47	0.23	0.08	5	7	7	3.80	0.55	-13.85
TZEIORQ 47 x TZEIORQ 42	2859	53	1	137.19	59.04	1.05	1.53	5	6	7	3.23	0.64	-13.99
TZEIORQ 41 x TZEIORQ 47	3182	53	1	137.51	56.31	1.23	1.20	4	6	6	2.88	0.86	-14.81
TZEIORQ 45 x TZEIORQ 43	2367	53	2	143.07	63.02	0.16	2.58	5	7	7	2.18	0.62	-15.57
MEAN	**4382**	**53**	**1**	**157.08**	**73.25**	**0.36**	**0.89**	**4**	**5**	**5**	**2.57**	**0.79**	
SED	**343.47**	**0.46**	**1.12**	**4.72**	**3.34**	**0.33**	**0.31**	**0.31**	**0.24**	**0.40**	**0.56**	**0.05**	

YIELD = Grain yield (kg ha^{-1}); DA= days to 50% anthesis ; DS= days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height (cm); EHT = ear height (cm); RL = root lodging; SL = stalk lodging; HC = husk cover (rated on a scale of 1-9); PASP = plant aspect (1-9); EASP = ear aspect (1-9); EROT = ear rot; EPP =ears per plant; MI= multiple trait base index.

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Table 5. 16 Grain yield and other agronomic traits of 25 early provitamin A quality protein maize hybrids (best 15 and worst 10) and four checks evaluated across drought, low N and optimal environments

HYBRID	YIELD	DS	ASI	PLHT	EHT	RL	SL	HC	PASP	EASP	EROT	EPP	STGR	MI
TZEIORQ 40 x TZEIORQ 26	5014	52	1	157.27	73.95	0.27	0.48	4	4	4	2.13	0.94	3	8.09
TZEIORQ 23 x TZEIORQ 44	4908	52	1	165.16	78.55	0.00	0.42	4	5	5	2.43	0.92	3	7.23
TZEIORQ 47 x TZEIORQ 23	4608	53	1	163.44	81.44	0.69	0.54	3	5	4	1.75	0.89	3	7.14
TZEIORQ 26 x TZEIORQ 47	5151	53	1	153.90	72.75	0.13	0.56	4	5	4	2.18	0.90	3	6.71
TZEIORQ 42 x TZEIORQ 20	4823	53	2	163.84	81.18	0.15	0.73	4	4	4	1.83	0.88	4	6.67
TZEIORQ 24 x TZEIORQ 41	4955	53	1	161.77	77.06	0.70	0.51	4	5	4	1.71	0.86	3	6.66
TZEIORQ 20 x TZEIORQ 45	4854	53	1	166.14	77.42	0.09	0.58	4	5	4	3.17	0.93	3	6.57
TZEIORQ 29 x TZEIORQ 43	4549	54	2	155.99	73.59	0.55	1.30	4	5	5	2.05	0.81	3	6.29
TZEIORQ 48 x TZEIORQ 43	4812	54	1	165.73	81.55	2.04	0.56	4	5	4	2.23	0.86	3	6.05
TZEIORQ 7 x TZEIORQ 42	4822	52	1	162.54	78.97	0.00	0.45	4	5	4	2.39	0.96	4	6.01
TZEIORQ 43 x TZEIORQ 5	5031	52	2	156.81	74.73	0.38	0.58	4	5	4	2.18	0.95	3	5.87
TZEIORQ 6 x TZEIORQ 29	4416	54	1	158.69	75.60	0.38	0.43	4	5	4	1.90	0.89	3	5.45
TZEIORQ 29 x TZEIORQ 24	4642	54	1	159.49	80.68	0.01	0.48	3	5	4	1.43	0.88	4	5.29
TZEI 124 x TZEI 25 (check 2)	4779	54	1	168.91	74.22	0.51	0.38	4	4	4	1.77	0.85	3	5.19
TZEIORQ 26 x TZEIORQ 13	5052	53	1	167.30	79.11	0.38	0.30	4	5	4	1.83	0.93	4	5.02
TZEIOR 127 x TZEIOR 57 (check 1)	4268	53	1	171.21	72.71	0.25	0.31	4	5	4	2.85	0.89	3	4.13
TZEIORQ 29 x TZEIORQ 40	4500	54	2	154.00	73.27	0.65	1.31	4	5	4	2.26	0.87	4	3.97
TZEIORQ 42 x TZEIORQ 6	3701	53	2	153.11	73.48	1.10	0.67	5	5	5	1.40	0.77	4	2.64
TZEIORQ 7 x TZEIORQ 59	4171	54	1	171.17	78.47	0.04	0.13	4	5	5	2.04	0.90	4	2.14
TZE Pop DT STR x TZEI 17 (check 4)	4102	54	1	159.06	74.99	0.13	0.61	4	5	5	2.44	0.82	3	1.96
TZEIORQ 11 x TZEIORQ 48	4198	53	1	172.96	83.44	2.01	0.33	4	5	5	2.43	0.83	4	1.02
TZEIORQ 2 x TZEIORQ 7	2877	55	1	156.21	75.93	0.42	0.26	4	5	5	2.63	0.76	4	-1.36
TZE Pop DT STR x TZEI 13 (check 3)	3213	56	2	163.80	76.86	0.13	0.85	4	5	5	2.27	0.72	4	-2.74
TZEIORQ 20 x TZEIORQ 48	3199	55	1	163.52	81.62	0.03	0.06	5	6	5	2.25	0.77	5	-5.39
TZEIORQ 42 x TZEIORQ 44	1316	56	2	124.78	55.19	0.87	0.97	5	7	7	3.23	0.61	4	-9.08
TZEIORQ 5 x TZEIORQ 7	2007	57	1	143.47	69.85	0.33	0.10	4	6	6	2.92	0.62	5	-11.33
TZEIORQ 43 x TZEIORQ 41	1507	56	2	126.55	55.06	0.51	1.18	5	7	6	2.54	0.61	5	-12.09
TZEIORQ 47 x TZEIORQ 42	1729	56	3	130.57	57.28	1.07	0.93	5	6	7	2.92	0.60	5	-13.99
TZEIORQ 45 x TZEIORQ 43	1376	55	2	127.22	56.86	0.21	2.06	6	7	7	2.24	0.53	6	-15.57
MEAN	**3951**	**54**	**1**	**156.71**	**73.99**	**0.48**	**0.62**	**4**	**5**	**5**	**2.26**	**0.82**	**4**	
SED	**226.09**	**0.41**	**0.58**	**2.96**	**2.12**	**0.28**	**0.19**	**0.2**	**0.16**	**0.24**	**0.39**	**0.04**	**0.21**	

YIELD = Grain yield (kg ha^{-1}); DA= days to 50% anthesis ; DS= days to 50% silking; ASI = anthesis-silking interval; PLHT = plant height (cm); EHT = ear height (cm); RL = root lodging; SL = stalk lodging; HC = husk cover (rated on a scale of 1-9); PASP = plant aspect (1-9); EASP = ear aspect (1-9); EROT = ear rot; EPP =ears per plant; STGR= stay green characteristic; MI= multiple trait base index.

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5.3.7 Identification of single cross hybrid testers

The criteria for choosing a single cross hybrid as a potential tester for the development of 3-way or double cross hybrids are as follows (a) the two inbred lines which constitute the single cross hybrid must belong to the same heterotic group, (b) they must possess high significant positive GCA for grain yield under stress, and (c) they must have high grain yield *per se* to serve as a seed parent in a successful three-way or double-cross hybrids to guarantee high seed yield (Pswarayi and Vivek, 2008). Based on these criteria, four single cross hybrids (TZEIORQ 59 x TZEIORQ 11, TZEQI 82 x TZEIORQ 59, TZEIORQ 2 x TZEQI 82 and TZEIORQ 11 x TZEIORQ 29) had their parental inbred lines place in the same heterotic group (group I) according to the SNP-based DArT markers method. The parental lines of the four identified hybrids recorded significant ($P < 0.05$) positive GCA-male and female effects for grain yield across drought and low-N environments as specified in section 5.3.3 (Table 5.11). Furthermore, TZEIORQ 2 x TZEQI 82 among the five hybrids had relatively better yielding ability across stress environments to qualify its use as seed parents in a successful three-way and double-cross hybrid for high seed production. TZEIORQ 2 x TZEQI 82 was therefore identified as a potential early maturing PVA-QPM single cross tester.

5.3.8 Assessment of inbred-hybrid relationships for grain yield

The correlation between mid-parent values and the means of measured traits of the hybrids were significant for grain yield, plant and ear heights, plant and ear aspects, ears per plant and stay green characteristic under drought, and grain yield, days to 50% anthesis and silking, plant and ear heights, plant and ear aspects and stay green characteristic under low-N and across test environments (Table 5.17). Also, mid-parent values had significant correlations with means of measured traits of the hybrids for grain yield, days to 50% anthesis and silking, plant height, and plant aspect under optimal environments.

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Furthermore, grain yield recorded significant positive average heterosis of 513, 237, 235 and 315% under drought, low-N, optimal and across environments respectively (Table 5.17). Similarly, plant and ear heights had high significant positive heterosis under each and across all research environments. Significant negative heterosis was observed for days to 50% anthesis and silking under drought, low-N, optimal and across environments, while anthesis silking interval recorded significant negative heterosis under drought. Also, highly significant positive heterotic values were obtained for ear aspect and ears per plant under drought environments.

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Table 5. 17 Correlation coefficients of pairs of traits of 24 early provitamin A-quality protein maize parental lines, the grain yield of their hybrids and the average heterosis under drought, low N, optimal and across environments in Nigeria, 2016-2017

	Correlation coefficient (r)				Average heterosis (%)			
Trait	**Drought**	**Low-N**	**Optimal**	**Across Env**	**Drought**	**Low N**	**Optimal**	**Across Env**
Grain yield (kgha^{-1})	0.30**	0.23*	0.21*	0.26*	512.89***	236.61***	234.58***	314.63***
Days to 50% anthesis (days)	0.06	0.27**	0.29**	0.29**	-2.63*	-5.20***	-5.49***	-5.47**
Days to 50% silking (days)	0.04	0.30**	0.29**	0.29**	-5.23**	-5.53***	-5.40***	-6.25***
Anthesis silking interval	0.02	0.14	0.04	0.11	-38.90**	-102.07	1.76	-35.62
Plant height (cm)	0.45***	0.51***	0.46***	0.50***	114.05***	28.37***	35.02***	44.60***
Ear height (cm)	0.34**	0.23*	0.16	0.22*	137.19***	52.54***	58.98***	71.26***
Root lodging	0.08	0.07	0.13	0.11	22.02	187.42	160.88	28.63
Stalk lodging	0.07	0.04	0.01	0.03	0.424	10.38	34.95	16.1
Husk cover	0.12	0.09	0.17	0.01	11.88	30.45*	31.59	10.87
Plant aspect (1-9)[a]	0.24*	0.29**	0.14	0.31**	10.07	11.91	28.65**	11
Ear aspect (1-9)[b]	0.45***	0.33**	0.24*	0.32**	24.78***	-0.45	15.38	-4.44
Ear rot	0.07	0.13	0.1	0.06	28.91	-21.22	34.3	17.15
Number of ears per plant (No.)	0.26*	0.06	0.07	0.11	214.51***	18.41	12.27	31.28*
Stay green characteristic (1-9)[c]	0.23*	0.34**	-	0.21*	-9.81	-6.11	-	-9.45

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively, Env= environment.

[a] Plant aspect (on a scale of 1-9), where 1= excellent overall phenotypic appeal and 9= poor overall phenotypic appeal.

[b] Ear aspect (on a scale of 1-9), where 1= clean, uniform, large and well-filled ears and 9= rotten, variable, small and partially filled ears.

[c] Stay green characteristics (on a scale of 1-9), where 1= less than 10% overall dead leaf area and 9= more than 80% overall dead leaf area.

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5.3.9 Sequential path analysis for grain yield and other agronomic traits of the hybrids evaluated under drought, low N, optimal and across environments

Under drought, ear aspect, ears per plant, days to 50% anthesis and stalk lodging were identified by the stepwise multiple regression analysis as traits in the first order, with significant contributions to grain yield, explaining 91% of the total variation in grain yield of the early maturing PVA-QPM hybrids (Fig. 5.11). Of the four first order traits, ear aspect had the highest negative direct effect (-0.822) on grain yield, while a lower direct contribution of 0.069 was made by stalk lodging. Positive direct contributions were made by ears per plant (0.365) and stalk lodging (0.069). Five traits recorded indirect contributions to grain yield through one or two of the first order traits and were categorized as traits in the second order. Among the five traits, ASI and plant aspect indirectly contributed to grain yield through two of the first order traits, likewise plant aspect. Each of the remaining second order traits, days to 50% silking, stay green characteristic and plant height made their contributions to grain yield through a first order trait. ASI made the highest negative (-0.852) indirect contribution to grain yield through ears per plant, while plant aspect made the highest positive indirect contribution to grain yield through ear aspect under drought. Furthermore, each of the three third order traits (ear height, husk cover and root lodging) made indirect contributions to grain yield through all the five second order traits except root lodging which contributed to grain yield through three of the first order traits. Ear rot was the only fourth order trait and made relatively weak positive indirect contributions to grain yield through husk cover and root lodging.

Ear and plant aspects, ear height and days to 50% anthesis constituted the traits which directly contributed to grain yield, accounting for 93% of the overall variation in grain yield under low-N (Fig. 5.12). Ear aspect recorded the highest negative (-0.756) direct contribution to grain yield, followed by days to 50% anthesis (-0.121) and plant aspect (-0.086) while ear height (0.152) had significant positive direct contributions to grain yield.

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There were six traits in the second order of which days to 50% made the highest positive (0.999) indirect contribution to grain yield followed by plant height (0.918). Stay green characteristic and anthesis silking interval positively and indirectly contributed to grain yield through ear aspect and days to 50% anthesis respectively. Although, ears per plant was among the three third order traits under low-N, it significantly contributed to grain yield through five out of the six second order traits.

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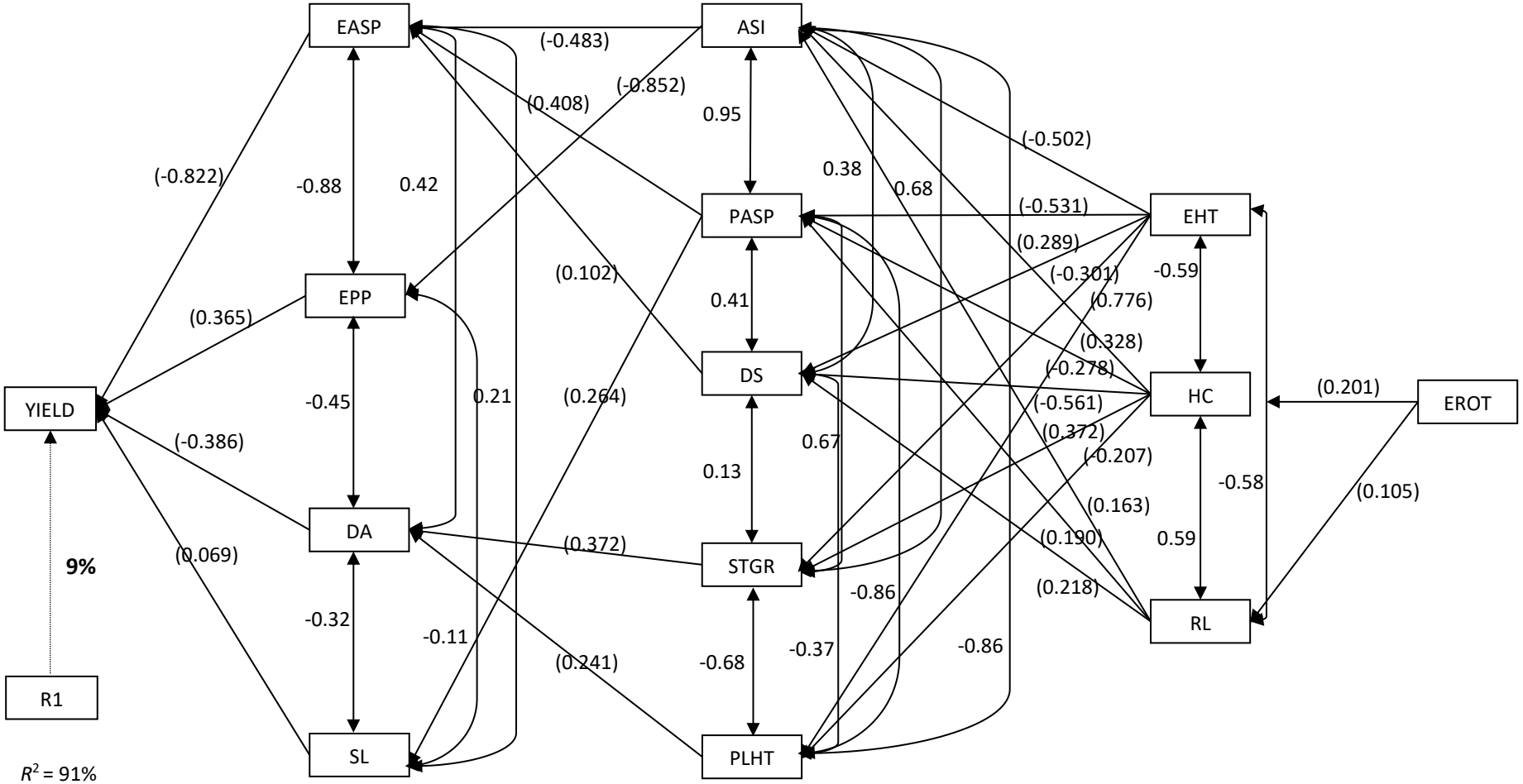

Figure 5. 11 Path analysis model diagram showing causal relationships of measured traits of early maturing provitamin A quality protein maize hybrids evaluated under drought during the 2016/2017 and 2018 dry seasons at Ikenne and Kawada respectively, in Nigeria. Bold value is the residual effect; values in parenthesis are direct path coefficients while other values are correlation coefficients. R^2= co-efficient of determination; R1= residual effects; YIELD= grain yield; EASP= ear aspect; EPP= ears per plant; DA= days to 50% anthesis; SL= stalk lodging; ASI= anthesis–silking interval; PASP= plant aspect; DS= days to 50 % silking; STGR= stay green characteristics; PLHT= plant height; EHT= ear height; HC= husk cover; RL= root lodging; EROT= ear rot.

