CHARACTERIZATION AND HETEROSIS AMONG EXTRA-EARLY MATURING ORANGE MAIZE INBRED LINES UNDER DROUGHT AND STRIGA INFESTATION

By

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN PLANT BREEDING DEGREE

WEST AFRICA CENTRE FOR CROP IMPROVEMENT

COLLEGE OF BASIC AND APPLIED SCIENCES

UNIVERSITY OF GHANA

LEGON

DECEMBER, 2018

DECLARATION

I hereby declare that except for references to work of other researchers, which have been duly cited, 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

Striga hermonthica and drought are major threats to maize (*Zea mays* L.) productivity and production in savannas of sub-Saharan Africa (SSA), a sub-region known to be plagued with vitamin A deficiency (VAD). Incorporating both drought tolerance and *Striga* resistance into high yielding orange maize cultivars for *Striga* endemic and drought-prone areas of the savanna agro-ecologies of SSA will increase acceptability of orange cultivars by farmers and aid in alleviating VAD and food shortage in the sub-region. The specific objectives of this research were to determine: (i) the performance of extra-early maturing maize inbred lines for tolerance to *Striga* and drought, (ii) the genetic diversity in extra-early maturing orange maize inbred lines using SNP-based DArTseq markers, (iii) the combining abilities of extra-early maturing orange maize inbred lines and heterosis for tolerance to *Striga* and drought, (iv) the performance and stability of extra-early maturing hybrids for tolerance to *Striga* and drought, (v) the combining abilities of extra-early maturing orange maize inbred lines and heterosis for carotenoids in maize kernels. One hundred and eighty inbred lines comprising 152 orange inbreds and 28 yellow lines were evaluated under *Striga* infestation, managed drought stress, and optimal environments at Ikenne, Abuja and Mokwa in Nigeria using a 12 x 15 alpha lattice design. Thirty-four (34) out of 180 inbreds evaluated and 32 out of 152 orange inbreds combined *Striga* resistance and drought tolerance, using base indices for selection. Twenty-four (24) of the 34 selected based on the indices were also selected by the multivariate best linear unbiased predictors (BLUPs) across all environments. The genetic purity and diversity among the 152 orange inbreds were assessed using 4620 polymorphic SNPs. The results revealed that 92% of the inbreds were pure with heterogeneity < 5% while the remaining 8% had heterogeneity ranging from 5.1 to 20.2%. Roger's genetic distance for about 71% of the pairs of lines fell between 0.2001 and 0.2500. Ninety-two percent of the pairs of inbreds

also showed relative kinship values ranging from 0.300 to 0.500. The population structure analysis using STRUCTURE and neighbour-joining clustering assigned 71% of the inbreds in 4 distinct groups. Fifteen inbreds selected among the 152 evaluated plus TZdEEI 7 and TZdEEI 12 were used to generate 136 diallel single cross hybrids which were evaluated together with four experimental hybrid checks under *Striga*-infested, drought stress, and optimal environments at three locations in Nigeria (Ikenne, Abuja, and Mokwa) in 2016 and 2017 (total of 11 environments). The experimental design used was a 10 x 14 alpha lattice. General and specific combining ability components of the genetic variance were significantly different from zero for grain yield and most of the traits. Additive and non-additive genetic effects were both important with a predominance of the latter in controlling most of the measured traits including grain yield under *Striga*-infested, drought stress, and across test environments. However, additive genetic effects were found to be the primary type of gene action for the staygreen characteristic and *Striga* resistance indicator traits, suggesting that selection for these traits could easily be done based on predictions of GCA alone. Using base indices, 26% of the hybrids combined *Striga* resistance with drought tolerance. Stability assessment of the top 26 hybrids across test environments based on their genetic value indicated that TZEEIOR 12 x TZEEIOR 196 was the most stable, combining resistance to *Striga* and tolerance to drought with grain yield of 3885 kg ha^{-1} and 5411 kg ha^{-1} across environments and under optimal conditions, respectively. Hayman diallel analysis revealed predominance of dominant alleles in the parents with the ratio of dominant to recessive alleles being greater than 2 for β-carotene (2.36). Also, at the loci exhibiting dominance, the effects of dominant alleles were predominantly negative. In conclusion, dominance with negative genetic effect was found to be the gene action for carotenoids accumulation in the set of inbreds used.