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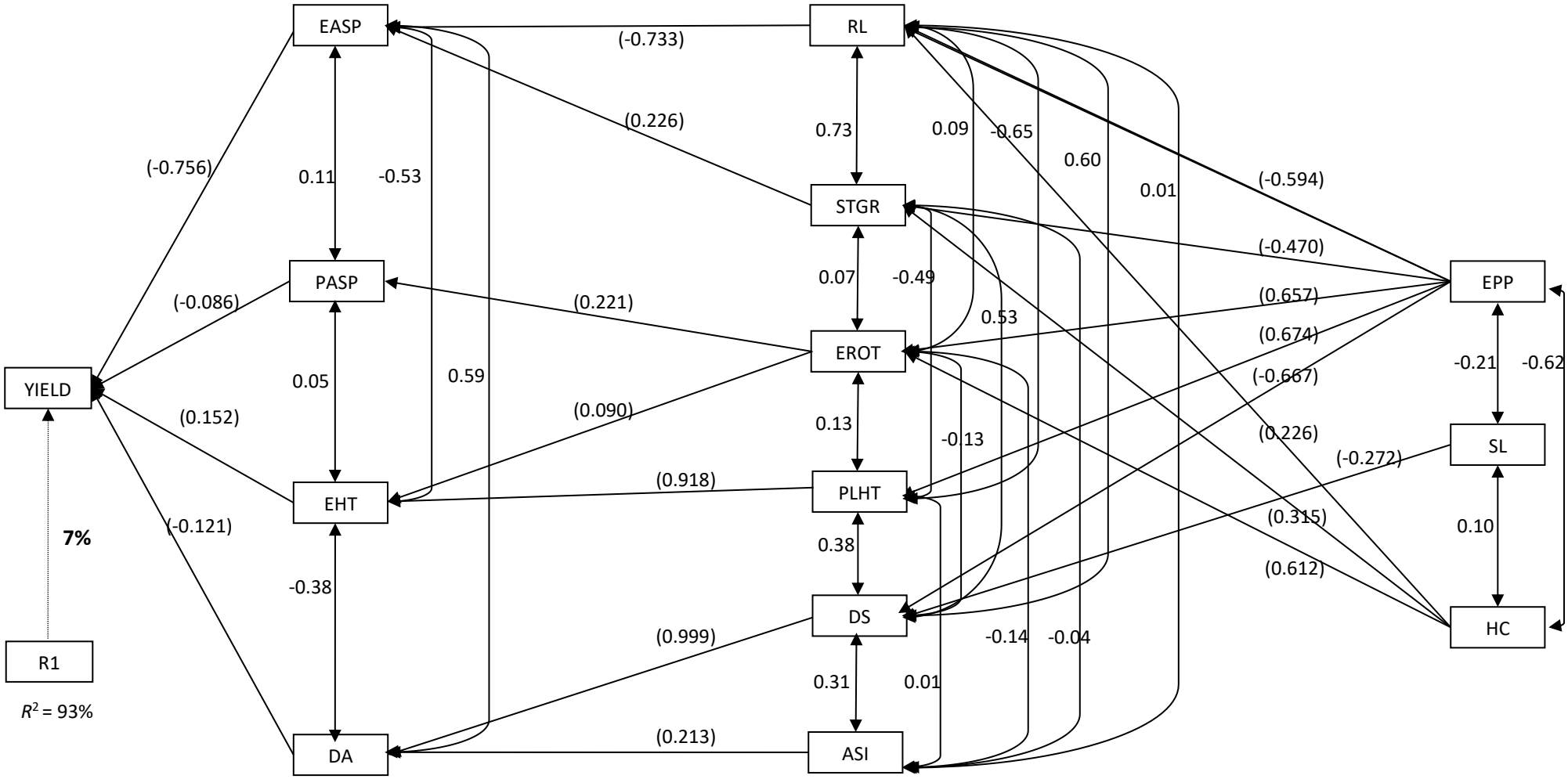

Figure 5. 12 Path analysis model diagram showing causal relationships of measured traits of early maturing provitamin A quality protein maize hybrids evaluated under low-N in the 2016 and 2017 growing seasons at Ile-Ife and Mokwa in Nigeria. Bold value is the residual effect; values in parenthesis are direct path coefficients while other values are correlation coefficients. R^2= co-efficient of determination; R1= residual effects; YIELD= grain yield; EASP= ear aspect; PASP= plant aspect; EHT= ear height; DA= days to 50% anthesis; RL= root lodging; STGR= stay green characteristics; EROT= ear rot; PLHT= plant height; DS= days to 50 % silking; ASI= anthesis–silking interval; EPP= ears per plant; SL= stalk lodging; HC= husk cover.

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Under optimal conditions, plant and ear aspects, ears per plant and ear rot had direct contributions to grain yield with 90% of the variation in grain yield of the early maturing PVA-QPM hybrids attributed to these traits (Fig. 5.13). Plant and ear aspects and ear rot made significant negative and direct contributions, while ears per plant made a positive direct contribution to grain yield. Ear rot as a first order trait had a negative contribution to grain yield. Husk cover indirectly contributed to grain yield through ear rot. Among the five traits in the second order category, plant height significantly made indirect contributions to grain yield through all the first order traits whereas, days to 50% silking and husk cover made indirect contributions to grain yield through three traits each in the first order category. Days to 50% anthesis and anthesis silking interval were among the three third order traits that indirectly contributed to grain yield through almost all the second order traits

Across drought, low-N and optimal environments, ear aspect, ears per plant, plant aspect, stalk lodging and stay green characteristics were identified as the first order contributors to grain yield of hybrids (Fig. 5.14). Ninety-six percent of the total variation in grain yield of hybrids were explained by these traits. Ear aspect consistently made a significant negative direct (-0.537) contribution to grain yield, with plant aspect and stay green characteristic also making negative direct contributions to grain yield but with minimal effects. On the contrary, ears per plant made a positive (0.168) direct contribution to grain yield with a relatively minimal effect. Six traits constituted the second order category from which a total of 14 indirect contributions were made to grain yield through the first order traits. Out of the 14 indirect contributions, four each were attributed to husk cover and plant height, two contributions each were made by days to 50% anthesis and ear rot, while days to 50% silking and root lodging made single contributions each to grain yield of the hybrids. Although ASI was one of the two third order traits, it had indirect contributions to grain yield of hybrids through 5 out of the 6 second order traits.

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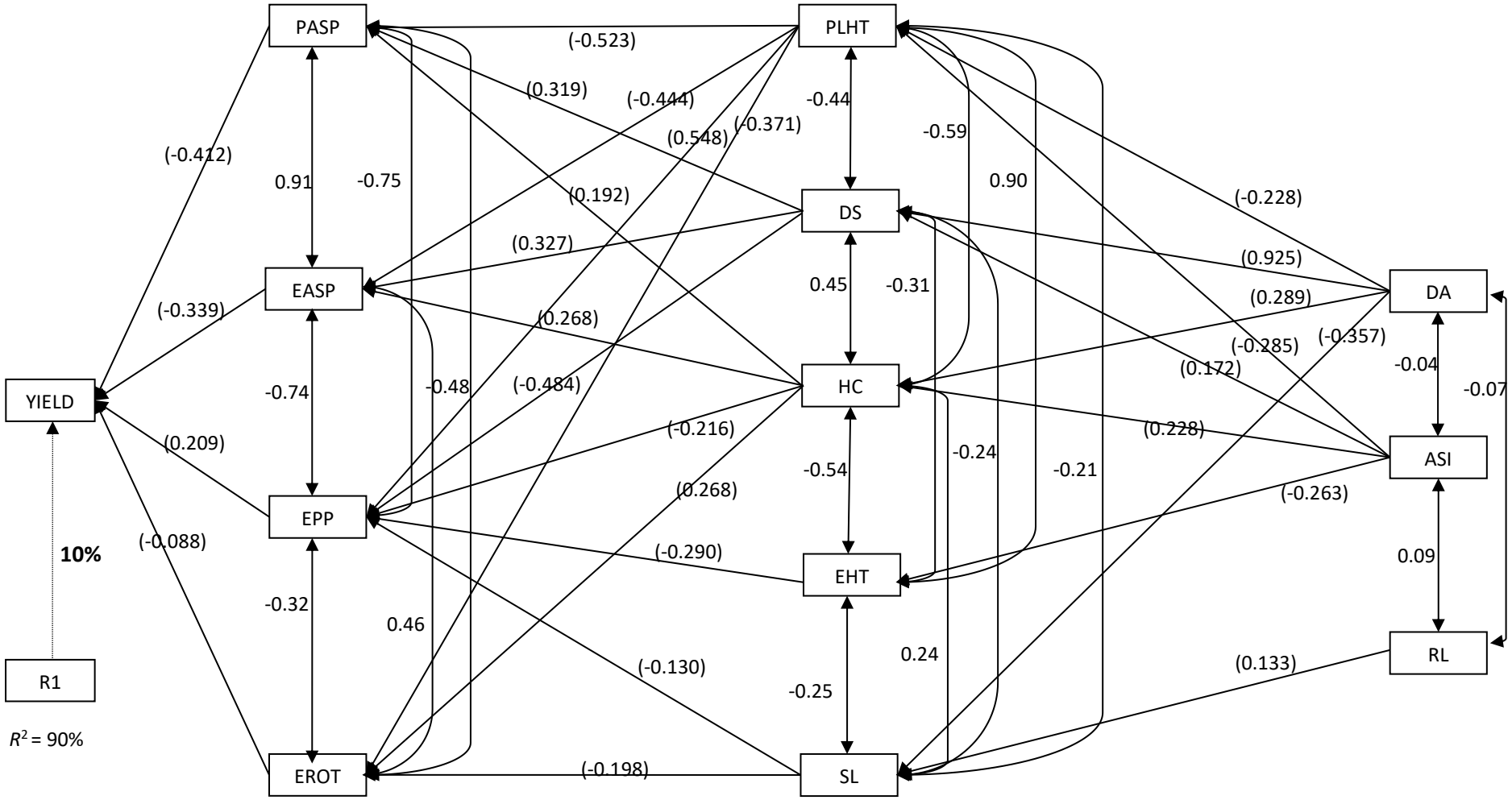

Figure 5. 13 Path analysis model diagram showing causal relationships of measured traits of early maturing provitamin A quality protein maize hybrids evaluated under optimal environments in the 2016 and 2017 growing seasons at Ikenne, Ile-Ife and Mokwa in Nigeria. Bold value is the residual effect; values in parenthesis are direct path coefficients while other values are correlation coefficients. R^2= co-efficient of determination; R1= residual effects; YIELD= grain yield; PASP= plant aspect; EASP= ear aspect; EPP= ears per plant; EROT= ear rot; PLHT= plant height; DS= days to 50 % silking; HC= husk cover; EHT= ear height; SL= stalk lodging; DA= days to 50% anthesis; ASI= anthesis–silking interval; RL= root lodging.

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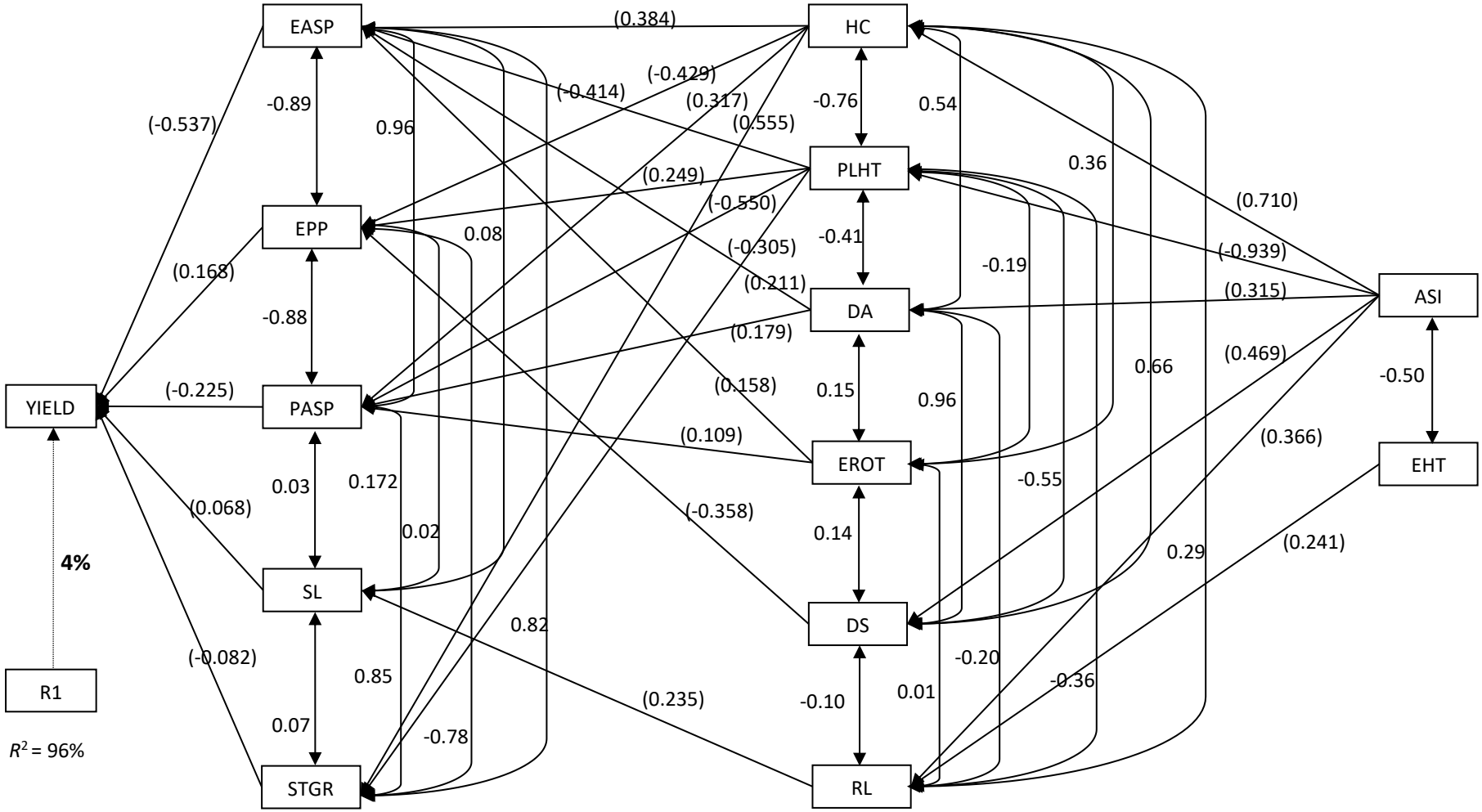

Figure 5. 14 Path analysis model diagram showing causal relationships of measured traits of early maturing provitamin A quality protein maize hybrids evaluated across drought, low-N and optimal environments in Nigeria. Bold value is the residual effect; values in parenthesis are direct path coefficients while other values are correlation coefficients. R^2= co-efficient of determination; R1= residual effects; YIELD= grain yield; EASP= ear aspect; EPP= ears per plant; PASP= plant aspect; RL= root lodging; STGR= stay green characteristics; HC= husk cover; PLHT= plant height; DA= days to 50% anthesis; EROT= ear rot; DS= days to 50% silking; SL= stalk lodging; ASI= anthesis–silking interval; EHT= ear height.

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5.3.10 Stability of hybrid performance across drought, low-N and optimal environments

The significant differences observed for genotype, environments and genotype x environment interactions for grain yield under drought, low-N, optimal and across environments in this study, necessitated the use of the GGE biplot procedure to decompose the genotype x environment interactions and to investigate the yield and stability of the hybrids across the test environments. The first and the second principal component axes (PC1 and PC2) explained 77.7% of the variation in grain yield of hybrids (Figs. 5.15 and 5.16).

The "which-won-where" view of the GGE biplot was employed to identify location-specific hybrids (Fig. 5.15). In the polygon view, the vertex entries in each sector represents the highest yielding genotypes in the locations that fall within the particular sector. The distance between the genotype and the biplot origin measures how it differs from the mean of all genotypes- an indication of the genotype's peculiarity. Thus, the vertex genotypes in each sector are more responsive to their locations than those within the polygon and located close to the biplot origin. The polygon view displayed seven sectors with entries 2, 20, 22, 13, 19, 4 and 12 representing the vertex hybrids (Fig. 15.15). Three environments, E2, E3, and E12 fell within the sector which had TZEIORQ 29 x TZEIORQ 43 (entry 2) as the vertex hybrid, implying that TZEIORQ 29 x TZEIORQ 43 was the ideal hybrid in terms of yield for those environments. Likewise, five environments E1, E4, E5, E6 and E10 were in the sector where TZEIORQ 26 x TZEIORQ 47 (entry 20) was the vertex hybrid and therefore TZEIORQ 26 x TZEIORQ 47 was the highest yielding hybrid in those environments. Two vertex hybrids, TZEIORQ 24 x TZEIORQ 41 (entry 22) and TZEIORQ 23 x TZEIORQ 44 (entry 13) were placed in environment E9 and were therefore the high yielding hybrids in that environment. Environments E7, E8 and E11 did not have vertex hybrids and therefore no highest yielding hybrid was identified in those environments. Entries 4, 12 and 19 were vertex hybrids but they were not identified with any of the environments used. The check, TZEIOR 127 x TZEIOR 57 (entry 26) was the least responsive genotype to environmental variability.

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The “mean performance vs. stability” GGE biplot view was used to identify the highest yielding and most stable hybrid(s) across the 12 test environments (Fig. 15.16). The thick single-arrow line that passes through the biplot origin (intercept of the vertical and horizontal axis) and the average tester (centre of the innermost concentric circle) is referred to as the average-tester coordinate axis (ATC). The double-headed arrow line (ATC ordinate) separates hybrids with below average means (to the left side of the line) from those with above average means (to the right side of the line). The average yield of the hybrids is determined by the imaginary projections from the positions of the hybrids onto the average-tester coordinate ordinate (ATC ordinate) axis or the double-headed arrow line, while stability of the hybrids is measured by their projections onto the average-tester coordinate abscissa (ATC abscissa) or the single-arrow line. The farther the hybrid is from the ATC ordinate in the right direction, the higher the grain yield while the shorter the absolute length of the projection of a hybrid to the ATC abscissa, the more stable it is. Based on this interpretation, the entry/tester GGE biplot identified TZEIORQ 24 x TZEIORQ 41 (entry 22) and TZEIORQ 26 x TZEIORQ 47 (entry 20) as the highest yielding hybrids with TZEIORQ 24 x TZEIORQ 41 as the most stable across all research environments (Fig. 15.16). The highest yielding and most stable hybrid TZEIORQ 24 x TZEIORQ 41 out-yielded the best commercial hybrid check TZEI 124 x TZEI 25 (entry 27) by 4% across test environments (Table 5.16).

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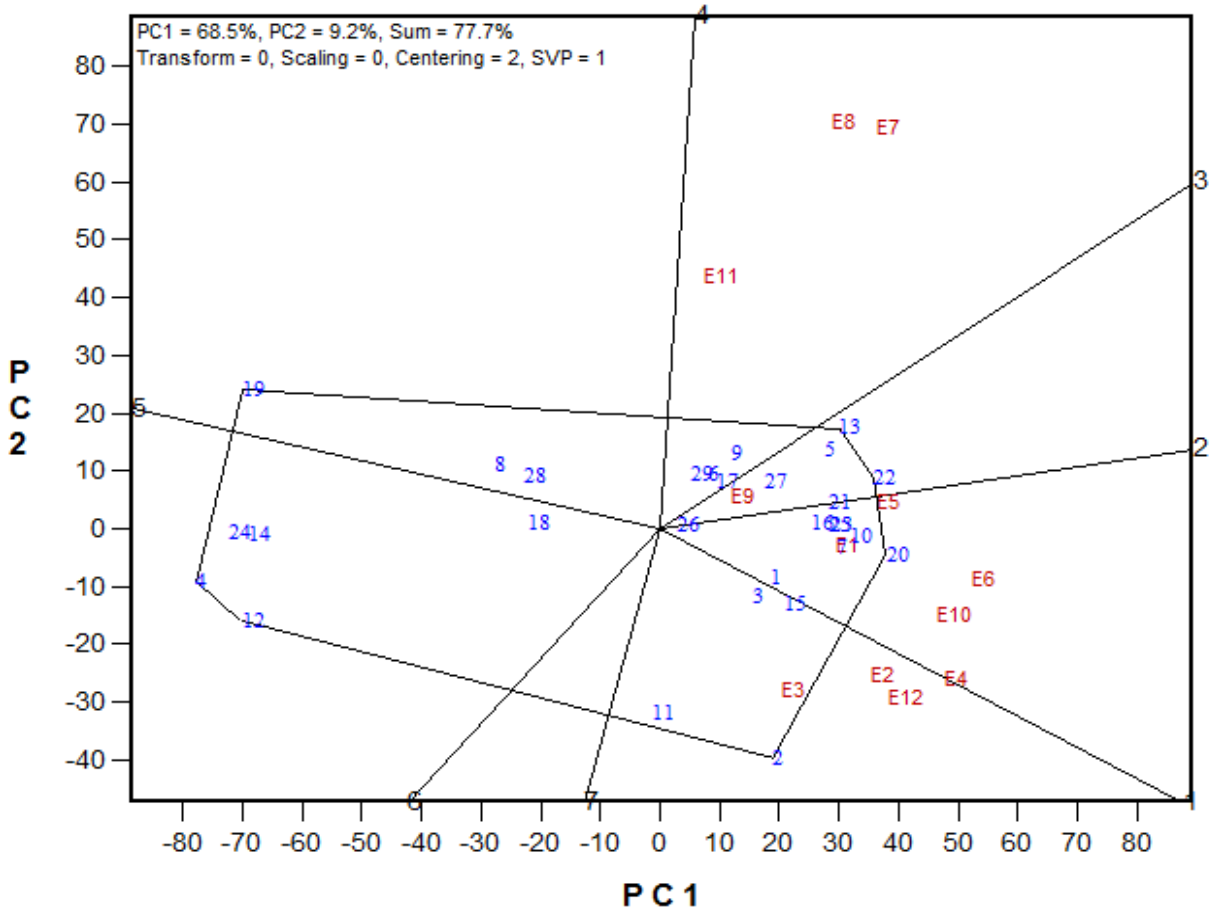

Entry	Hybrid
1	TZEIORQ 29 x TZEIORQ 24
2	TZEIORQ 29 x TZEIORQ 43
3	TZEIORQ 29 x TZEIORQ 40
4	TZEIORQ 45 x TZEIORQ 43
5	TZEIORQ 48 x TZEIORQ 43
6	TZEIORQ 11 x TZEIORQ 48
7	TZEIORQ 20 x TZEIORQ 45
8	TZEIORQ 20 x TZEIORQ 48
9	TZEIORQ 6 x TZEIORQ 29
10	TZEIORQ 42 x TZEIORQ 20
11	TZEIORQ 42 x TZEIORQ 6
12	TZEIORQ 42 x TZEIORQ 44
13	TZEIORQ 23 x TZEIORQ 44
14	TZEIORQ 47 x TZEIORQ 42
15	TZEIORQ 47 x TZEIORQ 23
16	TZEIORQ 7 x TZEIORQ 42
17	TZEIORQ 7 x TZEIORQ 59
18	TZEIORQ 2 x TZEIORQ 7
19	TZEIORQ 5 x TZEIORQ 7
20	TZEIORQ 26 x TZEIORQ 47
21	TZEIORQ 26 x TZEIORQ 13
22	TZEIORQ 24 x TZEIORQ 41
23	TZEIORQ 43 x TZEIORQ 5
24	TZEIORQ 43 x TZEIORQ 41
25	TZEIORQ 40 x TZEIORQ 26
26	Check 1-TZEIOR 127 x TZEIOR 57
27	Check 2-TZEI 124 x TZEI 25
28	Check 3-TZE Pop DT STR x TZEI 13
29	Check 4-TZE Pop DT STR x TZEI 17

Figure 5. 15 A ‘‘which won where” genotype plus genotype x environment interaction biplot of grain yield of 25 (best 15 and worst 10 based on the combined drought and low-N base index) selected early maturing provitamin A quality protein maize hybrids plus four checks evaluated across drought, low-N and optimal environments from 2016 to 2018 in Nigeria. E1= Ikenne under drought; 2016; E2= Ile-Ife under low N; 2016; E3= Mokwa under low N; 2016; E4= Ile-Ife under low N; 2017; E5= Mokwa under low N; 2017; E6= Kadawa under drought; 2018; E7= Ile-Ife under optimal; 2016; E8= Ikenne under optimal; 2016; E9= Mokwa under optimal; 2016; E10= Ile-Ife under optimal; 2017; E11= Ikenne under optimal; 2017; E12= Mokwa under optimal; 20117.

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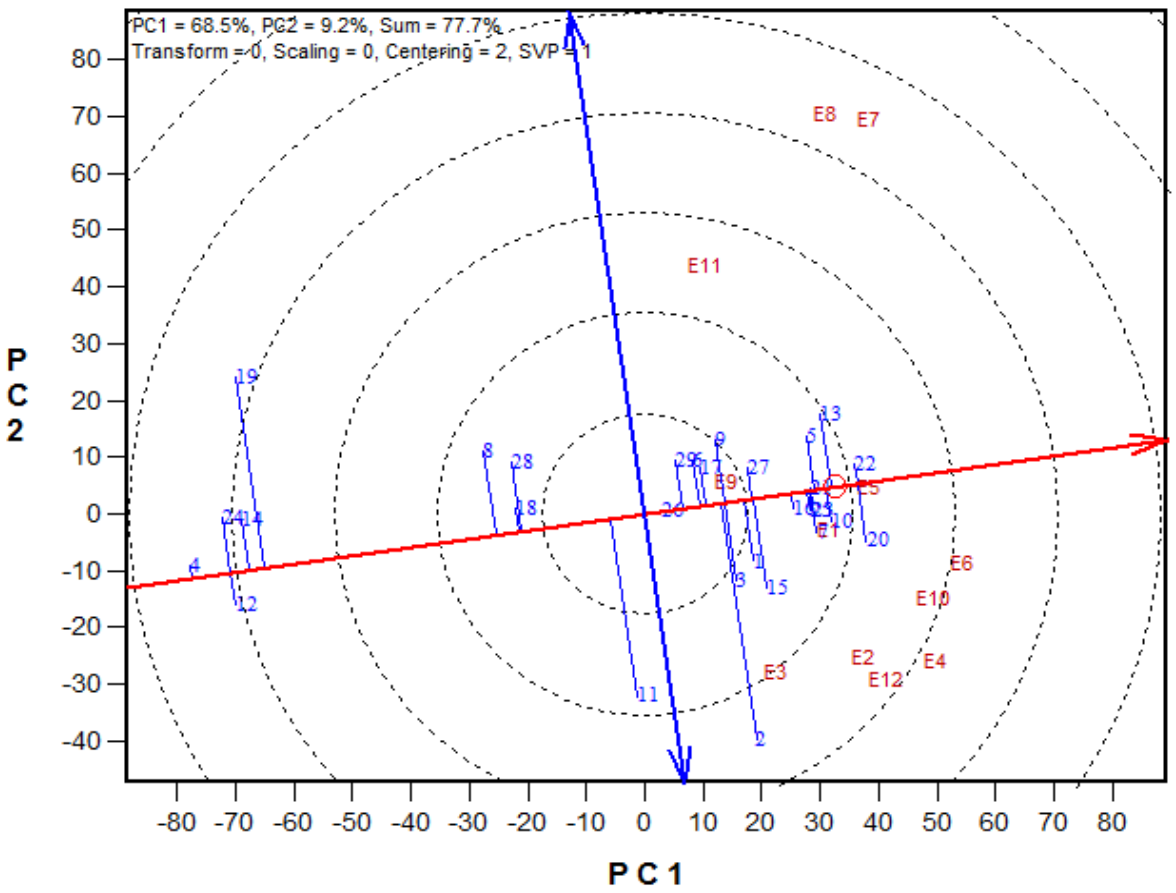

Entry	Hybrid
1	TZEIORQ 29 x TZEIORQ 24
2	TZEIORQ 29 x TZEIORQ 43
3	TZEIORQ 29 x TZEIORQ 40
4	TZEIORQ 45 x TZEIORQ 43
5	TZEIORQ 48 x TZEIORQ 43
6	TZEIORQ 11 x TZEIORQ 48
7	TZEIORQ 20 x TZEIORQ 45
8	TZEIORQ 20 x TZEIORQ 48
9	TZEIORQ 6 x TZEIORQ 29
10	TZEIORQ 42 x TZEIORQ 20
11	TZEIORQ 42 x TZEIORQ 6
12	TZEIORQ 42 x TZEIORQ 44
13	TZEIORQ 23 x TZEIORQ 44
14	TZEIORQ 47 x TZEIORQ 42
15	TZEI ORQ 47 x TZEIORQ 23
16	TZEIORQ 7 x TZEIORQ 42
17	TZEIORQ 7 x TZEIORQ 59
18	TZEIORQ 2 x TZEIORQ 7
19	TZEIORQ 5 x TZEIORQ 7
20	TZEIORQ 26 x TZEIORQ 47
21	TZEIORQ 26 x TZEIORQ 13
22	TZEIORQ 24 x TZEIORQ 41
23	TZEIORQ 43 x TZEIORQ 5
24	TZEIORQ 43 x TZEIORQ 41
25	TZEIORQ 40 x TZEIORQ 26
26	Check 1-TZEIOR 127 x TZEIOR 57
27	Check 2-TZEI 124 x TZEI 25
28	Check 3-TZE Pop DT STR x TZEI 13
29	Check 4-TZE Pop DT STR x TZEI 17

Figure 5. 16 An entry/tester genotype main effect plus genotype x environment biplot of grain yield for 25 (best 15 and worst 10 based on the combined drought and low-N base index) selected early maturing provitamin A quality protein maize hybrids plus four checks evaluated across drought, low-N and optimal environments from 2016 to 2018 in Nigeria. E1= Ikenne under drought, 2016, E2= Ile-Ife under low N, 2016, E3= Mokwa under low N, 2016, E4= Ile-Ife under low N, 2017, E5= Mokwa under low N, 2017, E6= Kadawa under drought, 2018, E7= Ile-Ife under optimal, 2016, E8= Ikenne under optimal, 2016, E9= Mokwa under optimal, 2016, E10= Ile-Ife under optimal, 2017, E11= Ikenne under optimal, 20 17, E12= Mokwa under optimal, 20117.

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5.4 Discussion

The significant mean squares of genotype (G) and environments (E) observed for grain yield and most the measured traits under drought, low-N, optimal and across environments indicated the presence of genetic variation among the hybrids (Badu-Apraku and Oyekunle, 2012) and that the test environments were unique and could reveal the genetic differences among the hybrids. The significant mean squares of GEI observed for grain yield and most traits under each and across environments suggested that the expression of the measured traits was influenced by the environmental differences. These results substantiated the need to conduct genotype evaluations across multiple environments (Najafian *et al*., 2010; Zali *et al*., 2011; Badu-Apraku *et al*., 2011a; 2011b), and to employ one of the available methods for assessing yield performance and stability of genotypes (Gauch and Zobel, 1997; Yan *et al*., 2000). The non-significant mean squares of ASI for G, E, GEI GCA-male, GCA-female and SCA suggested that the effect of ASI among the hybrids under low-N, optimal and across environments was minimal compared to that under drought. This implied that for the set of hybrids used, the inclusion of ASI in the base index under low-N was not important. These findings were consistent with the results of Badu-Apraku and Fakorede (2017) who reported ASI to be unimportant in selection for high grain yield under low-N in extra-early maize. However, these results contradicted the findings of Bolaños and Edmeades (1996) and Bänziger *et al*. (2000) who identified ASI as reliable trait for indirect selection for grain yield under low-N. The significant mean squares observed for ASI under drought corroborates the reports by Bolaños and Edmeades (1993), Bänziger *et al*. (2000), Messmer *et al*. (2009), Ziyomo and Bernardo (2013) and Badu-Apraku *et al*. (2011a) that ASI is a reliable indicator for drought tolerance. Furthermore, the non-significant mean squares of hybrid x research conditions interactions for grain yield and all traits across environments implied that, the rankings of the hybrids were consistent across research conditions, and that hybrid

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performance across drought, low-N and optimal conditions was significantly influenced by their genetic potentials. The results also implied that selection for a hybrid that performs well across drought or low-N will not carry a yield penalty in optimal conditions as reported by Bolaños and Edmeades, (1993). Moreover, the significant GCA-male x E, GCA-female x E and SCA observed for grain yield and most agronomic traits under drought, low-N, optimal and across environments suggested that GCA contributions of inbred parents were not consistent across environments. Similar result was reported by Badu-Apraku *et al*. (2013b) who found significant GCA x E interaction mean squares for grain yield and most traits under contrasting environments.

The preponderance of GCA (GCA-male + GCA-female) effects over SCA for grain yield and most agronomic traits under drought, low-N, optimal and across environments indicated that additive gene effect was more important than the non-additivet and that GCA largely contributed to the inheritance of the traits measured for the 96 early PVA-QPM hybrids evaluated. These results suggested that superior hybrids could be produced by crossing the parents with significant and positive GCA effects (Baker 1978; Badu-Apraku *et al*. 2013b). Similar results have been reported by several workers under drought (Betrán *et al*., 2003a; Badu-Apraku *et al*., 2004; Hallauer *et al*., 2010; Oyekunle and Badu-Apraku, 2013; Adebayo *et al*., 2014; Annor and Badu-Apraku, 2016) and under low-N (Lafitte and Edmeades, 1995; Kling *et al*., 1997; Adofo-Boateng *et al*., 2015; Ifie *et al*., 2015). However, these results are in contrast with the findings of other studies that non-additive genetic effects controlled the inheritance of grain yield and most agronomic traits of maize hybrids under drought (Meseka *et al*., 2013), and under low-N (Katsantonis *et al*., 1988; Betrán *et al*., 2003a; Meseka *et al*., 2006; Makumbi *et al*., 2011). The differences in the reports of the different authors may be due to the differences in the genetic materials studied, the level of inbreeding and the intensity of drought or low-N imposed. Another implication of the superiority of GCA over SCA is that

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recurrent selection in a population formed by intercrossing a set of inbreds that form the hybrids would be effective in increasing the frequency of the beneficial alleles.

The NCD II is preferred because it allows the use of more parents and facilitates estimation of maternal and paternal effects compared with the diallel (Hallauer and Miranda 1988). There is still a huge gap in the literature with respect to the contributions of male and female parents to grain yield and secondary traits in hybrids in studies involving NCD II, especially under drought (Derera *et al*., 2008) and low-N. In the present study, the ratio of GCA-male and GCA-female mean squares was used to estimate maternal and paternal effects (Kearsey and Pooni, 1996). Under drought, low-N, optimal and across environments, the non-significant differences observed among the contributions of GCA-male and GCA-female for grain yield and almost all traits implied that maternal and paternal effects were equally important in the inheritance of the traits measured for the hybrids. These results supported the findings of Annor and Badu-Apraku (2016), who found non-significant differences in the contributions of the GCA-male and GCA-female sum of squares for grain yield and most measured traits under drought as well as Derera *et al*. (2008) who found similar magnitude of GCA-male and GCA-female sum of squares for grain yield under optimal conditions. Similar result was reported by Khehra and Bhalla (1976). The results however, contradicted the report by Derera *et al*. (2008) that cytoplasmic effect influenced grain yield, prolificacy, ASI and ear aspect only under drought, as well as ASI, ears per plant and ear aspect under both drought and optimal conditions. Similar contradictory results were reported by Adebayo *et al*. (2014) that paternal effects modified the inheritance of ear aspect under drought, and by Oyekunle and Badu-Apraku, (2013) that cytoplasmic effects played a role in the inheritance of grain yield of hybrids under optimal environments. The few exceptions included the significantly greater GCA-female effects relative to GCA-male effects for stay green characteristic under drought and across environments, and also for root lodging under optimal conditions

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indicating that maternal effects modified the inheritance of the two traits in the early PVA-QPM hybrids under the respective environments. These results implied that inbreds with significant negative GCA-female effects for stay-green characteristic should be made female parents in their crosses to take maximum advantage of the effects of cytoplasmic inheritance on senescence in their progenies under drought and across environments. Also, ears per plant which had significantly larger GCA-male effect over GCA-female effects under optimal environments suggests that paternal effects conditioned prolificacy when production factors are not limited and that inbreds with significant positive GCA-male effects for ears per plant should serve as male parents to maximize prolificacy under optimal conditions. Contrary to this result, Derera *et al*. (2008) found that maternal effects conditioned prolificacy under optimal conditions. Moreover, the significantly larger GCA-female effects of ASI relative to that of GCA-male across research environments suggested that maternal effects controlled the inheritance of ASI in this set of inbreds and that inbreds with significant negative GCA-female effects for ASI should be made females in their crosses to contribute to reduced anthesis and silking days in their offspring in the three contrasting environments.

To a large extent, moderate to very high heritability estimates were obtained for grain yield, plant and ear aspects, ears per plant, stay green characteristics, days to 50% anthesis and silking, plant and ear heights under drought, low-N, optimal and across test environments suggesting that genetic gains could be achieved through direct phenotypic selection for these traits. In contrast, the relatively low heritability estimates observed for ASI under each and across all environments, as well as root and stalk lodging, husk cover and ear rot under drought, low-N and optimal environments indicated that the proportions of phenotypic variance of these traits are inadequately associated with genetic factors and that direct phenotypic selection for these traits may be ineffective. The relatively high heritability estimates observed for grain yield under drought (0.74) and low-N (0.75) compared to that of

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optimal condition (0.56) implied that direct selection for grain yield under drought and low-N could be more effective than under optimal environments. Contrary to this result, several authors have obtained low heritability estimates for grain yield particularly under stress partly due to the quantitative nature of the inheritance of the trait, and also the severity of the stress imposed (Bolaños and Edmeades, 1993; Edmeades, 1996; Bänziger *et al*., 2000; Badu-Apraku *et al*., 2011a).

General combining ability effects or additive gene effects of inbred lines for a trait are useful in determining the contributions of the parental lines to their hybrids. For example, inbred lines which have highy significant positive GCA effects for grain yield under drought or low-N have high probability of contributing favourable alleles for grain yield to the progenies in a recurrent selection programme to develop drought and/ low-N tolerant populations. Additionally, such inbreds could be used to improve existing populations and to develop drought and/ low-N hybrid and synthetic varieties for commercialization. The observed significant positive GCA-male and GCA-female effects for grain yield for inbred lines TZEIORQ 29, TZEIORQ 11, TZEIORQ 59, TZEQI 82 and TZEIORQ 2 across drought, low-N and optimal environments suggested that these inbred lines would contribute favourable alleles for improved grain yield to their progenies across test environments when used either as male or female parents. Similarly, the observed significant positive GCA-female effects for grain yield observed for inbreds TZEIORQ 48, TZEIORQ 43 and TZEIORQ 70 across the test environments indicated their high potentials to contribute favourable alleles for grain yield to their offspring when used as female parents. A similar inference could also be drawn for TZEIORQ 13 and TZEIORQ 24 which displayed significant positive GCA-male effects for grain yield across research environments. Furthermore, the significant negative GCA-male and GCA-female effects for the stay green characteristic displayed by inbreds TZEIORQ 29, TZEIORQ 59 and TZEQI 82 across the test environments is an indication that

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these inbreds, when used either as males or females, would contribute desirable alleles for delayed senescence, increased photosynthesis and hence increased assimilates production to the offspring.