DEDICATION

To my wife, Pawoubadi LAKOUNYO.

To my children, Bolanigni Ulrich and Ningnè Félicité.

To my father, Evignénou Seth and my mother, Koulikpama Akoua.

ACKNOWLEDGMENTS

I would like to thank the West Africa Agricultural Productivity Program-TOGO (WAAPP-TOGO) for providing the scholarship throughout the period of my study. I am also grateful to DAAD for their financial support, and through them, a special thank you to Professor Nohr Donatus for providing funds for my lab analysis in the University of Hohenheim and to Julian Wald and Alex Koza for their technical support.

I would like to sincerely thank Professor Eric Danquah who gave me the opportunity to be partly supported by DAAD.

I would like to also thank my supervisors at WACCI, Professor Isaac Asante, Dr John Eleblu, and Dr Beatrice Elohor Ifie, for their contribution to the completion of this study. I also thank them for reading all parts of this thesis and for making numerous suggestions for improvement and clarity.

I am very grateful to Dr Baffour Badu-Apraku for accepting me into his project, and for his supervision, and ideas during the past three years. I also thank him for providing all the facilities needed and for the quality of the training under his supervision at IITA.

I thank Professor Hans-Peter Piepho and Dr Jens Möhring for reviewing my scripts for data analysis and for providing me with the necessary documentation.

My IITA-MIP colleagues over the past three years were a frequent source of support and inspiration, particularly Dr Konate Laban, Ebenezer Obeng-Bio, and many others.

I would like to thank my parents for always encouraging me to do what I loved.

Finally, my lovely wife Jeannine Lakounyo provided me with support and understanding, and I thank her with all my heart.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CIMMYT	International Maize and Wheat Improvement Center
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization of the United Nations
GD	Genetic distance
GGE	Genotype plus genotype by environment interaction
IITA	International Institute of Tropical Agriculture
MAS	Marker-assisted selection
MIP	Maize improvement program
NJ	Neighbour-joining trees
PIC	Polymorphic information content
SNP	Single nucleotide polymorphisms
SSA	sub-Saharan Africa
UPGMA	Unweighted pair group method with arithmetic average
WA	West Africa
WAP	Week after planting
WCA	West and Central Africa

CHAPTER ONE

GENERAL INTRODUCTION

Maize (*Zea mays* L.) is the predominant staple crop in most countries of sub-Saharan Africa (SSA) where vitamin A deficiency is a serious human health problem (WHO, 2009). The prevalence of vitamin A deficiency was estimated to be among the highest (48%) in SSA with an occurrence of 95% of reported death from diarrhea and measles in 2013 as a result of the weakened immune system due to vitamin A deficiency (Stevens *et al.,* 2015). Defined as a liver retinol reserve of < 0.1 µmol g^{-1}liver (Tanumihardjo, 2011), vitamin A deficiency is most severe among pre-school aged children and pregnant women (Rice *et al.*, 2004). In SSA, the annual *per capita* food maize consumption averages 36 kg (Atlin *et al.*, 2011) making it the most consumed cereal in the sub-region. Besides, maize is a carotenogenic species with natural variation of total carotenoids ranging from 0.00 to 19 µg g^{-1} DW (HarvestPlus, 2017). Therefore, maize has been targeted as a crop for provitamin A biofortification as a more sustainable and cost-effective way to deal with vitamin A deficiency in SSA. So far, two genes out of 58 known to play key roles in biosynthesis and accumulation of carotenoids in maize have been successfully utilized in marker-assisted breeding. Combinations of favourable alleles at both *lycE* and *crtRB1* loci allowed increases up to 22.6 µg g^{-1} (Menkir *et al.,* 2017) mostly in maize inbreds. At the same time, the current released cultivars contain an average of 6 to 8 µg g^{-1} of provitamin A (HarvestPlus, 2017) which is far below the breeding target of 15 µg g^{-1}. Hence, in-depth understanding of the mode of inheritance of such a complex quantitative trait in maize is needed to identify a better choice of breeding scheme and decide whether to go for open pollinated varieties (OPVs) or hybrids using the readily available superior inbreds as parents.