Classification of the newly developed early maturing PVA-QPM inbreds into appropriate heterotic groups is important for the exploitation of maximum heterosis through crossing of inbred lines from opposing heterotic groups (Terron *et al*. 1997). This would increase the chances of developing novel and superior early PVA-QPM hybrids and synthetics with combined drought and low-N tolerance for commercialization in SSA. The heterotic grouping based on GCA of multiple traits (HGCAMT) method proposed by Badu-Apraku *et al*. (2013b) and the SNP-based DArT markers method were compared in the present study and the SNP-base DArT markers method was more efficient. The DArTseq markers method recorded significantly higher breeding efficiency than the HGCAMT method under drought, low-N, optimal and across research conditions. These results were consistent with the report by Badu-Apraku *et al*. (2016a) who found the SNP-based methods to be more efficient than the HGCAMT method. The results suggested that, maximum heterosis (more productive crosses) could be exploited from the early PVA-QPM inbred set under drought, low-N and optimal conditions by selecting parental lines from opposing heterotic groups based on the DArTseq markers method. Inbreds classified into the same heterotic group by the molecular markers could be recombined to form heterotic populations that could be improved through recurrent selection. The SNP-based DArT maker method identified three heterotic groups and the order of the grouping followed the available pedigree records. It was striking to note that almost all the inbreds classified into the heterotic group II by the SNP-based method exhibited tolerance to combined drought and low-N. These inbreds could be crossed to the inbreds from opposing heterotic groups to identify high yielding hybrids. This could be expected because several reports have demonstrated high correlation between genetic distance and hybrid

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performance in maize (Lee *et al*., 1989; Smith *et al*., 1990; Betrán *et al*., 2003a; Xu *et al*., 2004; Makumbi, 2005; Kiula *et al*., 2008).

Using the criteria proposed by Pswarayi and Vivek (2008) for the identification of a tester, inbred line TZEIORQ 29 was identified as the best male and female tester in heterotic group I, while TZEIORQ 24 was the best male tester in heterotic group II out of the 24 parental lines used in the NCD II combining ability study. This implied that, TZEIORQ 29 could be used either as a male or female parent to classify the remaining lines into heterotic groups to develop high yielding hybrids and synthetics. Similarly, TZEIORQ 24 could be used as a male parent to group the other non-parental inbreds into heterotic groups. In addition, the two testers could be utilized to classify newly developed PVA-QPM inbred lines from other populations into heterotic groups for the development of high yielding hybrids and synthetic varieties under drought, low-N and optimal environments. It was therefore, not surprising to find TZEIORQ 29 x TZEIORQ 24 as the best hybrid under drought, and among the top performing hybrids across drought, low-N and optimal environments in Nigeria.

Assessment of the 96 early PVA-QPM single cross hybrids plus 4 checks under drought, low-N and optimal conditions was necessary to identify high yielding hybrids under each and across the 3 research conditions. The results revealed varying degrees of tolerance under drought, with a wide range of grain yield reduction of 2 to 80%, and an average of 35%. The average grain yield reduction recorded under drought at flowering was within the reported range of 40-90% (Grant *et al*, 1989; Nesmith and Ritchie, 1992; Menkir and Akintunde, 2001; Badu-Apraku *et al*., 2005; Derera *et al*., 2008, Badu-Apraku *et al*., 2011b). These results indicated that the induced drought at flowering was severe enough to effectively discriminate among the drought tolerant and susceptible hybrids thus facilitating the identification and selection of outstanding hybrids. Applying a selection intensity of 10%, the drought base index which combines increased grain yield and prolificacy with short ASI and delayed leaf

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senescence, as well as excellent plant and ear aspects under drought (Badu-Apraku *et al*., 2011a) was used to identify the ten best hybrids. In decreasing order of drought tolerance, the ten hybrids were TZEIORQ 29 x TZEIORQ 24 > TZEIORQ 24 x TZEIORQ 41 > TZEIORQ 40 x TZEIORQ 26 > TZEIORQ 20 x TZEIORQ 45 > TZEIORQ 6 x TZEIORQ 29 > TZEIORQ 26 x TZEIORQ 47 > TZEIORQ 47 x TZEIORQ 23 > TZEIORQ 7 x TZEIORQ 42 > TZEIORQ 40 x TZEIORQ 5 > TZEI-124 x TZEI 25. It is worth noting that the grain yield of the 10 top performing hybrids were not significantly different from each other with the exception of the best commercial hybrid check, TZEI 124 x TZEI 25, which ranked 10th and TZEIORQ 47 x TZEIORQ 23 which ranked 7th. The highest yielding hybrid TZEIORQ 29 x TZEIORQ 24 out-yielded the best commercial hybrid check, TZEI 124 x TZEI 25 by 34%. TZEI 124 x TZEI 25 is a normal yellow single-cross hybrid released in Ghana as "CSIR-Denbea", in Mali as "Tamalaka" and in Nigeria as "Sammaz 41". This result consequently indicated that the nine top performing early maturing PVA-QPM hybrids (which are lacking in the sub-region) would not only be invaluable for increased maize production and productivity in drought prone areas of SSA, but also their consumption would help address the health disorders emanating from vitamin A deficiency and protein energy malnutrition (Bressani, 1992; Pixley *et al*., 2013; Badu-Apraku and Fontem-Lum, 2010). These results also indicated the substantial improvements made in the early maturing PVA-QPM hybrids in drought tolerance in the sub-region.

Under low-N conditions, the average grain yield reduction of 36% recorded was within the ranges, 10-50% and 20-50% reported by Wolfe *et al*. (1988) and Bänziger *et al*. (1999) respectively. These results implied that the low-N condition imposed was severe enough to effectively differentiate among the low-N tolerant and susceptible hybrids. With a selection intensity of 10%, the low-N base index which combines high grain yield, more ears per plant, short ASI, excellent plant and ear aspects and prolonged stay green characteristics under low-

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N (Badu-Apraku *et al*., 2011a) was effectively used to identify the best hybrids. In decreasing order of low N tolerance, the ten best hybrids were ranked as follows; TZEIORQ 29 x TZEIORQ 43 > TZEIORQ 43 x TZEIORQ 5 > TZEIORQ 40 x TZEIORQ 26 > TZEIORQ 42 x TZEIORQ 20 > TZEIORQ 70 x TZEIORQ 2 > TZEIORQ 43 x TZEIORQ 2 > TZEIORQ 42 x TZEIORQ 11 > TZEIORQ 43 x TZEIORQ 26 > TZEIORQ 48 x TZEIORQ 43 > TZEIORQ 26 x TZEIORQ 47. Although the two best performing hybrids were not significantly different from each other, the highest yielding and the best hybrid TZEIORQ 29 x TZEIORQ 43 significantly out-yielded the best hybrid check, TZEIOR 127 x TZEIOR 57 by 25% under low N. The best hybrid check under low N was a PVA non-QPM single cross hybrid identified as promising in previous studies and was promoted to the regional trials in the IITA-MIP. These results therefore, indicated that the 2 top performing early PVA-QPM hybrids would be very crucial for increased maize production and productivity under low-N environments in the sub-region. The results highlighted the enormous progress made in the early maturing PVA-QPM hybrids for low N tolerance.

Although it is expected that selected drought and/ low-N tolerant hybrids would also display acceptable grain yield levels under optimal conditions, it may not always be the case. Therefore, an important consideration in the present study was to ensure that hybrids that showed superior performance under drought and/ low-N would not carry yield penalties under optimal conditions. The combined drought and low-N base index using was used to rank the hybrids under optimal conditions while placing more emphasis on the performance under stress. The highest yield *per se* under optimal conditions was recorded by the commercial hybrid check TZEI 124 x TZEI 25 (5895 kg ha^{-1}), but it was not significantly different from the top two hybrids TZEIORQ 40 x TZEIORQ 26 and TZEIORQ 23 x TZEIORQ 44. These two hybrids were also among the top performers under drought and/ low-N environments implying that they did not carry yield penalties under optimal conditions (Bolaños and

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Edmeades, 1993) and that grain yields of those hybrids would be consistent across contrasting environments.

Moreover, the combined assessment of performance across drought, low-N and optimal environments revealed consistent grain yield performance of selected hybrids under each and across research environments. For example, grain yield of the highest yielding hybrid, TZEIORQ 26 x TZEIORQ 47 was not significantly different from the best performing hybrid, TZEIORQ 40 x TZEIORQ 26 as well as the best check, TZEI 124 x TZEI 25. The best 10 hybrids selected across research environments, TZEIORQ 40 x TZEIORQ 26, TZEIORQ 23 x TZEIORQ 44, TZEIORQ 47 x TZEIORQ 23, TZEIORQ 26 x TZEIORQ 47, TZEIORQ 42 x TZEIORQ 20, TZEIORQ 24 x TZEIORQ 41, TZEIORQ 20 x TZEIORQ 45, TZEIORQ 29 x TZEIORQ 43, TZEIORQ 48 x TZEIORQ 43 and TZEIORQ 7 x TZEIORQ 42 were the best 10 top-performing hybrids under optimal environments, while 8 and 7 hybrids were among the best 10 under drought and low N conditions respectively. These results further confirmed that the best hybrids selected across the 3 research conditions would consistently display superior grain yield performance across seasons and in varying production environments in SSA. The relatively fewer days to anthesis and silking, and ASI under low-N conditions compared to the values obtained under drought suggested that the impact of the drought imposed (2-80% grain yield reduction) was severer than that of the low-N (4-75% grain yield reduction). These results also implied that the drought conditions had higher discriminating ability among the hybrids than those of low-N and that identification of outstanding hybrids could be facilitated under drought than under low-N environments. What is interesting about the result is that there is high expectation of drought tolerant hybrids to also possessing adequate levels of low-N tolerance. TZEIORQ 2 x TZEQI 82 was identified as potential single cross hybrid tester across drought, low-N and optimal environments. This

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hybrid would be useful in the development of early PVA-QPM 3-way and double cross hybrids for commercialization in the sub-region.

Assessment of the relationship between hybrids and their parental lines for grain yield and other important agronomic traits under drought, low-N and optimal environments was necessary to ascertain the possible improvement in those traits of the hybrids under each and across environments based on the performance of parents. The significant correlation recorded between mid-parent values and the means of the corresponding hybrids for grain yield, plant height, ear aspect and stay-green characteristic under drought, low-N, optimal and across environments suggested that initial screening and selection of drought and low-N tolerant inbred lines for the development of superior hybrids would be effective. The significant but weak correlation observed between mid-parent values and mean of hybrids for grain yield under low-N and optimal environments indicated the need for using secondary traits to support grain yield in predicting hybrid performance based on their parents under low-N and optimal conditions. These results corroborated the findings of Meseka *et al*. (2006). However, the result is not entirely consistent with the report by Betrán *et al*. (2003a) who observed significant and strong correlation between mid-parent and hybrid means under low-N but a weak correlation under optimal conditions. On the other hand, the non-significant correlation between parental lines and their hybrids for ears per plant under low-N and optimal conditions suggested that predicting hybrid performance on the basis of prolificacy may not be effective under low-N and optimal environments. Similar results were reported by Oyekunle and Badu-Apraku (2013) under optimal environments. The inconsistencies in the results of the different studies could be attributed to the different parental inbreds used as well as the levels of inbreeding (Meseka *et al*. 2006). The high significant positive heterosis observed for grain yield, plant height and ear height under drought, low-N, optimal and across test environments indicated that although additive gene effect controls grain yield and most other traits in this

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set of inbreds, there is high potential for the exploitation of superior hybrid performance to increase grain yield under each and across test environments. These results, coupled with the significant negative heterosis observed for days to 50% anthesis and silking under drought, low-N, optimal and across environments implied that the hybrids matured faster than their parents, produced taller and more vigorous plants which translated into high grain yield. Similar results were reported by Ofori *et al*. (2015) under optimal conditions and Meseka *et al*. (2006) under low-N conditions.

Grain yield in maize is influenced by complex interactions among secondary traits and environment. Knowledge of the relationship among the key secondary traits and grain yield as influenced by environment is crucial for determining the traits with direct and indirect contributions to grain yield. The step-wise multiple regression and sequential path co-efficient analysis enhances the understanding of the various contributions by different secondary traits to grain yield (Dewey and Lu, 1959). It effectively demonstrated the relative importance of the yield related traits involved in the analysis and measures their direct and indirect contributions to the main response variable, grain yield.

Under drought, the high percentage (r^2 = 91%) of the total variation explained suggested that the traits employed in the path co-efficient analysis effectively illustrated their relative contributions to grain yield. The direct contributions of ear aspect, ears per plant, days to 50% anthesis and stalk lodging to grain yield of the early PVA-QPM hybrids indicated that these traits were the major regulators of grain yield under stressful moisture conditions. These results further highlighted the importance of ear aspect and ears per plant used in the drought tolerance base index (Badu-Apraku *et al*., 2011a, 2013b; Bänziger *et al*., 2000). This implied that selection of genotypes under drought based on these traits would lead to genetic gains (Edmaedes *et al*., 1999; Talabi *et al*., 2017). Days to 50% anthesis preceded ASI and stay green characteristics with a direct negative contribution to grain yield under drought,

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suggesting that earliness is an important contributor to drought tolerance in the hybrids. However, ASI and stay green characteristic had indirect contributions to grain yield through ears per plant and days to 50% anthesis, respectively. The results were in disagreement with other reports (Edmeades *et al*., 1999; Bänziger *et al*., 2000; Badu-Apraku *et al*., 2011a, 2013b; Edmeades, 2013, Talabi *et al*., 2017) indicating that ASI and stay green characteristics were among the key secondary traits under drought. Furthermore, the magnitude direct contributions of ear aspect, ears per plant, days to 50% anthesis and stalk lodging to grain yield implied that the hybrids combine excellent and increased number of ears per plant with relatively shorter maturity periods to increase grain yield under drought with a minimal level of stalk lodging.

Under low-N, ear aspect and days to 50% anthesis consistently had direct contributions to grain yield as occurred under drought substantiating the importance of these traits in controlling grain yield of the hybrids under the two stressful environments. The results also illustrated the importance of plant aspects as a key trait influencing grain yield under low-N as reported by Badu-Apraku *et al*. (2011a). The direct effect of ear height on grain yield might have accounted for the indirect effects observed for stalk and root lodging under low-N. Although stay green characteristics and ASI were not among the first order traits under low-N, an indirect contribution to grain yield was observed for stay green characteristics through ear aspect, and also for ASI through days to 50% anthesis. These results were however, in contrast with the results of other studies (Edmeades *et al*., 1999; Bänziger *et al*., 2000; Badu-Apraku *et al*., 2011a, 2013b; Edmeades, 2013) which identified ASI and stay green characteristics among the most reliable secondary traits under low-N. The high percentage (r^2 = 93%) of the total variation attributable to grain yield implies that the path co-efficient analysis and the set of traits used were effective in partitioning the total variation into the components.

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Under optimal environments, the direct positive contributions to grain yield by plant aspect, ear aspect and ears per plant indicated that the hybrids combined desirable plant architecture, excellent ears and prolificacy for increased grain yield. However, the direct minimal and negative contribution made by ear rot to grain yield implied that high grain yield was associated with lower ear rot under optimal conditions and this was indirectly regulated by tight husk cover. The inclusion of ASI in the third order category of secondary traits indicated that its contribution to grain yield under optimal conditions is not large enough to differentiate between high and low yielding hybrids.

Across drought, low-N and optimal environments, a very high percentage (r^2 = 96%) of the total variation in grain yield was accounted for demonstrating that the path co-efficient analysis effectively partitioned the variations attributable to the measured traits to grain yield across environments thus confirming that it is a useful tool for determining the contributions of secondary traits to grain yield in contrasting environments. The identification of the four traits, ear aspect, ears per plant, plant aspect and stay green characteristic as the first order contributors to grain yield of hybrids indicated the reliability of these traits for selection for improved grain yield under the contrasting environments confirming earlier reports by the following, (Bänziger and Lafitte, 1997; Edmeades *et al*., 1999; Bänziger *et al*., 2000; Betrán *et al*., 2003a; Badu-Apraku *et al*., 2011a, 2013b; Edmeades, 2013, Talabi *et al*., 2017). The observed direct positive minimal contribution of stalk lodging to grain yield suggested that high yielding hybrids bearing larger and more ears could slightly experience stalk lodging as a trade-off across stress and non-stress environments. The indirect contribution of ASI through almost all the second order traits despite its categorization as a third order trait across the contrasting environments demonstrated the importance of ASI to grain yield especially, under stressful environments. The lack of direct or indirect contribution by ear height to grain yield

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across environments indicated that ear placement has no relationship with *per se* grain yield and that variation in ear height would not be useful for selecting high yielding hybrids.

An important objective in this study was to identify high and stable yielding hybrids across drought, low-N and optimal environments for further testing and commercialization. Such hybrids with broad adaptation would be most preferred by farmers, especially in SSA because of the significant variation in seasons and production environments. On the contrary, in a few instances, hybrids with location-specific adaptation may be necessary. Significant GEI is advantageous when the objective is to develop location specific varieties characterized by narrow adaptation. However, it is a disadvantage when developing varieties for broad adaptation (Badu-Apraku and Fakorede, 2017). The observed significant GEI mean squares for grain yield and most other measured traits under drought, low-N, optimal and across environments indicated that the expression of the traits varied with environments. This observation therefore, warranted the use of the "which-won-where" and the "mean performance vs. stability" GGE biplot views to identify hybrids with location-specific and broad adaptations, respectively. From the "which-won-where" view of the GGE biplot, the first and the second principal component axes (PC1 and PC2) explained 77.7% of the variation in grain yield of the hybrids indicating that PC1 and PC2 sufficiently approximated the environment-centred data. The hybrid, TZEIORQ 29 x TZEIORQ 43 (entry 2) was the highest yielding in environments E2 (2016 low-N, Ile-Ife), E3 (2016 low-N, Mokwa) and E12 (2017 optimal, Mokwa). These resulte suggested that TZEIORQ 29 x TZEIORQ 43 would display superior performance in nitrogen deficient environments at Ile-Ife and Mokwa without compromising yield under optimal environments at Mokwa. Also, E2, E3 and E12 could form a mega-environment because they shared the same winning hybrid. However, several years of testing in these three locations would be needed to ascertain whether they are indeed mega-environments or not. Yan *et al*. (2000, 2007, and 2010) pointed out that the decision as to

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whether location-groups could be considered as mega-environments or not is based on the consistency of the location groupings and of the winning genotypes in the individual location-groups across years. Hybrid TZEIORQ 26 x TZEIORQ 47 (entry 20) was the highest yielding in environments E1 (2016 drought, Ikenne), E4 (2017 low-N, Ile-Ife), E5 (2017 low-N, Mokwa), E6 (2018 combined drought and heat, Kadawa) and E10 (2017 optimal, Ile-Ife). These results indicated that TZEIORQ 26 x TZEIORQ 47 would be high yielding across drought and low-N environments at Ikenne, Kadawa, Ile-Ife and Mokwa with an acceptable performance under optimal conditions at Ile-Ife. What was also interesting with these results was that TZEIORQ 26 x TZEIORQ 47 would be the preferred hybrid under drought at Kadawa located in the semi-arid/Sudan Savanna agro-ecology of Nigeria. Additionally, TZEIORQ 24 x TZEIORQ 41 (entry 22) and TZEIORQ 23 x TZEIORQ 44 (entry 13) were the highest yielding hybrids only in environment E9 (2017 optimal, Mokwa) suggesting that these two hybrids were the most promising for the non-stressful environment at Mokwa. Moreover, the "mean performance vs. stability" GGE biplot view identified TZEIORQ 24 x TZEIORQ 41 (entry 22) and TZEIORQ 26 x TZEIORQ 47 (entry 20) as the highest yielding hybrids across the 12 test environments. Of the two hybrids, TZEIORQ 24 x TZEIORQ 41 was the most stable across the test environments. This hybrid should be tested extensively to confirm the consistency of performance and commercialized in SSA.

5.5 Conclusions

Additive genetic effects were more important than the non-additive for grain yield and most of the measured agronomic traits of the 96 early PVA-QPM hybrids under drought, low-N, optimal and across environments. There were no significant differences in the GCA-male and GCA-female effects for grain yield and most traits under drought, low-N, optimal and across environments and that maternal and paternal effects were equally important in the inheritance of most traits. The more efficient heterotic grouping method, SNP-based DArT markers,

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classified the parental inbreds into three heterotic groups based primarily on pedigrees of origin and TZEIORQ 29 was identified as the best male and female tester for heterotic group I, while TZEIORQ 24 was the best male tester for heterotic group II. Significant correlations were found between mid-parent values and the means of the corresponding hybrids for grain yield, plant height, ear aspect and stay green characteristic under drought, low-N, optimal and across environments suggesting that initial screening and selection of drought and low-N tolerant inbreds for the development of superior hybrids would be effective. Four traits comprising ear aspect, ears per plant, plant aspect and stay green characteristic were identified as the first order contributors to grain yield of hybrids indicating the reliability of these traits for selection to improve grain yield under the contrasting environments. The hybrid TZEIORQ 2 x TZEQI 82 was identified as a single cross hybrid tester across drought, low-N and optimal environments. TZEIORQ 26 x TZEIORQ 47 would be the most preferred hybrid for drought and low-N stressful environments of Nigeria. The hybrid TZEIORQ 24 x TZEIORQ 41 was the highest yielding and most stable across stressful and non-stressful environments and should be further tested to confirm the consistency of performance for commercialization in SSA.

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CHAPTER SIX

6.0 Study of the gene action controlling carotenoids and validation of provitamin A candidate genes in early maturing provitamin A-quality protein maize inbreds

6.1 Introduction

Vitamin A is an essential micro-nutrient required by human beings for improved eye sight and enhanced immune system. Unfortunately, humans are unable to synthesise vitamin A and must rely on external sources. The provitamin A (PVA) maize has the potential of supplying more than the daily dietary requirement of 15.0 µg g^{-1} DW PVA in humans to alleviate vitamin A deficiency (VAD) compared to about 2.0µg g^{-1} DW in the commonly cultivated and consumed yellow maize (Pixley *et al*., 2013). In spite of this potential, progress has been slow for the improvement of PVA carotenoids in developed maize varieties in SSA. This is partly due to the inconsistent reports on the gene action controlling the inheritance of the PVA trait. Several workers have indicated the preponderance of additive over non-additive genetic effects in determining PVA concentrations in maize (Egesel *et al*., 2003; Suwarno, 2012; Suwarno *et al*., 2015; Menkir *et al*., 2014; Owens *et al*., 2014), suggesting that recurrent selection for PVA content should be effective (Coors, 1999). Furthermore, positive correlations among individual carotenoids (Kurilich and Juvik, 1999; Menkir *et al*., 2008; Owens *et al.*, 2014), and between grain yield and PVA content (Suwarno *et al*., 2015; Menkir *et al*., 2014) have been reported. These findings suggest that simultaneous increases in PVA carotenoids and grain yield may be effective (Pfeiffer and McClafferty, 2007; Bouis and Welch, 2010; Menkir *et al*., 2014). On the contrary, other studies have indicated the importance of non-additive gene action over additive in controlling the PVA carotenoid concentrations (Halilu *et al.*, 2016), with grain yield showing no significant genotypic correlations with carotenoid content, suggesting that the two traits can be improved concurrently.

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Three genes, phytoene synthase1 (*PSY1*), lycopene epsilon cyclase (*LcyE*), and β-carotene hydroxylase1 (*crtRB1*) have been identified to regulate the key steps involved in the accumulation of PVA carotenoids (Wurtzel *et al*., 2012). Among the three genes, *crtRB1* (-*5'TE and 3'TE*) is the most functional for increased β-carotene levels (Babu *et al*., 2013). Two of the three significant polymorphic sites of *LcyE* (-5'TE and 3' indel) have been validated using 26 different tropical segregating populations Babu *et al*. (2013). In addition, the Kompetitive allele-specific PCR (KASP) SNP, snpZM0015 located inside the *crtRB1* gene on chromosome 10 has been optimized and recommended for accelerating PVA improvement in maize (Intertek Group Plc., Sweden, unpublished). Based on the inconsistent knowledge of the inheritance of the PVA trait and the availability of functional markers to accelerate PVA accumulation in maize, this study was conducted to generate information on the gene action conditioning the PVA carotenoids and to validate the functional PVA genes in the early PVA-QPM inbred population. This information is needed to guide breeding strategies for developing superior early PVA-QPM hybrids. The objectives of the present study were to:

i. assess the combining ability of selected inbreds for PVA carotenoids,
ii. identify superior hybrids that combine elevated levels of PVA and tryptophan, and
iii. identify inbred lines harbouring the functional *crtRB1* and *LcyE* genes to serve as donor parents of the favourable alleles.

6.2 Materials and methods

6.2.1 Genetic materials

Based on the grain yield performance of the 96 early maturing PVA-QPM hybrids evaluated under drought, low-N and optimal environments in Chapter five, 54 hybrids involving 18 parental lines were selected for the present study. In addition, TZEIOR 127 x TZEIOR 57 was used as a hybrid check. The hybrid check did not have quality protein

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background because drought and low-N tolerant single crosses with elevated levels of PVA and quality protein were not available. The inbred lines involved in the generation of the single cross hybrids were the inbreds assayed for their levels of PVA and tryptophan in chapter three. These inbred lines, which relatively good general combining abilities across contrasting environments included TZEIORQ 29, TZEIORQ 45, TZEIORQ 48, TZEIORQ 20, TZEIORQ 6, TZEIORQ 44, TZEIORQ 42, TZEIORQ 59, TZEIORQ 23, TZEIORQ 47, TZEIORQ 7, TZEIORQ 13, TZEIORQ 2, TZEIORQ 5, TZEIORQ 26, TZEIORQ 24, TZEIORQ 43 and TZEIORQ 40. For the PVA candidate genes validation, all the 70 inbreds evaluated in Chapter three were used. The pedigree information of the inbreds are described in Chapter three section 3.2.1.

6.2.2 Production of hybrid kernel samples for carotenoids and tryptophan analyses

The 54 NCD II hybrids plus the check were planted under well-watered growing conditions in January, 2018 at IITA Ibadan to produce kernel samples for carotenoids and tryptophan analyses. The spacing, controlled self-pollination, sampling procedure, extraction and analyses of PVA carotenoids and tryptophan contents were carried out as described in Chapter three Sections 3.2.7 to 3.2.9. Analysis of samples for PVA carotenoids and tryptophan were done with two replications for statistical analysis.

6.2.3 Statistical analysis of carotenoids and tryptophan contents of the early maturing provitamin A-quality protein maize hybrids

The carotenoids and tryptophan data for the selected hybrids were transformed using natural logarithm to allow statistical analysis, as the ratios would not have followed a normal distribution curve without the transformation. The North Carolina Design II ANOVA for carotenoids and tryptophan of the hybrids were performed using PROC GLM in SAS version 9.4 (SAS Institute, 2012), and means were separated using standard error of difference (SED).

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Broad sense heritability estimates were computed for the carotenoid and tryptophan contents as illustrated in section 3.2.4. The general linear model used for the NCD II mating design was:

$$X_{ijkl} = \boldsymbol{\mu} + \boldsymbol{S_l} + \boldsymbol{g_i(S_l)} + \boldsymbol{g_j(S_l)} + \boldsymbol{h_{ij}(S_l)} + \boldsymbol{r_k(S_l)} + \boldsymbol{e_{ijkl}}$$

Where:

X_{ijkl}= The observed value of the progeny of the ith female, jth male in the kth replication within set l.

$\boldsymbol{\mu}$ = population mean,

$\boldsymbol{S_l}$= average effect of the lth set,

$\boldsymbol{g_i(S_l)}$= GCA effect common to all hybrids of the ith female nested within lth set,

$\boldsymbol{g_j(S_l)}$= GCA effect common to all hybrids of the jth male nested within lth set,

$\boldsymbol{h_{ij}(S_l)}$= SCA effect of hybrid from the ith female and jth male nested within lth set,

$\boldsymbol{r_k(S_l)}$ = effect of the kth replication nested within the lth set,

$\boldsymbol{e_{ijkl}}$= the experimental error (Singh and Chaudhary, 1985).

The hybrid component of the variation was decomposed into variation due to male sets, female sets and female × male interactions sets in the NCD II ANOVA. The F-test for male, female and female × male mean squares was calculated using the experimental error. The main effects of the male sets and female sets is the GCA effect while the female × male sets interaction represents SCA effects (Hallauer and Miranda, 1988). The form of ANOVA used for the NCD II and repeated over environments is presented in Table 6.1.

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Table 6. 1 Form of the analysis of variance of North Carolina Design II

source of variation	degrees of freedom(df)*	mean squares(ms)	expected mean squares
Sets (S)	s-1		
Replications (S)	s(r-1)		
Males (S)	s(m-1)	M_1	$\sigma_e^2 + r\sigma^2_{mf} + rf\sigma^2_m$
Females (S)	s(f-1)	M_2	$\sigma_e^2 + r\sigma^2_{mf} + rm\sigma^2_f$
Males x Females (S)	s(m-1)(f-1)	M_3	$\sigma_e^2 + r\sigma^2_{mf}$
Error	s(r-1)(mf-1)	M_4	σ_e^2
Total	srmf-1		

*s, r, m, and f refer to the number of sets, replications within sets, males and females, respectively.

The proportionate contribution of PVA and other carotenoids was computed as percentage of the sum of squares for the crosses attributable to general combining ability (GCA) and specific combining ability (SCA) as follows:

Contribution of GCA-male = [ssm / (ssm + ssf + ssmf) x 100]

Contribution of GCA-female = [ssf / (ssm + ssf + ssmf) x 100]

Contribution of SCA (%) = [ssmf / (ssm + ssf + ssmf) x 100]

where:

ssm = sum of squares due to males within sets,

ssf = sum of squares due to females within sets,

ssmf = sum of squares due to male x female within sets interaction, (Hallauer and Miranda, 1988).

The relative GCA-males, GCA-females and SCA effects for grain yield and other traits were computed from the adjusted means using line x tester approach (Singh and Chaudhary, 1985)

GCA-male = $\overline{X}_j - \overline{Y}$

GCA-female = $\overline{X}_i - \overline{Y}$

SCA = $\overline{X}_{ij} - \overline{X}_i - \overline{X}_j + \overline{Y}$

where:

$\overline{X}_j$ = the mean of hybrids with a given male averaged over replicates and females,

$\overline{X}_i$ = the mean of hybrids with a given female averaged over replicates and males,

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$\overline{X}_{ij}$ = the mean of a given hybrid averaged over replicates and females,

$\overline{Y}$ = the experimental mean.

Standard errors for GCAs effects were calculated as described by Cox and Frey (1984):

$SE_{GCA} = [MS_f (f-1) / mfr]^{½}$ or $[MS_m (m-1) / mfr]^{½}$

$SE_{SCA} = [MS_{fm}(m-1)(f-1) / mfr]^{½}$

Where, MS_f, MS_m and MS_{fm} are the respective female within set, male within set and female x male within set interaction mean squares and were multiplied by the appropriate proportion of total number of observations (female x male x replicate). The significance of the GCA-female, GCA-male, and SCA effects of the individual inbred lines were determined using the respective standard error of difference (SED). In addition, the F test or variance ratio was used to compare the mean squares of the GCA male and female as suggested by Kearsey and Pooni (1996) and to determine the relative significance of cytoplasmic effects.

Correlations among PVA and other carotenoids, kernel colour, tryptophan and grain yield of the hybrids were calculated to examine the relationships among the traits and to provide information on the breeding strategies to adopt to improve the traits. Correlation coefficients were computed using the Spearman rank correlation method implemented in SAS version 9.4 (SAS Institute, 2012).

6.2.4 Validation of provitamin A functional genes in 70 early maturing provitamin A-quality protein maize inbred lines using allele specific markers

6.2.4.1 Leaf sample collection and DNA extraction

Maize leaf samples were collected from 10 plants (one leaf per plant) of each inbred line at 2 weeks after planting. Freeze drying of leaf samples, genomic DNA samples isolation, DNA quality determination and concentrations used were as described in chapter four, section 4.2.2.

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6.2.4.2 PCR based genotyping

PCR based functional markers of the *crtRB1* gene were tested across the 70 inbred lines. Primer sequence, PCR reaction conditions and thermal cycling profiles were modified based on the method of Yan *et al*. (2010) and Harjes *et al*. (2008). The primer pair, forward: crtRB1_3TE_T_F1-TCTTTTCACCGCCCTTTT; and reverse: crtRB1_3TE_T_R1c – AACAGCAATACAGGGGAC used to amplify the crtRB1-3'TE marker, and primer pair, forward:–CGTGACCATATGTACACTCTC; reverse: -TCACCGGATATGGCACTGG used to amplify the LcyE_5'TE were ordered from Integrated DNA Technology Inc (IDT, Belgium). PCR amplifications of 1 µl sample DNA (30 ng/µl) were performed using OneTaq Quick-Load 2x Master Mix with Standard Buffer (New England BioLabs Inc. UK) with a mixture composed of 5 µl of 2x Master Mix with Standard Buffer, 0.5 µl of each primer, and ultra-pure water making up to 10 µl total reaction volume. PCR thermal cycling profile was 1 cycle of initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 1min, and extension at 68°C for 1 min. This was followed by 1 cycle of final extension at 68°C for 5 min and hold at 4°C. Fragments in the PCR products were resolved on 2% agarose gel. The polymorphic sites of the crtRB1-3'TE gene used has a 325/1250 bp indel, with 595 bp amplicon being the favourable allele, while 920 and 1845 bp are the unfavourable alleles. Also, the polymorphic sites of the LcyE-5'TE gene used has a 401/1567 bp indel, with 595 bp amplicon being the favourable allele and 1845 bp being the unfavourable allele. The names of polymorphic sites of the 3 PCR-base functional markers and the nature of polymorphisms are indicated in Table 6.2 as per their respective references (Azmach *et al*., 2013).

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Table 6.2 Nomenclature and allelic series of the three functional markers according to their references

Gene	Polymorphic site/Marker(gene name-polymorphism)	Nature of Polymorphism	Allelic series and notations ϕ
LCYE (Harjes *et al*., 2008)	*LcyE*-5'TE	401/1567 bp indel	**1**, 2, 3, **4**
crtRB1 (Yan *et al*., 2010)	*crtRB1-5'TE*	397/206 bp indel	1, **2**, 3
	crtRB1-3'TE	325/1250 bp indel	**1**, 2, 3

ϕ Allelic variants indicated in bold face underlined letters represent the best favourable alleles as described in the references.

6.2.4.3 Kompetitive allele specific PCR (KASP) genotyping

Genomic DNA isolated from leaf tissue of the 70 maize inbred lines was used as template for the KASP genotyping reaction. Sample DNA was diluted to a working concentration of 30 ng/ µl for use in the KASP genotyping reaction. KASP assay, snpZM0015, was used to investigate the presence and/or absence of the favourable alleles for the crtRB1 gene. KASP reaction was performed in a 96-well plate in a reaction volume of 10 µl consisting 5 µl template DNA and 5 µl of the prepared genotyping mix (2x KASP master mix and primer mix). Protocols for the preparation and running of KASP reactions are presented in the KASP manual (http://www.kbioscience.co.uk, accessed on 2nd July 2018). KASP assay kit was purchased from LGC Genomics (LGC Group, UK). All amplification reactions were performed using the Roche LightCycler 480 II (LC480 II) System (Roche-Life Science, USA) at the Bioscience Centre of IITA Ibadan, Nigeria. Amplification condition was as follows: 1 cycle of KASP special Taq activation at 94°C for 15 min, followed by 36 cycles of denaturation at 94°C for 20 s, and annealing and elongation at 60°C (dropping 0.6°C per cycle) for 1 min. Endpoint detection of the fluorescence signal was acquired for 1 min at 30°C using the same instrument. Genotyping results (amplifications for favourable and unfavourable alleles) were analysed using Klustercaller software (LGC Group, UK) and genotyping data

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were visualized as cluster plots and downloaded using SNPviewer software (LGC Group, UK). Allele calls for the SNP was made based on validation result provided by Intertek (Intertek Group Plc., Sweden), as homozygous AA for favourable allele, homozygous GG for unfavourable allele, or heterozygous AG for both alleles.

6.3 Results

6.3.1 Analysis of Variance of provitamin A carotenoid and tryptophan concentrations

The NCD II analysis of variance of the nutritional data revealed significant ($P < 0.05$ or $P < 0.01$) variation among set and hybrid mean squares for all PVA carotenoids as well as tryptophan (Table 6.3). Significant differences were observed among GCA-male, GCA-female, and SCA effects for all PVA carotenoids, and tryptophan except the SCA effects of β-cryptoxanthin, β-carotene and PVA. Broad sense heritability estimates varied from 43% for β-cryptoxantine to 95% for lutein among the quantified carotenoids, and 96% for tryptophan.

Table 6. 3 Mean squares of carotenoids and tryptophan of 54 selected early maturing provitamin A quality protein maize hybrids along with one check

		Mean squares of Carotenoids§ (μg g^{-1}dry weight)							
Source	**DF**	**Lut**	**Zeax**	**β-cryp**	**α-caro**	**β-caro**	**ϮPVA**	**Tcaro**	**Tryp**
Set	5	78.13**	94.39**	1.76*	0.24**	6.49**	9.67**	383.83**	0.00085**
Rep (Set)	6	26.40	30.10	2.46	0.02	10.09	11.27	151.62*	0.00013**
Hybrid	54	52.69**	23.58**	1.36*	0.15**	2.16*	4.07**	94.95**	0.00111**
Male (Set)	12	71.16**	22.61**	2.04**	0.23**	2.49**	5.23**	117.27**	0.00085**
Female (Set)	12	86.95**	27.94**	1.76*	0.21**	3.65**	6.29**	77.02**	0.00120**
Female*Male (Set)	24	21.53**	7.26**	0.39	0.05**	0.33	0.83	35.95**	0.00086**
Error	48	2.07	2.01	0.56	0.01	0.42	0.53	7.79	0.00002
Heritability		0.95	0.75	0.43	0.91	0.82	0.72	0.85	0.96

*, **, = significant at $P < 0.05$ and 0.01, respectively; §Carotenoids are abbreviated as Lut= lutein, Zeax= Zeaxanthin, β-cryp= β-cryptoxanthin, α-caro= alpha-carotene, β-caro= β-carotene, ϮPVA= provitamin A and Tcaro= total carotenoid; Tryp= tryptophan.