The savanna agro-ecology of sub-Saharan Africa is characterized by high solar radiation and low night temperatures, which are known to represent favourable conditions for C4 plants such as maize. Nowadays, savanna agro-ecology is considered as the maize grain basket of the whole sub-region due to the introduction and the wide spread use of extra-early and early maturing maize. However, maize production is constrained by several biotic and abiotic stresses in savanna zones, which under field conditions occur most often simultaneously. Prominent among these threats are drought and the parasitic weed, *Striga hermonthica* Del. Benth. In the marginal areas where the annual rainfall is mostly below 500 mm, maize yield loss from drought stress may be much higher than the average estimated losses of 15% of total production *per annum*. Grain yield losses can even be greater if the drought stress occurs at the most drought-sensitive stages of crop growth, such as flowering and grain filling periods. For example, drought stress can reduce yield by 21% when it occurs at the grain filling period, by 50% at flowering (Denmead and Shaw, 1960), and by as much as 90% (NeSmith and Ritchie, 1992) when it occurs a few days before tassel emergence to the beginning of grain filling period. Furthermore, 30 to 80% of maize yield losses have been estimated to occur in 2.5 million hectares infested by *Striga* in SSA (AATF, 2006). Global warming and the accompanying increase in unpredictable intensity and frequency of rainfall patterns, in addition to the fact that more than 80% of maize in SSA is rainfed (Edmeades *et al.*, 2017), call urgently for more effective improvement of maize yields under drought stress. Breeding maize for resistance to *Striga* and tolerance to drought is an effective means of combating these threats and tremendous efforts towards this has resulted in varieties resistant or tolerant to each stress (Badu-Apraku and Fakorede, 2017). However, extra-early orange maize cultivars combining resistance to *Striga* and tolerance to drought with elevated levels of carotenoids are lacking.

Success in breeding maize to address the increasing demand for resistant/tolerant cultivars to adverse environmental changes reside in the availability of maize genetic variability and its efficient use through different methods. To this end, inbred lines are routinely developed from numerous source populations and used to develop new cultivars or base populations. Hence, these inbred lines constitute a sample of the existent maize genetic variability. Exploring this variability aids in achieving breeding goals such as developing new varieties that combine desirable multiple traits.

Molecular characterisation of genotypes under selection using SNPs has the advantage of assessing the variability at the base level and provides the basis of differentiating and classifying genotypes into heterotic groups (Yang *et al.*, 2011; Dao *et al.*, 2014; Wu *et al.*, 2016). It also aids in identifying genetic purity of genotypes (Semagn *et al.*, 2012; Ertiro *et al.*, 2017), especially advanced maize inbred lines, to assure their identity and guarantee the heterotic effects in crosses. In order to maximize the gains in selection for increased levels of carotenoids, resistance to *Striga*, and tolerance to drought in hybrid development, in-depth understanding of inheritance and heterosis for provitamin A carotenoids and the aforementioned major constraints are needed. The main objective of this research was to analyse the genetic tolerance of maize inbred lines and hybrids to *Striga* and drought. The specific objectives of this research were to study:

i. the performance of extra-early maturing maize inbred lines for tolerance to *Striga* and drought;
ii. the molecular diversity in extra-early maturing orange maize inbred lines using SNP markers;
iii. the combining abilities of extra-early maturing orange maize inbred lines and heterosis for tolerance to *Striga* and drought;

iv. the performance and stability of extra-early maturing hybrids in tolerance to *Striga* and drought;

v. the combining abilities of extra-early maturing orange maize inbred lines and heterosis for carotenoids in maize kernels.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The complex "*Striga*-drought" and maize yield stability in the savannas of West Africa

Savanna zones are said to comprise about 75% of the total land mass of West Africa (WA) (Badu-Apraku and Fakorede, 2017). They are, in addition, characterized by higher solar radiation with increased daytime temperature and lower night time temperature (Kassam *et al.,* 1975), which are ideal for increased photosynthesis rate of up to 20-40% (Kassam and Kowal, 1973) compared to the forest agro-ecologies which are characterized by cloudy cover especially during the growing season. These are probably reasons why a large proportions of the maize productions in West and Central Africa (WCA) has shifted to the savanna agro-ecology of WCA (Bolaños and Edmeades, 1996; Badu-Apraku and Fakorede, 2017). Adoption of maize in the savannas has been made possible by the development and availability of extra-early and early-maturing varieties in addition to the fact that maize yields are higher than those of the traditional crops namely, sorghum (*Sorghum bicolor* L.) and millet (*Pennisetum typhoides* L.). Despite the appropriate environmental conditions that the savannas offer for increased maize production and productivity, the combined effect of drought and *Striga*, under field conditions, can be disastrous, thereby hindering food security of the sub-region.

Drought and *Striga* are the major abiotic and biotic constraints to maize production in WCA, especially, in savannas zones of West Africa (WA). They play a key role in determining maize yield stability, productivity, and production in this area of maize production considered as the grain basket of WA.

On one hand, more than 80% of agriculture in SSA is rainfed (Edmeades *et al.,* 2017). Consequently, rainfall represents one of the most important factors of all climatic variables in

agricultural activity. Updated and precise global yield losses due to drought are lacking. However, it is commonly admitted that drought causes about 17% yield losses per year in maize in the tropics (Edmeades *et al.*, 1999) while losses can approach 60% in individual seasons as reported by Rosen and Scott (1992), in regions such as Southern Africa.

On the other hand, under maize cultivation, the most deleterious effects of *Striga* in SSA have been estimated to occur in 2.5 million hectares with grain losses of 30 to 80%, representing a value of approximately US $1 billion per year (AATF, 2006). Fifteen countries among which eight of eastern and southern Africa (Malawi, Kenya, Tanzania, Zimbabwe, Mozambique, Ethiopia, Uganda, Zambia) and seven of western Africa (Nigeria, Ghana, Benin, Togo, Cameroun, Cote d'Ivoire, Burkina Faso) account for 92% of the continent's *Striga* infested maize fields. The levels of infestation are often so high that maize can suffer total yield loss, thus compelling farmers to abandon their fields. The *Striga* problem is intimately associated with low levels of soil fertility which results in the sub-region from many factors including intensification and the reduced fallow periods (Schmidt-Vogt *et al.*, 1991).

In summary, it is clear that *Striga* and drought individually pose an ominous obstacle to maize production in an area known for its potentially high productivity and production. Moreover, it is obvious that the combined effect of the two stress factors will hamper all efforts at alleviating food insecurity, hunger and hidden hunger in SSA.

Breeding for combined drought tolerance and *Striga* resistance in maize more efficiently under the changing environments become imperative because there are no economically viable technologies to facilitate crop production under these stresses (Farooq *et al.*, 2009).

2.2 Overview of agricultural drought and *Striga hermonthica*

2.2.1 Definition of drought in agriculture

Agricultural drought is defined on the basis of soil water deficit and occurs when there is not enough soil moisture to support crop production (Modanesi *et al.,* 2018). Different models predicted a substantial impact on soil moisture and temperature conditions with increased frequency and duration of droughts as a consequence of climate change (Stocker *et al.,* 2013). In such a context, the vulnerability of agriculture in SSA, a sub-region which is already known to be threatened by recurrent drought, is expected to further increase (Kamali *et al.,* 2018). However, in a previous study, Lobell *et al.* (2011) reported that an increment of 1°C in mean temperatures will result in about 65% and 100% maize yield losses under well-watered conditions and in drought-prone areas, respectively. The most severe effects of drought stress on maize plant occur when drought coincides with 7 to 10 days period before and after flowering (Edmeades *et al.*, 2017). Before flowering, under stress, ear growth slows down more than tassel growth resulting in a delay in silk emergence relative to pollen shed, giving rise to what is known as anthesis-silking interval (ASI) (Edmeades *et al.*, 2017). When drought occurs at flowering period, complete abortion of ears can be observed which consequently may lead to barrenness. Also, when the stress extends throughout grain filling, reduced number of kernels per ear and poor grain filling are observed (Edmeades *et al.*, 2017).