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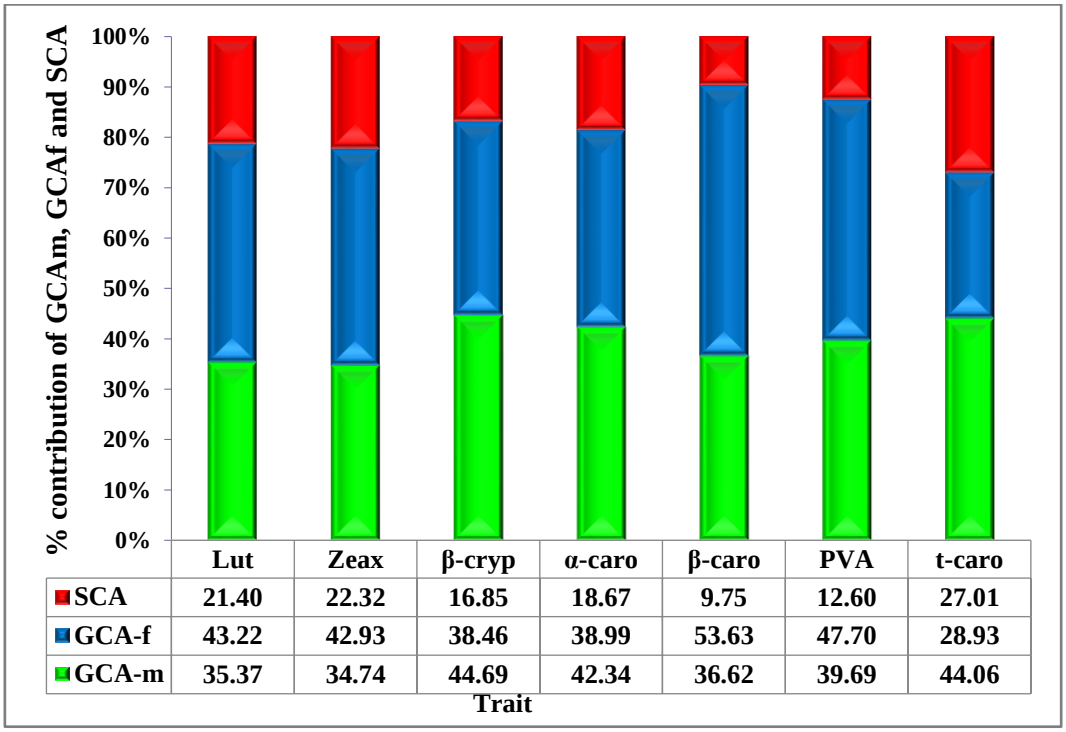
6.3.2 Proportionate contributions of combining ability effects of provitamin A carotenoids of the early provitamin A-quality protein maize inbred lines

The proportionate contributions of GCA (GCA-male and GCA-female) and SCA effects of PVA and other carotenoids are presented in Figure 6.1. The GCA effects were greater than SCA effects for PVA and all carotenoids. The GCA effects compared to their respective genotypic sum of squares ranged from 73% for total carotenoids to 90% for β-carotene while SCA ranged from 10% for β-carotene to 27% for total carotenoids. GCA effects accounted for 87% for PVA, while the three PVA carotenoids, β-cryptoxanthin, α-carotene and β-carotene had GCA effects of 83, 81 and 90% respectively. Although the variation among GCA-male and GCA-female effects was not significant for tryptophan and all carotenoids, it was worth noting that the GCA-female effects for PVA and β-carotene were relatively larger than the GCA-male effects (Fig. 6.1).



	Lut	Zeax	β-cryp	α-caro	β-caro	PVA	t-caro
SCA	21.40	22.32	16.85	18.67	9.75	12.60	27.01
GCA-f	43.22	42.93	38.46	38.99	53.63	47.70	28.93
GCA-m	35.37	34.74	44.69	42.34	36.62	39.69	44.06

Figure 6.1 Proportion of total genotypic sum of squares for carotenoids of early maturing provitamin A quality protein maize inbred lines attributable to general combining ability and specific combining ability effects. Lut= Lutein, Zeax= Zeaxanthin, β-cryp= β-cryptoxanthin, α-caro= alpha-carotene, β-caro= β-carotene, PVA= provitamin A and tcaro= Total carotenoids.

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6.3.3 General combining ability (GCA-male and GCA-female) effects for provitamin A carotenoids of the selected inbred lines

Out of the eighteen selected parental lines, only TZEIORQ 29 recorded highly significant (P< 0.01 and < 0.05) positive GCA-male and GCA-female effects for PVA, β-carotene, α-carotene and β-cryptoxanthin (Table 6.4). The inbred line TZEIORQ 29 had significant (P< 0.01 and < 0.05) negative GCA-male and GCA-female effects for Zeaxanthin which is a non-PVA carotenoid. Also, TZEIORQ 13 had significant positive (P< 0.05) GCA-female effects for PVA and β-carotene. However, significant (P< 0.05) negative GCA-male and GCA-female effects for total carotenoids were detected for TZEIORQ 13.

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Table 6.4 General combining ability effects of carotenoids (µg g^{-1} Dry weight) for selected early provitamin A quality protein maize inbred lines

	Lutien		Zeaxanthin		β-cyptoxanthin		α-carotene		β-carotene		Provitamin A		Total carotenoids	
Inbred	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**	**GCA-m**	**GCA-f**
TZEIORQ 29	2.35	5.09	-3.51*	-4.13**	1.39**	1.23**	0.47**	0.38**	1.33**	1.48*	2.26**	2.27**	2.03	4.04
TZEIORQ 45	1.02	-2.29	2.79*	0.79	-0.48	-0.84	-0.11	-0.23	-0.16	-0.56	-0.46	-1.10	3.06	-3.14
TZEIORQ 48	-3.36	-2.80	0.71	3.34*	-0.91*	-0.39	-0.36*	-0.15	-1.17*	-0.92	-1.80*	-1.19	-5.10	-0.91
TZEIORQ 20	-2.52	-3.24	1.50	2.35	0.47	0.35	0.01	0.07	0.36	0.09	0.59	0.30	-0.20	-0.38
TZEIORQ 6	-1.60	-2.57	-1.23	-1.38	-0.16	0.29	-0.10	-0.04	-0.31	-0.31	-0.44	-0.18	-3.40	-4.00
TZEIORQ 44	4.12	5.81*	-0.26	-0.97	-0.31	-0.65	0.10	-0.03	-0.05	0.22	-0.16	-0.12	3.59	4.38
TZEIORQ 42	1.78	0.52	0.67	-0.79	0.01	-0.03	-0.02	-0.19	0.37	0.13	0.37	0.02	2.80	-0.36
TZEIORQ 59	2.85	3.05	0.62	0.76	-0.30	-0.15	0.01	0.07	0.03	0.39	-0.13	0.35	3.20	4.12
TZEIORQ 23	-4.63	-3.57	-1.29	0.03	0.29	0.18	0.03	0.12	-0.40	-0.52	-0.24	-0.37	-6.00*	-3.77
TZEIORQ 47	3.86	4.97	2.37	0.26	0.07	-0.42	0.11	0.06	-0.12	-0.86	-0.03	-1.04	6.29*	4.01
TZEIORQ 7	0.29	-0.08	0.59	1.68	-0.05	0.39	0.01	-0.24	-0.65	-0.41	-0.67	-0.34	0.20	1.34
TZEIORQ 13	-4.15	-4.90	-2.96*	-1.95	-0.03	0.03	-0.11	0.19	0.77	1.28*	0.70	1.38*	-6.48*	-5.35*
TZEIORQ 2	1.52	-0.49	1.14	1.06	-0.07	-0.05	-0.08	-0.03	0.12	0.14	0.05	0.10	2.63	0.63
TZEIORQ 5	-2.66	-0.94	-0.89	-1.38	-0.10	-0.2	-0.01	-0.06	-0.21	-0.26	-0.26	-0.38	-3.87	-2.83
TZEIORQ 26	1.14	1.43	-0.25	0.33	0.17	0.25	0.09	0.09	0.09	0.11	0.22	0.28	1.23	2.20
TZEIORQ 24	-3.90	2.58	0.90	-2.25	0.59	-0.10	0.14	-0.10	-0.33	-0.64	0.03	-0.74	-2.61	-0.52
TZEIORQ 43	1.62	-1.30	-0.69	1.17	-0.34	-0.01	-0.11	-0.02	0.05	0.10	-0.18	0.08	0.52	-0.06
TZEIORQ 40	2.28	-1.28	-0.21	1.08	-0.25	0.10	-0.03	0.13	0.29	0.54	0.15	0.66	2.08	0.58
SED	2.52	2.78	1.29	1.44	0.39	0.36	0.13	0.12	0.43	0.52	0.62	0.68	2.95	2.39

*, ** Significant at 0.05 and 0.01 probability levels respectively; GCA-m, GCA-f = general combining ability of male and female effects respectively.

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6.3.4 Identification of superior hybrids combining elevated levels of provitamin A and tryptophan with drought and low-N tolerance

Zeaxanthin and lutein were the dominant carotenoids identified with average mean values of 15.72 and 14.90 µg g^{-1} respectively, followed by β-carotene, 4.45 µg g^{-1}, β-cryptoxanthin, 3.12 µg g^{-1} and α-carotene, 1.04 µg g^{-1} (Table 6.5). Generally, the α-carotene contents of the hybrids were very low compared with the levels of the other carotenoids. PVA levels varied from 3.02 µg g^{-1} for TZEIORQ 6 x TZEIORQ 48 to 9.82 µg g^{-1} for TZEIORQ 29 x TZEIORQ 40 with a mean of 6.54 µg g^{-1}. Tryptophan contents varied from 0.013% for TZEIORQ 23 x TZEIORQ 6 to 0.198% for Obatanpa (the check) with a mean of 0.118%, and Obatanpa was 37% higher in tryptophan content than the second best inbred, TZEIORQ 42. Over 98% of the hybrids had > 0.075% tryptophan in sample in whole grain. Grain yield across environments of the selected hybrids ranged from 1727 kg ha^{-1} for TZEIORQ 23 x TZEIORQ 6 to 5151 kg ha^{-1} for TZEIORQ 26 x TZEIORQ-47 with a mean of 4319 kg ha^{-1}. Grain yield and PVA contents of the top six hybrids including the best check, TZEIOR 127 x TZEIOR 57 based on PVA ranking were not significantly different from each other. Similarly, grain yield and PVA content of the highest yielding and most stable hybrid, TZEIORQ 24 x TZEIORQ 41 was not significantly different from the best check. Generally, the drought and low-N tolerant hybrids had relatively higher levels of PVA than the susceptible hybrids (Table 6.5). Grain yield significantly and positively ($P< 0.01$ and < 0.05) correlated with PVA, β-cryptoxanthin, α-carotene and β-carotene but not with total carotenoids, lutein, Zeaxanthin and tryptophan. The estimated correlation coefficients among PVA and its component carotenoids were highly significant and positive ($P< 0.001$) but not with tryptophan (Table 6.6). Significant ($P< 0.05$) correlation was obtained between PVA and total carotenoids. Similarly, the non-PVA carotenoids, lutein and Zeaxanthin showed highly significant positive ($P< 0.001$) correlations with total carotenoids but not with PVA and its carotenoids.

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Table 6. 5 Contents of carotenoids and tryptophan of 27 early provitamin A quality protein maize hybrids (22 best and 4 worst) plus one check, and their grain yield across drought, low-N and optimal environments along with their reactions to the two stresses

	Carotenoids[a] (µg g^{-1}dry weight)								Yield Across	§Reaction to		
Hybrid	**Lut**	**Zeax**	**β-cryp**	**α-caro**	**β-caro**	**ϮPVA**	**Tcar**	**Tryp (%)**	**Env (kg ha^{-1})**	**Drought**	**Low N**	**Combined**
TZEIORQ 29 x TZEIORQ 40	25.61	14.23	4.19	1.44	7.01	9.82	52.47	0.139	4500.42	T	T	T
TZEIORQ 29 x TZEIORQ 43	22.73	13.63	4.06	1.31	7.09	9.78	48.82	0.136	4548.68	T	T	T
TZEIORQ 29 x TZEIORQ 24	13.85	12.54	4.94	1.46	5.64	8.84	38.44	0.132	4642.06	T	T	T
TZEIOR 127 x TZEIOR 57 (check)	11.17	19.11	4.95	1.44	5.26	8.46	41.94	-	4267.89	T	T	T
TZEIORQ 20 x TZEIORQ 29	15.09	10.99	4.64	1.55	5.32	8.42	37.59	0.130	4183.61	T	T	T
TZEIORQ 6 x TZEIORQ 29	17.27	10.23	4.83	1.50	5.11	8.27	38.94	0.104	4415.98	T	T	T
TZEIORQ 13 x TZEIORQ 42	12.56	14.04	2.21	0.98	6.01	7.61	35.80	0.122	4548.50	T	T	T
TZEIORQ 13 x TZEIORQ 59	13.02	15.46	2.22	1.01	5.90	7.52	37.62	0.146	4384.51	T	T	T
TZEIORQ 13 x TZEIORQ 23	9.23	11.96	2.94	0.97	5.28	7.23	30.37	0.134	4513.81	T	T	T
TZEIORQ 44 x TZEIORQ 29	22.28	9.59	3.08	1.28	4.98	7.17	41.21	0.126	4687.49	T	T	T
TZEIORQ 24 x TZEIORQ 41	15.25	16.91	2.79	1.29	5.00	7.03	41.42	0.081	4954.71	T	T	T
TZEIORQ 45 x TZEIORQ 24	11.62	22.04	3.30	1.16	4.74	6.97	42.85	0.093	4608.05	T	T	T
TZEIORQ 40 x TZEIORQ 26	15.70	19.34	3.32	1.30	4.60	6.90	44.25	0.139	5014.33	T	T	T
TZEIORQ 7 x TZEIORQ 42	17.01	19.38	3.44	0.57	4.52	6.53	44.92	0.112	4821.66	T	T	T
TZEIORQ 59 x TZEIORQ 20	18.86	18.89	2.73	1.05	4.61	6.50	46.14	0.107	4262.57	T	T	T
TZEIORQ 43 x TZEIORQ 26	16.90	20.77	3.19	1.18	4.25	6.44	46.30	0.092	4792.13	T	T	T
TZEIORQ 48 x TZEIORQ 40	15.55	22.32	2.75	0.96	4.58	6.43	46.15	0.133	5111.23	T	T	T
TZEIORQ 20 x TZEIORQ 45	12.47	19.29	2.98	1.01	3.90	5.90	39.65	0.084	4854.25	T	T	T
TZEIORQ 48 x TZEIORQ 43	13.24	19.59	2.55	0.82	4.05	5.73	40.25	0.102	4812.20	T	T	T
TZEIORQ 42 x TZEIORQ 20	12.71	15.74	2.95	0.36	3.94	5.60	35.70	0.143	4822.56	T	T	T
TZEIORQ 23 x TZEIORQ 44	14.23	15.82	2.79	1.11	3.39	5.34	37.34	0.120	4908.37	T	T	T
TZEIORQ 47 x TZEIORQ 23	13.63	15.88	2.64	1.04	3.04	5.11	36.23	0.118	4608.27	T	T	T
TZEIORQ 26 x TZEIORQ 47	17.27	13.56	2.64	1.04	3.03	4.87	37.54	0.105	5151.23	T	T	T
TZEIORQ 5 x TZEIORQ 7	11.62	11.85	2.20	0.83	2.59	4.10	29.08	0.109	2007.11	S	S	S
TZEIORQ 23 x TZEIORQ 6	14.19	14.40	2.01	0.65	2.54	3.87	33.79	0.031	1726.66	S	S	S
TZEIORQ 20 x TZEIORQ 48	10.32	18.08	1.82	0.57	2.47	3.66	33.25	0.116	3198.92	S	S	S
TZEIORQ 6 x TZEIORQ 48	9.18	9.93	1.72	0.47	1.93	3.02	23.23	0.121	2895.67	S	S	S
†OBATANPA (check)	-	-	-	-	-	-	-	0.198	-	-	-	-
Mean	14.90	15.76	3.11	1.47	4.47	6.56	39.30	0.118	4342.33	-	-	-
SED	1.51	1.60	0.62	0.06	0.86	0.93	3.45	0.004	226.09	-	-	-

[a]Carotenoids are abbreviated as Lut= lutein, Zeax= Zeaxanthin, β-cryp= β-cryptoxanthin, α-caro= alpha-carotene, β-caro= β-carotene, ϮPVA= provitamin A and Tcaro= total carotenoids, Tryp= tryptophan, Env= environments, T= Tolerant, S= Susceptible.

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Table 6. 6 Phenotypic correlation among traits of selected provitamin A quality protein maize hybrids under optimal environments in Nigeria, 2018

Trait	ϮPVA	β-cryp	α-caro	β-caro	Tcaro	Lut	Zeax	Tryp
β-cryp	0.73***							
α-caro	0.63***	0.72***						
β-caro	0.94***	0.51***	0.47**					
Tcar	0.33*	0.29*	0.39**	0.32*				
Lut	0.04	0.02	0.21	0.09	0.83***			
Zeax	0.15	0.24	0.13	0.04	0.54***	0.14		
Tryp	0.075	0.057	0.182	0.052	0.244	0.115	0.128	
YIELD	0.40**	0.38**	0.31*	0.33*	0.18	0.04	0.21	-0.092

Carotenoids are abbreviated as ϮPVA= provitamin A; β-cryp= β-cryptoxanthin; α-caro= alpha-carotene; β-caor= β-carotene; Tcaro= Total carotenoid; Lut= Lutein; Zeax= Zeaxanthin; Tryp= Tryptophan; Yield= grain yield across drought, low N and optimal environments.