2.2.2 Overview of *Striga hermonthica*

The genus *Striga*, previously assigned to the family Scrophulariaceae, is now grouped within the family Orobanchaceae Vent. based on several recent phylogenetic evidence (Spallek *et al.*, 2013). It comprises about 30 obligate root-parasitic annual plants, commonly known as witchweeds presumably because most of their life cycles occur underground, the period during which severe

symptoms appear before the parasite emerges (Spallek *et al.*, 2013). Approximately, 80% of the characterized *Striga* species are known to be endemic to Africa and more precisely, originated from the region between the Semien Mountains of Ethiopia and the Nubian Hills of Sudan (Atera *et al.*, 2011) which is also described as the centre of origin of sorghum (*Sorghum bicolor* L.). *S. hermonthica, S. asiatica, S. gesnerioides,* and to some extent *S. aspera* and *S. forbesi* Benth are, in decreasing order, the five *Striga* species of economic importance in SSA which cause serious damage to the sub-region cereal production (Spallek *et al.*, 2013).

Striga hermonthica (Delile) Benth. is an obligate hemiparasite. That is, *S. hermonthica* is photosynthetically active but the establishment of parasitism with a host is essential to its survival (Spallek *et al.*, 2013). It is also obligately allogamous and closely related to *S. aspera* with which it can intermate and produce viable and virulent offspring (Safa *et al.*, 1984). This exchange and recombination of genetic material between *S. hermonthica* and *S. aspera* make their respective populations extremely diverse and continually changing. *S. hermonthica* infests 57% of the total area in SSA under cereal production (Sauerborn, 1991). In Togo, Mali and Nigeria, maize growing areas infested with *S. hermonthica* represent about 30–40% (De Groote *et al.*, 2008). This large coverage of infestation which results in about 30–90% yield loss (Musyoki *et al.*, 2015) makes *S. hermonthica* the most economically important parasitic weed. Yield losses largely depend on *Striga* density (Mbuvi *et al.*, 2017), host species and genotype, land use system, soil nutritional status and rainfall patterns (Atera *et al.*, 2013). The geographic distribution and level of infestation is projected to steadily increase in SSA (Ejeta and Gressel, 2007) because there are no means to properly control the parasite and also because its seeds are easily propagated due to their tiny size (Atera *et al.*, 2012). Furthermore, habitat suitable for its growth are predicted to expand as a consequence of changes in climatic conditions (Mohamed *et al.*, 2006).

Germination of *Striga* seeds requires a preconditioning phase which is characterized by high temperature (20-40°C), adequate moisture for 7-14 days (Parker and Riches, 1993) and germination stimulants known as Strigalactones which are exuded from a susceptible host. Strigalactones are plant hormones which are synthesized from carotenoids and serve as regulators in several developmental processes to adapt root architecture to nutrient availability. They also play key role in plant-arbuscular mycorrhizal (AM) fungus symbiosis (Charnikhova *et al.*, 2017). In maize, in addition to the classical strigolactones such as 5deoxystrigol (5-DS) and sorgomol (Yoneyama *et al.*, 2015), two chemical structures for the strigolactones were recently described and termed zealactones (Charnikhova *et al.*, 2017). Once a germinated *Stiga* plant is able to establish a xylem-to-xylem connections with its host, it extracts water and nutrients throughout its life cycle. This results in a range of drought-like and nutrients deficiency symptoms on the host. Commonly, the infested host plants become stunted, show chlorosis, and can even die in severe cases (Dörr, 1997). It has been estimated that each single plant of *S. hermonthica* can cause 5% yield loss on a host plant (Parker and Riches, 1993).

2.3 Breeding tolerance in maize for drought

2.3.1 Requirements for breeding for tolerance to drought

Breeding for drought tolerance, like any other trait, requires two basic things (Edmeades, 2013): genetic variation and selection environment. The genetic variation should be identifiable and heritable within a breeding population. It is usually revealed in the significant levels of interactions between genotype and the stress level in response to drought stress (Edmeades, 2013). The choice of selection environment is made among the target environments and must ensure that stress intensity, timing, and frequency can be reliably managed to expose genetic variation for traits season after season (Barker *et al.*, 2005). However, in most maize target environments, drought

occurs randomly and so breeders nowadays prefer the "hotspot" approach in which the stress is managed in the environments through irrigation. Managed drought stress environments are rain-free testing sites where water stress is induced and its timing and intensity are managed through irrigation.