6.3.5 Inbred lines carrying alleles of LcyE and crtRB1 markers proposed for elevated provitamin A in maize endosperm

Among the 3 PCR-based functional markers used, the crtRB1_3'TE_T_F1/R1C showed clear amplification with the expected band size (595 bp, labelled in red) for eight (~11%) out of the 70 inbred lines tested (Fig. 6.2). The eight inbred lines carrying the functional gene (*crtRB1*) were entries 8 to 14, and 70 representing inbred lines TZEIORQ 10, TZEIORQ 12, TZEIORQ 13, TZEIORQ 14, TZEIORQ 15, TZEIORQ 16, TZEIORQ 17 and TZEI 129. They had moderate PVA levels in respective order of 5.53, 6.26, 7.90, 5.92, 7.48 5.40, 8.93 and 6.41 µg g^{-1}. TZEIORQ 55 which had the highest PVA level of 15.38 µg g^{-1} (entry 68) was identified as heterozygous, having both the favourable and the unfavourable alleles (with faint bands). On the other hand, the *crtRB1-5'TE* and the *LcyE-5'TE* primers did not amplify with their respective band sizes of 400 bp and 250 bp across the entire panel of the inbred lines. The KASP SNP (snpZM0015) effectively distinguished between favourable and unfavourable alleles for the *crtRB1* gene in the 70 inbreds (Fig. 6.3). All the eight inbreds that were amplified for the *crtRB1* gene using the gel-based *crtRB1-3'TE* marker were also

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identified by the KASP SNP, snpZM0015 for the same gene [A:A (blue)= Lines with favourable allele]. Correspondingly, snpZM0015 found TZEIORQ 55 carrying the heterozygous alleles [G:A (green)= Heterozygous] as revealed by the PCR-gel based *crtRB1-3'TE* marker. Fifty-seven inbreds had the unfavourable allele [G:G (red)= inbred lines with the unfavourable allele], while no amplification was observed for two of the inbred lines [? (pink)= inbreds that did not amplify]. The 2 non-template controls [NTC (black)= no template controls] effectively checked the amplification and efficiency of the KASP SNP (snpZM0015) by clustering together (Fig. 6.3).

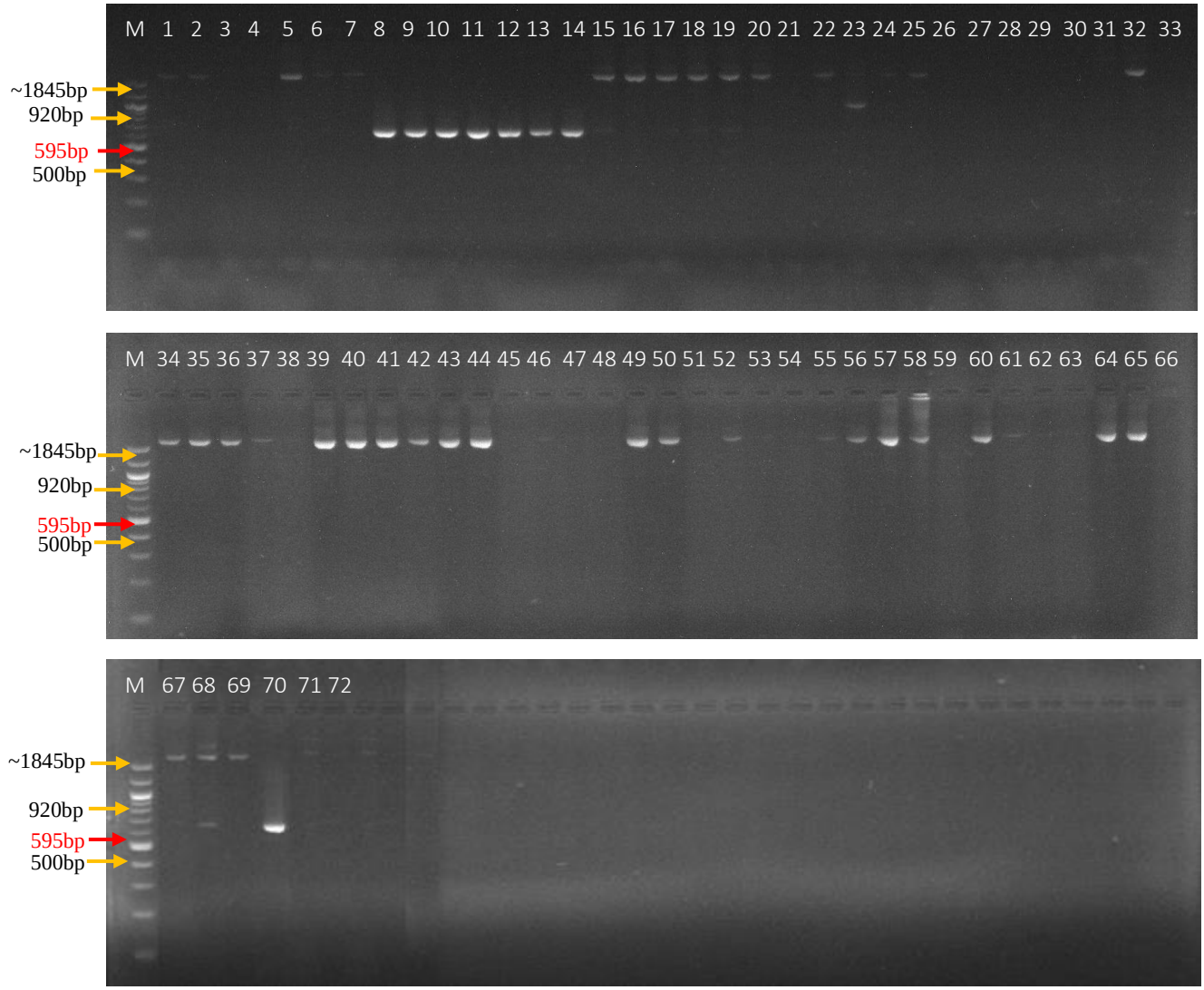

Figure 6. 2 PCR amplification of selected early maturing provitamin A quality protein maize inbred lines using crtRB1_3TE_F1/R1C primer resolved on 2% agarose gel. M = 100bp DNA Ladder Marker; Favourable allele = 595bp; Unfavourable allele = 1845 bp; Lanes 1-70 = Inbred lines with entry number as labelled; Lanes 71 and 72= NTCs.

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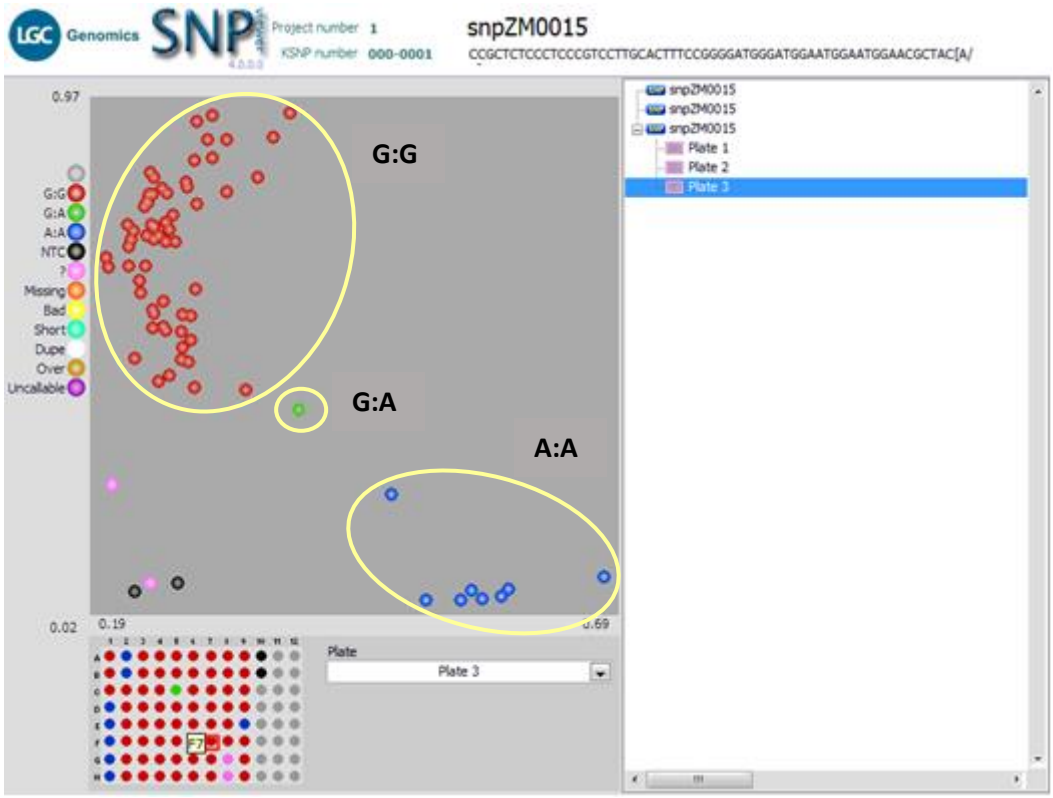

Figure 6. 3 SNP-viewer Image of 70 early maturing provitamin A quality protein maize inbred lines genotyped using KASP snpZM0015. A:A (blue) = inbreds with favourable allele; G:G (red)= inbreds with unfavourable allele; G:A (green)= Heterozygous for the allele; ? (pink) = inbreds that did not amplify; NTC (black)= no template controls; snpZM0015 = KASP SNP targeting the crtRB1 gene.

6.4 Discussion

The significant mean squares of sets and hybrids observed for all carotenoids and tryptophan indicated that the sets within which parental inbreds were placed were unique and that there is genetic variation among the hybrids to allow selection. This was in agreement with earlier reports indicating the existence of genetic variation in carotenoids in yellow maize lines and hybrids in the tropics (Pfeiffer and McClafferty, 2007; Menkir *et al*., 2008; Burt, 2010; Suwarno *et al*., 2015). The high heritability estimates found for carotenoids and tryptophan of the hybrids demonstrated the relevance of the genetic component of the total variation observed for the traits and that the traits could be transferred to subsequent

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generations. These findings however, contradicted the report by Halilu *et al*. (2016), who found low heritability estimates (< 0.30) for α-carotene, β-carotene, β-cryptoxanthin and PVA. The significant GCA (GCA-male + GCA-female) effects for PVA and other carotenoids, as well as SCA effects for most carotenoids suggested that both additive and non-additive gene effects were important in the inheritance of these traits. However, the preponderance of GCA effects over SCA for PVA and other carotenoids indicated that additive gene action was more important than the non-additive in the inheritance of PVA carotenoids and that GCA was the major contributor to the heritable variation in PVA carotenoids of the hybrids. These findings were consistent with the results of other studies (Egesel *et al*., 2003; Suwarno, 2012; Suwarno *et al*., 2015; Menkir *et al*., 2014; Owens *et al*., 2014). This observation suggested that favourable alleles of PVA could be pyramided in inbred lines to develop hybrids and synthetics. A similar result was reported by Menkir *et al*. (2017). The results also implied that recurrent selection for PVA content should be effective (Coors, 1999). These findings however contradicted the report by Halilu *et al*. (2016), who found that non-additive genetic variance was higher than additive genetic variance for all the carotenoids measured including PVA, suggesting a preponderance of non-additive gene action over the additive. Furthermore, it was surprising to find non-significant contributions of GCA-male and GCA-female effects for tryptophan, PVA and its component carotenoids implying that cytoplasmic genes did not have significant influence on the inheritance of these traits in the set of inbred lines. With respect to tryptophan, this result is inconsistent with the findings of Varadaraju and Joel (2017) who reported high tryptophan levels in hybrids to be primarily related to the contributions of maternal inbred parents indicating that quality protein content was under maternal genetic control. The inconsistency in the results of the different studies may be attributed to the differences in the genetic materials and the stage of inbreeding of the inbred lines used.

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The significant positive GCA-male and GCA-female effects observed for the inbred TZEIORQ 29 for PVA and its component carotenoids suggested that it contributed to increased levels of these carotenoids in its crosses either as a male or a female. The inbred TZEIORQ 29 could therefore be exploited for PVA favourable alleles in the development of superior hybrids and synthetics and for the improvement of the early maturing PVA-QPM inbred population. The significant positive GCA-female effect recorded for TZEIORQ 13 for PVA and β-carotene indicated that it could be used as a female parent to make gains in PVA and β-carotene levels of its hybrids. However, the significant negative GCA-male and GCA-female effects for total carotenoids detected for TZEIORQ 13 implied that it contributed as a female parent in reducing total carotenoids in its hybrids. This result could be a shortfall for TZEIORQ 13 because high levels of total carotenoids could be an advantage if the influx of total carotenoids favours the accumulation of PVA carotenoids in the carotenoid biosynthetic pathway.

The range of PVA concentrations, 6.50 – 9.82 µg g^{-1} dry weight (DW) recorded for the top 15 hybrids in the present study was moderate compared with the target of 15 µg g^{-1} DW set by HarvestPlus (HarvestPlus, 2004; Ortiz-Monasterio *et al*., 2007; Harjes *et al*., 2008). This result suggested that there is the need to introgress favourable PVA alleles from high PVA temperate sources to improve the tropically adapted early PVA-QPM inbred lines. This would help to facilitate the accumulation of PVA carotenoid concentrations in existing early PVA-QPM hybrids and to even exceed the set target (15 µg g^{-1} dry weight). Exceeding the PVA set target is a practically feasible approach to maximize the benefits of consuming PVA maize because studies have revealed that the loss in PVA during storage, milling, and preparation of different traditional food items could be up to 70% (Muzhingi *et al.*, 2008; Mugode *et al*., 2014; Pillay *et al*., 2014; De Moura *et al*., 2015) and the degree of loss widely varies among maize genotypes. The range of values of tryptophan content (> 0.075 in sample

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in whole grain) recorded for the hybrids indicated that all the hybrids possess the quality protein trait as reported by Vivek *et al*. (2008) and Teklewold *et al*. (2015). TZEIORQ 29 had relatively high PVA content of 12.10 µg g^{-1}, recorded significant positive GCA-male and female effects for PVA and its carotenoids, was a good combiner for PVA carotenoids either as a male or female, and was consistently found as a parent among the top hybrids in terms of PVA levels with the exception of the check, TZEIOR 127 x TZEIOR 57. This result underscored the preponderance of additive gene action over that of non-additive for PVA carotenoids in the set of inbred lines studied. TZEIORQ 13 was good only as a female combiner for PVA and β-carotene. All the top 15 hybrids were tolerant across drought and low-N environments. Grain yields and PVA contents of the top hybrids in terms of PVA levels as well as the highest yielding and most stable hybrid, TZEIORQ 24 x TZEIORQ 41 were not significantly different from the best check TZEIOR 127 x TZEIOR 57. Moreover, all the top hybrids had PVA contents less than 15.00 µg g^{-1}. However, due to the lack of single cross drought and low-N tolerant hybrids with elevated levels of PVA and quality protein in SSA, and since the hybrid check (in advanced stages of testing) do not possess quality protein background, it is worth to further test the identified hybrids for commercialization in SSA while breeding efforts continue to focus on meeting the set target.

The observed significantly positive correlations among individual PVA carotenoids as reported by Kurilich and Juvik (1999), Menkir *et al*. (2008) and Owens *et al*. (2014) and also between PVA concentration and grain yield of the hybrids as reported by Suwarno *et al*. (2015) and Menkir *et al*. (2014) might have accounted for the drought and low-N tolerance observed for all top performing hybrids in terms PVA content. This observation emphasized that simultaneous increases in accumulation of PVA and other carotenoids may be effectively accomplished without compromising grain yield potential and related important agronomic traits (Pfeiffer and McClafferty, 2007; Bouis and Welch, 2010; Menkir *et al*., 2014).

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Moreover, a PVA functional gene(s) validation study was conducted using known functional markers to investigate the relationship between favourable alleles of PVA and its carotenoid content. In the present study, four functional markers, *crtRB1-5'TE, crtRB1-3'TE, LcyE-5'TE* and KASP snpZM0015 were used to validate the most functional PVA genes, *crtRB1 and LcyE* (Yan *et al*., 2010; Babu *et al*., 2013; Azmach *et al*., 2013; Harjes *et al*., 2008) for increased β-carotene levels in a panel of 70 early maturing PVA-QPM inbred lines at the IITA Bioscience Laboratory. According to Anderson and Lübberstedt (2003) and Varshney *et al*. (2005), functional markers are DNA markers capable of revealing polymorphisms in allele combinations which are functionally responsible for the differences in phenotypes. From the results, the *crtRB1-3'TE* was the most polymorphic functional marker and identified eight inbreds, TZEIORQ 10, TZEIORQ 12, TZEIORQ 13, TZEIORQ 14, TZEIORQ 15, TZEIORQ 16, TZEIORQ 17 and TZEI 129 carrying the favourable alleles of the *crtRB1* gene. These inbreds could serve as donor parents of favourable alleles for the *crtRB1* gene. These results agreed with the findings of Yan *et al*. (2010), Babu *et al*. (2013) and Azmach *et al*. (2013) who validated the *crtRB1-3'TE* functional marker in a PVA maize germplasm. However, the PVA contents of the eight inbreds identified to harbour favourable alleles of the *crtRB1* gene were moderate suggesting a situation of reduced gene expression (Hood, 2004; Mocellin and Provenzano, 2004) which could be due to gene silencing occurring during either transcriptional or translational processes (Redberry, 2006). The results also indicated the need to further increase different favourable alleles of PVA in the background of the early PVA-QPM inbred lines. Inbred TZEIORQ 55 which recorded the highest PVA level of 15.38 µg g^{-1} DW had the heterozygous form of the *crtRB1* alleles. Further stages of inbreeding may be required to fix the *crtRB1* alleles of TZEIORQ 55. The results of the KASP marker, snpZM0015 was in concordance with that of *crtRB1-3'TE*, identifying the same eight inbreds to harbour the favourable alleles of the *crtRB1* functional gene, and detected TZEIORQ 55 as

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heterozygous for the *crtRB1* alleles. It was striking to find the *crtRB1-5'TE* and *LcyE-5'TE* completely monomorphic for the alleles of the crtRB1 and LcyE genes in the entire set of inbred lines, yet some of the inbreds recorded moderate to high PVA contents. These results contradicted the findings of Yan *et al*. (2010), Harjes *et al*. (2008) and Azmach *et al*. (2013) who reported that *crtRB1-5'TE* and *LcyE-5'TE* are reliable markers for detecting the favourable alleles of the *crtRB1* and *LcyE* functional genes. These results therefore suggested that genes other than the *crtRB1* and *LcyE* genes such as *PSY1* (Harjes *et al*., 2008) were responsible for the increased concentrations of the PVA carotenoids observed for some of the inbred lines. For instance, it is surprizing to find that TZEIORQ 55 and TZEIORQ 29 which recorded 15.38 and 12.10 μg g^{-1} DW PVA respectively, did not clearly harbour the favourable alleles of *crtRB1* or *LcyE* gene in their backgrounds based on the performance of the markers used. Elucidating the observation that other PVA genes may be at play, Owens *et al*. (2014) reported the *zep1* and *lut1* genes to be together with the *crtRB1* and *LcyE* genes resulting in increased levels of PVA carotenoids. Morover, the authors indicated that although *crtRB1*, *LcyE*, *zep1* and *lut1* were clearly important for detecting favourable alleles for PVA carotenoids, they indicated that these four genes may not be sufficient for efficient breeding in all contexts and genetic backgrounds. They therefore proposed that favourable alleles at specific loci of four other genes (*y1*, *zds1*, *crtRB3* and *ccd1*) in the PVA biosynthetic pathway could be relevant for the accumulation of PVA concentrations. With this knowledge of the functional PVA genes and the phenotyping results of the carotenoids, TZEIORQ 55 and TZEIORQ 29 could serve as donor parents of PVA favourable alleles other than those of *crtRB1* and *LcyE* for the improvement of the PVA trait in the set of inbred lines.

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6.5 Conclusions

Additive gene action was more important than the non-additive in the inheritance of all carotenoids and that GCA was the major contributor to the heritable variation in carotenoids of the early PVA-QPM hybrids phenotyped. It was found that cytoplasmic genes did not have significant influence on the inheritance of carotenoids and tryptophan in the set of inbred lines studied. Inbred line TZEIORQ 29 with significant positive GCA-male and female effects for PVA and its component carotenoids could be exploited for the PVA favourable alleles to develop high PVA hybrids and synthetics, and also for the improvement of the early PVA-QPM inbred lines. Inbred line TZEIORQ 13 with significant positive GCA-female effect for PVA and β-carotene suggested that it could be useful as a female parent for gains in breeding for high PVA maize. The moderate range of PVA contents observed for the hybrids suggested that there is the need to introgress favourable PVA alleles from temperate sources to improve the tropically adapted early PVA-QPM inbred lines and to facilitate the accumulation of PVA in available hybrids. The top hybrids TZEIORQ 29 x TZEIORQ 40, TZEIORQ 29 x TZEIORQ 43, TZEIORQ 29 x TZEIORQ 24, TZEIORQ 20 x TZEIORQ 29 and TZEIORQ 6 x TZEIORQ 29 with moderately high PVA levels should be further tested and commercialized in SSA to combat vitamin-A deficiency and protein energy malnutrition in the sub-region. Significant positive correlation between PVA carotenoids and grain yield of hybrids was observed indicating that simultaneous increases in accumulation of PVA and other carotenoids may be effectively accomplished without compromising grain yield of the hybrids.

In the PVA candidate genes validation study, the *crtRB1-3'TE* was more functional and identified eight inbreds, TZEIORQ 10, TZEIORQ 12, TZEIORQ 13, TZEIORQ 14, TZEIORQ 15, TZEIORQ 16, TZEIORQ 17 and TZEI 129 to harbour the favourable alleles of the *crtRB1* gene. The KASP SNP, snpZM0015 gave consistent result with the *crtRB1-3'TE*.

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The eight inbreds identified could serve as donor parents of favourable alleles for the *crtRB1* gene. Knowledge of the functional PVA genes and the phenotyping results of the carotenoids indicated that TZEIORQ 55 and TZEIORQ 29 could donate favourable alleles other than those of *crtRB1* and *LcyE* for the improvement of PVA levels in available hybrids, synthetics and the PVA-QPM inbred lines.

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CHAPTER SEVEN

7.0 Conclusions and Recommendations

7.1 Conclusions

Maize production and productivity in SSA are limited by several abiotic constraints including drought and low-N which constitute the two most important abiotic stresses in the sub-region. Genetic enhancement of maize under drought and low-N could improve grain yield in farmers' fields. In terms of nutritive value, the QPM has the potential to provide more than twice each of the levels of lysine and tryptophan to prevent protein energy malnutrition (PEM) especially among infants. Similarly, the PVA maize can provide more than 15 µg g^{-1} dry weight of PVA to combat VAD and its health-related problems. Due to the existence of genetic variations for earliness, drought and low-N tolerane as well as elevated levels of PVA and quality protein in maize, the IITA-MIP has developed a large number of inbreds with these desirable traits. The inbreds were evaluated under drought (17 mm of irrigation water provided per week up to 25 DAP), low-N (30 kg N per ha) and optimal conditions (90 kg ha^{-1}) in Nigeria for two years. A set of 19 inbreds were also assayed for PVA and tryptophan levels. The objective was to identify inbred lines that could be used in hybrid combinations to produce superior early maturing drought and low-N tolerant hybrids with elevated levels of PVA and quality protein (with enhanced levels of lysine and tryptophan). DArTseq markers were used to examine the extent of genetic diversity in the set of early maturing PVA-QPM inbred lines. Since *per se* performance of inbreds is not a good indicator of superior performance in their hybrids, it was necessary to assess the combining ability of the inbred lines for grain yield and other agronomic traits under drought, low-N and optimal conditions and also for PVA accumulation under optimal environments. The yield and stability of the hybrids across environments, as well as the levels of PVA and tryptophan were also examined.

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In addition, candidate genes variable for PVA accumulation in the inbreds were investigated using allele-specific markers.

Days to 50% anthesis and silking, plant and ear heights, and plant and ear aspects complemented grain yield to identify 33 drought and low-N tolerant inbreds. Almost all the inbreds had desirable levels of tryptophan (> 0.075%) in sample in whole grain. With the exception of TZEIORQ 55 and TZEIORQ 29 which had 15.38 and 12.10 μg g^{-1} PVA contents, low to moderate levels of PVA were recorded for most of the inbreds including those tolerant to drought and low-N indicating the urgent need to introgress favourable alleles of PVA into the inbred lines to enhance progress in developing high PVA-QPM hybrids adapted to drought and low-N environments in SSA.

The multivariate analyses using DArTseq markers to estimate the genetic diversity and population structure and applying the UPGMA clustering algorithm, the model-based structure analysis, and the principal component analysis consistently identified five unique clusters. The five groups essentially followed pedigree information indicating the existence of genetically distinct clusters in the early PVA-QPM inbred lines and that inbreds that clustered together share similar genetic backgrounds containing common alleles which are identical in state.

Results of the combining ability study of grain yield and other agronomic traits revealed that additive genetic effects were more important than the non-additive for grain yield and most of the measured traits of the hybrids under drought, low-N, optimal and across environments. Maternal and paternal GCA effects were equally important in the inheritance of measured traits. Additionally, the more efficient heterotic grouping method, SNP-based DArT markers, classified the inbreds into three heterotic groups with TZEIORQ 29 identified as the best tester either as a male or female for heterotic group I, while TZEIORQ 24 was the best male tester for heterotic group II. Significant correlations were observed between mid-

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parent values and the means of the corresponding hybrids for grain yield, plant height, ear aspect and stay green characteristic under drought, low-N, optimal and across environments suggesting that initial screening and selection of drought and low-N tolerant inbreds for the development of superior hybrids would be effective. Four traits consisting of plant and ear aspects, ears per plant and stay green characteristic were identified as the first order contributors to grain yield of hybrids underscoring the reliability of these traits for the improvement of grain yield under the different test environments. The hybrid TZEIORQ 2 x TZEQI 82 was identified as the single cross testers across drought, low-N and optimal environments. TZEIORQ 26 x TZEIORQ 47 was the most stress tolerant hybrid out-yielding the best check under drought and yielding equal to it under low-N, while TZEIORQ 24 x TZEIORQ 41 was the highest yielding and most stable hybrid across stress and non-stress environments yielding as much as the best check.

The combining ability study of the PVA and tryptophan contents revealed GCA as the major contributor to the heritable variation in PVA carotenoids. It was found that cytoplasmic genes did not have significant influence on the inheritance of carotenoids and tryptophan in the set of parental lines used. TZEIORQ 29 was the only inbred which had significantly ($P< 0.05$) positive GCA-male and female effects for PVA and its component carotenoids. In addition, TZEIORQ 13 had significant ($P< 0.05$) positive GCA-female effect for PVA and β-carotene.

Moderate range of PVA contents were observed for most of the hybrids with TZEIORQ 29 x TZEIORQ 40, TZEIORQ 29 x TZEIORQ 43, TZEIORQ 29 x TZEIORQ 24, TZEIORQ 20 x TZEIORQ 29 and TZEIORQ 6 x TZEIORQ 29 ranking as the top performers but were not significantly different from the check, TZEIOR 127 x TZEIOR 57. The significant positive correlation observed between PVA carotenoids and grain yield of hybrids

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indicateed that simultaneous increases in accumulation of PVA and other carotenoids may be effectively accomplished without compromising grain yield of the hybrids.

In the PVA candidate genes validation study, the *crtRB1-3'TE* was the most polymorphic functional marker and was highly consistent with the KASP SNP (snpZM0015). The two markers identified eight inbred lines (TZEIORQ 10, TZEIORQ 12, TZEIORQ 13, TZEIORQ 14, TZEIORQ 15, TZEIORQ 16, TZEIORQ 17 and TZEI 129) containing favourable alleles of the *crtRB1* functional gene. The *crtRB1-5'TE* and *LcyE-5'TE*, however, did not amplify for any of the inbred lines indicating that none of the inbreds carried both genes. Despite the moderate to high PVA contents of TZEIORQ 29 and TZEIORQ 55, they were not identified to harbour the favourable alleles of *crtRB1* and *LcyE* genes suggesting that other genes were responsible for the increased levels of PVA for these inbreds. Moreover, the preponderance of additive genetic effects over non-additive in the inheritance of PVA indicated that TZEIORQ 29 and TZEIORQ 55 could donate favourable alleles other than those of *crtRB1* and *LcyE* for the improvement of PVA concentrations in the available hybrids, synthetics and the PVA-QPM inbred lines.

7.2 Recommendations

1. Heritability, and genetic correlations of secondary traits with grain yield across drought, low-N and optimal environments should be used as a reliable approach to identify drought and low-N tolerant inbred lines.
2. The inbreds TZEIORQ 55 and TZEIORQ 29 were tolerant to low-N and had high PVA and tryptophan contents. These inbreds should be used for the development of low-N tolerant hybrids and synthetics with elevated levels of PVA and tryptophan, and for the improvement of the early PVA-QPM inbred lines.

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3. The inbreds such as TZEIORQ 29 and TZEIORQ 24 which recorded significantly positive GCA effects for grain yield across the contrasting environments could be useful for developing drought and/or low-N tolerant hybrids and synthetics.
4. TZEIORQ 26 x TZEIORQ 47 would be the preferred hybrid for drought environments especially in the semi-arid/Sudan Savanna agro-ecologies of SSA.
5. The highest yielding and most stable hybrid, TZEIORQ 24 x TZEIORQ 41 should be further tested to confirm the consistency of performance for commercialization in SSA.
6. The moderate PVA contents observed for the inbreds and hybrids suggested that there is the need to introgress favourable alleles of PVA to improve the early PVA-QPM inbred lines and to facilitate the accumulation of PVA in outstanding hybrids.
7. In terms of PVA content, the top five hybrids TZEIORQ 29 x TZEIORQ 40, TZEIORQ 29 x TZEIORQ 43, TZEIORQ 29 x TZEIORQ 24, TZEIORQ 20 x TZEIORQ 29 and TZEIORQ 6 x TZEIORQ 29 with moderate PVA but high tryptophan levels should be further tested and commercialized in SSA to combat VAD and PEM in the sub-region.
8. The eight inbreds identified to possess the *crtRB1* gene could serve as donor parents of favourable alleles of the gene in further breeding for high PVA maize.

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APPENDICES

Appendix 1. Reaction of the inbred lines to drought and low-N stresses

Reaction of TZEIORQ 24 (left) and TZEIORQ 41 (right) to drought condition at Ikenne during the 2016/2017 dry season in Nigeria.

Reaction of TZEIORQ 41 (left) and TZEIORQ 48 (right) to low-N condition at Ile-Ife during the 2017 growing season in Nigeria.

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Appendix 2. Sample ears and their corresponding provitamin A carotenoid profiles (µg g^{-1}) and tryptophan contents (% in unit sample in whole grain)

TZEIORQ 24

β-cryp	α-car	β-car	PVA	Tryp
3.71	1.16	4.17	6.60	0.084

TZEIORQ 43

β-cryp	α-car	β-car	PVA	Tryp
1.79	0.45	3.99	5.11	0.102

TZEIORQ 59

β-cryp	α-car	β-car	PVA	Tryp
2.11	1.00	4.64	6.19	0.122

TZEIORQ 5

β-cryp	α-car	β-car	PVA	Tryp
2.76	0.89	3.35	5.18	0.108

TZEIORQ 20

β-cryp	α-caro	β-car	PVA	Tryp
3.84	1.31	5.78	8.36	0.095

TZEIORQ 55

β-cryp	α-car	β-car	PVA	Tryp
6.61	1.71	11.23	15.38	0.099

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Appendix 2. (continued) Sample ears and their corresponding provitamin A carotenoid profiles (µg g^{-1}) and tryptophan contents (% in unit sample in whole grain)

TZEIORQ 45

β-cryp	α-car	β-car	PVA	Tryp
1.46	0.99	3.88	5.10	0.106

TZEIORQ 6

β-cryp	α-car	β-car	PVA	Tryp
3.18	0.94	3.44	5.50	0.119

TZEIORQ 48

β-cryp	α-car	β-car	PVA	Tryp
1.56	1.00	2.18	3.47	0.122

TZEIORQ 29

β-cryp	α-car	β-car	PVA	Tryp
6.11	2.10	7.69	12.10	0.110

TZEIORQ 7

β-cryp	α-car	β-car	PVA	Tryp
3.36	1.11	3.37	5.61	0.095

TZEIORQ 15

β-cryp	α-car	β-car	PVA	Tryp
1.25	1.61	6.54	7.48	0.076

TZEIORQ 13

β-cryp	α-car	β-car	PVA	Tryp
1.40	0.54	6.72	7.70	0.134

TZEIORQ 26

β-cryp	α-car	β-car	PVA	Tryp
2.88	0.89	3.40	5.28	0.093

TZEIORQ 2

β-cryp	α-car	β-car	PVA	Tryp
1.83	0.50	2.65	3.82	0.103

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Appendix 2. (continued) Sample ears and their corresponding provitamin A carotenoid profiles (µg g^{-1}) and tryptophan contents (% in unit sample in whole grain)

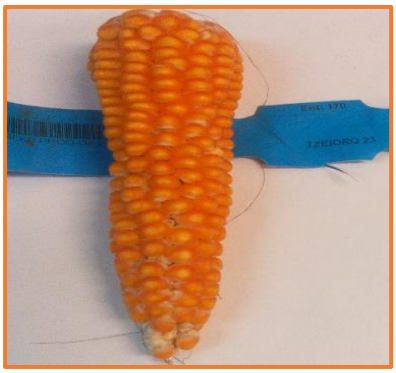
TZEIORQ 41

β-cryp	α-car	β-car	PVA	Tryp
-	-	-	-	0.089

TZEIORQ 42

β-cryp	α-car	β-car	PVA	Tryp
4.99	1.27	5.21	8.24	0.122

TZEIORQ 23



β-cryp	α-car	β-car	PVA	Tryp
2.82	0.91	3.20	5.06	0.096

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Appendix 3. Probabilities for assigning an individual inbred line into a group

	Probabilities for clustering					
Inbred	**Cluster I**	**Cluster II**	**Cluster III**	**Cluster IV**	**Cluster V**	**Group**
TZEIORQ22	1	0	0	0	0	1
TZEIORQ39	1	0	0	0	0	1
TZEIORQ40	1	0	0	0	0	1
TZEIORQ41	1	0	0	0	0	1
TZEIORQ42	1	0	0	0	0	1
TZEIORQ43	1	0	0	0	0	1
TZEIORQ44	1	0	0	0	0	1
TZEIORQ45	1	0	0	0	0	1
TZEIORQ46	1	0	0	0	0	1
TZEIORQ47	1	0	0	0	0	1
TZEIORQ68	0.737	0.085	0.065	0.051	0.063	1
TZEIORQ69	0.74	0.103	0.081	0.001	0.076	1
TZEIORQ70	1	0	0	0	0	1
TZEIORQ57	0	1	0	0	0	2
TZEIORQ58	0	1	0	0	0	2
TZEIORQ59	0	1	0	0	0	2
TZEIORQ60	0	1	0	0	0	2
TZEIORQ61	0	1	0	0	0	2
TZEIORQ62	0	1	0	0	0	2
TZEIORQ63	0	1	0	0	0	2
TZEIORQ65	0	1	0	0	0	2
TZEIORQ10	0	0	1	0	0	3
TZEIORQ11	0	0	1	0	0	3
TZEIORQ12	0	0	1	0	0	3
TZEIORQ13	0	0	1	0	0	3
TZEIORQ14	0	0	1	0	0	3
TZEIORQ15	0	0	1	0	0	3
TZEIORQ16	0	0	1	0	0	3
TZEIORQ17	0	0.001	0.999	0	0	3
TZEIORQ29	0	0.001	0.999	0	0	3
TZEIORQ30	0	0	0.999	0	0	3
TZEIORQ31	0	0	1	0	0	3
TZEIORQ32	0	0	1	0	0	3
TZEIORQ33	0	0	1	0	0	3
TZEIORQ34	0.078	0.066	0.712	0.015	0.13	3
TZEQI85	0	0	0	1	0	4
TZEQI91	0	0	0	1	0	4
TZEQI74	0	0.001	0	0.999	0	4
TZEQI82	0	0	0	1	0	4

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Appendix 3. continued. Probabilities for assigning an individual inbred line into a group

	Probabilities for clustering					
Inbred	**Cluster I**	**Cluster II**	**Cluster III**	**Cluster IV**	**Cluster V**	**Group**
TZEI129	0.083	0.042	0.109	0.761	0.005	4
TZEI24	0	0	0	1	0	4
TZEIORQ18	0	0	0	0.002	0.998	5
TZEIORQ19	0	0	0	0	1	5
TZEIORQ2	0	0	0	0	1	5
TZEIORQ20	0	0	0	0	1	5
TZEIORQ21	0	0	0	0	1	5
TZEIORQ23	0	0	0	0	1	5
TZEIORQ24	0	0	0	0	1	5
TZEIORQ25	0	0	0	0	1	5
TZEIORQ26	0	0	0	0.29	0.709	5
TZEIORQ27	0	0	0	0	1	5
TZEIORQ28	0	0	0	0	1	5
TZEIORQ3	0	0	0	0	1	5
TZEIORQ35	0	0	0	0	1	5
TZEIORQ36	0	0	0	0	1	5
TZEIORQ37	0	0	0	0	1	5
TZEIORQ48	0	0	0	0	1	5
TZEIORQ5	0	0	0	0	1	5
TZEIORQ52	0	0	0	0	1	5
TZEIORQ53	0	0	0	0	1	5
TZEIORQ6	0	0	0	0	1	5
TZEIORQ7	0	0	0	0	1	5
TZEIORQ71	0	0	0	0.113	0.887	5
TZEIORQ73	0	0	0	0	1	5
TZEIORQ8	0	0	0	0	1	5
TZEIORQ9	0	0	0	0	1	5
TZEIORQ54	0.197	0.163	0.347	0.074	0.219	Mixed
TZEIORQ55	0.12	0.127	0.424	0.074	0.255	Mixed
TZEIORQ66	0.111	0.113	0.448	0.052	0.276	mixed
TZEIORQ72	0.111	0.232	0.433	0.044	0.18	mixed

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Appendix 4. Reaction of the single cross hybrids to drought and low-N stresses

Reaction of TZEIORQ 29 x TZEIORQ 43 (left), TZEIORQ 42 x TZEIORQ 20 (right) and TZEIORQ 40 x TZEIORQ 41 (middle) to drought condition at Ikenne during the 2016/2017 dry season in Nigeria.

Reaction of TZEIORQ 29 x TZEIORQ 24 (left), TZEIORQ 26 x TZEIORQ 47 (right) and TZEIORQ 13 x TZEIORQ 15 (middle) to low-N condition at Ile-Ife during the 2017 growing season in Nigeria.