2.3.2 Plant physiological variables addressed in breeding for tolerance to drought

Genetic improvement of grain yield in a water-limited environment targets at least one of the three variables reviewed by Passioura (1977) which are: (i) the amount of water captured by the plant (W), (ii) the efficiency with which that water is converted to biomass (water use efficiency, WUE), and (iii) the harvest index (HI), in other words, the proportion of biomass forming grain. For instance, genetic control of root morphology (root depth and root biomass) is known to be the most effective means of increasing W. However, reports have shown that there is genetic variation for root depth and that to increase the amount of water captured by the roots, deeper roots are preferred to roots biomass increase (Edmeades *et al.*, 2006). In addition to increasing the amount of water captured, the more the roots go deeper into the soil, the better the plant stand (resistance to root lodging). Maize breeding also targets delayed leaf senescence also known as "staygreen" characteristic or leaf death as the best way of increasing WUE but, at the same time this trait seems to also assure root health and increases duration of kernel filling (Edmeades, 2013). However, a weak association between grain yield and staygreen characteristic has been reported under drought (Chapman and Edmeades, 1999).

2.3.3 The use of secondary traits

Emphasis on secondary traits in breeding for tolerance to drought stems from the fact that heritability and genetic variance for grain yield decline under stress as interplant and interplot variability that occurs under stress increase (Edmeades, 2013). The importance of the secondary

traits can be revealed by examining their genetic correlations with GY under drought stress, or by estimating their correlated response after selecting for grain yield under stress (Edmeades *et al.,* 1997), and also through path analysis (Badu-Apraku *et al.,* 2011; Talabi *et al.,* 2017). Edmeades *et al.* (2017) defined an appropriate secondary trait as a trait that is (1) genetically associated with grain yield under drought; (2) highly heritable; (3) stable and feasible to measure; and, (4) not associated with yield loss under ideal growing conditions. Commonly used secondary traits under drought are reduced barrenness, ASI, staygreen characteristic, and leaf rolling (Edmeades *et al.,* 2017). The barrenness is expressed in terms of number of ears per plant (EPP) while the staygreen characteristic is assessed through senescence. Some other traits such as osmotic adjustment and ability to remobilize stem reserves are also often used in studies of drought tolerance mechanism (Ludlow and Muchow, 1990). However, in a breeding programme, the criteria of cost and easy to be measured attached to a trait is of great importance and most of these traits do not meet (Chapman and Edmeades, 1999). It has also been pointed out that, traits that are known to increase the current photosynthesis rate are more valuable than that related to remobilization of the reserves (Schussler and Westgate, 1995; Westgate *et al.,* 1997). Furthermore, development of tassel, roots, and stems during flowering and grain filling periods can negatively affect ear development and therefore, decrease in their demand of resources during the periods can favour the development of ears and result in increased grain yield under stress. In addition to the commonly used secondary traits, potential traits to be considered in breeding for tolerance to drought in maize are disease resistance and ear aspect. Because the capability of a plant to withstand any stress decreases when diseased while ear aspect directly affects grain yield.

2.4 Breeding for resistance/tolerance in maize to *Striga*

2.4.1 Genetics of resistance to *Striga*

2.4.1.1 Resistance/tolerance in maize

With regard to the type of mechanism involved in the interaction between maize plant and *Striga*, three types of genotypes can be distinguished (Berner *et al.,* 1995). Low stimulators are genotypes that are less efficient in stimulating *Striga* to germinate. They are also known as pre-attachment resistant genotypes. Their resistance may involve the *lgs1* gene found in sorghum (Gobena *et al.*, 2017). This gene is located in a conserved region; therefore, studies are underway to use reverse genetics to find lgs1 in maize. Genotypes that stimulate *Striga* to germinate and allow it to attach, but slow its growth, delay its emergence, and reduce its vigour are known as post-attachment resistant genotypes. Finally, genotypes that stimulate *Striga* to germinate and allow it to attach, grow, and reproduce normally, but do not suffer much from the "intoxication effect" are known as tolerant genotypes. In field conditions, resistance is known to be associated with number of emerged *Striga* plants and yield under infestation while tolerance is associated with *Striga* damage, number of ears per plant, and yield under infestation. These traits have been combined together in a base index for selecting performant genotypes under *Striga* infestation, in the IITA Maize Improvement Programme (MIP).

2.4.1.2 Gene action/ inheritance of resistance to *Striga*

Studies on the genetics of maize to *Striga hermonthica* have yielded contradictory reports on the gene action involved in maize resistance to *Striga* even though all agreed that resistance is quantitatively inherited. For instance, Kim (1994); Berner *et al.* (1995); and Badu-Apraku (2007) found that additive gene effects were more important than nonadditive gene effects for host plant damage and grain yield under *Striga* infestation. On the contrary, Kim (1991) and Gethi and Smith

(2004), for example, concluded that nonadditive gene effects were more important than additive gene effects for host plant damage while additive gene effects were predominant for number of emerged *Striga* plants. Moreover, moderate heritability estimates were reported for host plant damage and grain yield while low heritability estimates were reported for *Striga* emergence under *S. hermonthica* infestation (Badu-Apraku *et al.,* 2007). For instance, Akanvou *et al.* (1997) estimated narrow-sense heritability of 0.33, 0.14, and 0.32, respectively for *Striga* damage, number of emerged *Striga* plants, and grain yield under *Striga* infestation.

2.4.1.3 Genes sources

At the beginning of the research in maize resistance to *Striga hermonthica* in IITA, the initial screening for resistance allowed identification of resistant inbred lines among the existing germplasm which were used as resistant gene sources to improve two composites : TZL Comp.1 and TZE Comp.5 (Badu-Apraku and Fakorede, 2017). These two materials have been the basis of the great achievement in developing and delivering resistant open-pollinated varieties, inbred lines and hybrids in all maturity groups. Furthermore, resistance identified in the perennial teosinte (*Zea diploperennis*) and in improved temperate maize have been used to increase the genes for resistance to *Striga*, which has been found to be quantitatively inherited.

2.5 Vitamin A deficiency in SSA and Biofortification in maize

2.5.1 The scope of the problem

The incidence of vitamin A (VAD) deficiency was estimated to be among the highest (48%) in SSA with an occurrence of 95% of the reported death from diarrhea and measles in 2013 (Stevens *et al.,* 2015). For instance, more than 7.2 million pregnant women and approximately 127 million pre-school aged children have been reported vitamin A deficient in developing countries (West , 2002). About 5-10 million children develop xerophthalmia from VAD every year and up to

500,000 among them become blind (Sommer, 1995). Also, VAD account for more than 600,000 of childhood deaths (West and Darnton-Hill, 2008).

Children affected by VAD often develop corneal blindness and their growth is retarded (West and Darnton-Hill, 2008). VAD also affects the immune system and can therefore result in several diseases such as measles and diarrhea which can lead to an increased risk of mortality (Rice *et al.*, 2004).

Two main reasons explain the high prevalence indices of VAD in developing countries: the almost exclusive consumption of white maize compared to the yellow maize and poverty. For human consumption in most parts of Africa there is a high preference of white maize compared to yellow maize (De Groote *et al.*, 2008, Pillay *et al.*, 2011). Also, during hunger periods the imported relief food were mainly from yellow maize, therefore, people perceive yellow maize to be inferior to white maize in addition to its unfavourable taste and texture (Muzhingi *et al.*, 2008). However, several studies have proved the contrary. For instance, in a study conducted in Mozambique, consumers preferred orange maize to white maize because of its aroma (Stevens and Winter-Nelson, 2008). More recently, another study conducted in rural Zambia demonstrated that when nutrition information is properly provided, consumers show higher preference to orange than white maize (Meenakshi *et al.*, 2012). These studies in summary highlight the importance of appropriate consumer education in the efforts towards alleviation of VAD through adoption and consumption of orange maize in Africa.

Many other strategies such as supplementation, food fortification, and diet diversification have been effective in addressing VAD (Mora, 2003) their impacts remain negligible due to the very high rate of poverty and other complicating factors in developing countries (Graham *et al.*, 2001).

2.5.2 Provitamin A biofortification in maize

Biofortification is a cost-effective, sustainable, and long-term means of breeding nutrients into food crops. It can help to alleviate VAD more efficiently because a provitamin A biofortified staple crop like maize will increase the daily micronutrient intakes throughout the lifecycle of individuals (Bouis *et al.*, 2011).

Currently, agronomic, conventional breeding, and transgenic biofortification are three common approaches used. However, the conventional breeding biofortification is the most used in maize as regard the provitamin A because of its naturally great diversity in provitamin A carotenoids content. Maize is a carotenogenic species with a known genetic diversity of carotenoids content and profiles (Burt *et al.*, 2011). Carotenoids represent more than 600 characterized structures of pigments synthesized in plant (Messias *et al.*, 2015). They are essential for growth and development, play key roles in the process of photosynthesis, protect plants against photooxidative damage, and are also precursors of abscisic acid synthesis (Gallagher *et al.*, 2004). Carotenoids are responsible for the observed range of colours (light yellow to dark orange) in maize kernels. They are primarily concentrated in the vitreous portion of the endosperm (Weber, 1987). Several studies found association between darker orange colour and higher total carotenoids, particularly lutein and zeaxanthin in maize (Burt *et al.*, 2011; Almeida Rios *et al.*, 2014) but not necessarily with higher provitamin A carotenoids (Harjes *et al.*, 2008). However, the estimated β-carotene level in majority of yellow maize is on average 1.7 µg g^{-1} (Harjes *et al.*, 2008).

The phytoene formation from geranylgeranyl pyrophosphate is the first step in biosynthesis of carotenoids. This first step is mediated by phytoene synthase (PSY). Three genes are known to encode PSY in maize. They are *psy1*, *psy2*, and *psy3*. The gene *psy1* also known as Y1 has been reported to highly determine carotene synthesis and levels of carotenoids in the maize endosperm

(Li *et al.*, 2008). For instance, white endosperm phenotype was observed to be due to a loss-of-function allele of psy1 which prevents accumulation of carotenoids in the endosperm (Gallagher *et al.*, 2004). Also, high polymorphism has been reported for the *psy1* in different varieties of maize (Palaisa *et al.*, 2003). The triploid maize endosperm (3n = 30) results in four possible phenotypic classes at the Y_1 locus depending on the number of dominant (Y_1) and recessive (y_1) alleles which are: $y_1y_1y_1$, $y_1y_1Y_1$, $y_1Y_1Y_1$, and $Y_1Y_1Y_1$.

2.5.3 Achievement towards conventional breeding for carotenoids in maize

The advent of genomics and bioinformatics have enabled the identification of additional genes in the maize carotenoid biosynthetic pathway (Wurtzel *et al.*, 2012). For example, Owens *et al.* (2014) performed a genome-wide association study in a panel of maize inbreds ranging from light yellow to dark orange in grain colour and found the existence of 58 candidate genes involved in biosynthesis of carotenoids in maize. Among them, eight candidate genes, *y1, zds1, lcyE, crtRB3, lut1, crtRB1, zep1*, and ccd1, have been reported to play key roles in carotenoids synthesis and accumulation and are all in chromosome regions associated with QTL for carotenoids (Chander *et al.*, 2008; Kandianis, 2010; Chandler *et al*,. 2013). Except for *crtRB3* and lut1, these genes were also associated with QTL for the intensity of the orange colour (Chandler *et al.*, 2013). Several studies revealed significant allelic variation for lycopene epsilon cyclase (*lcyE*) (Harjes *et al.*, 2008) and β-carotene hydroxylase 1 (*crtRB1*) (Yan *et al.*, 2010) which are known to significantly influence synthesis and accumulation of carotenoid in maize grains. There are, respectively, four and three polymorphism sites at *lcyE* and *crtRB1* known to be correlated with high levels of β-carotene. Variation at *crtRB1* include 5'TE, InDel4, and 3'TE with the largest effect attributable to the rare allele (insertion of 206 bp at 5'TE), found only in temperate germplasm at 2.9% (Yan *et al.*, 2010).

Babu *et al.* (2013) validated the effects of 2 polymorphisms (*lcyE* 5′TE, *lcyE* 3′Indel) of *lcyE* and the *crtRB1*-3′TE of *crtRB1* in 26 diverse tropical genetic backgrounds. When the transcript level of *lcyE* is reduced (favourable alleles), there is an increase of β-branch carotenoids. Also, favourable alleles of *crtRB1* with reduced transcript levels decrease hydroxylation of β-carotene. The increase of β-branch carotenoids and the decrease in hydroxylation of β-carotene result in higher provitamin A carotenoids levels in maize kernels (Harjes *et al.*, 2008; Yan *et al.*, 2010). For example, the levels of β-carotene and total provitamin A content have been shown to increase to up to two-tenfold as a result of *CrtRB1*-3′TE alone while favourable alleles at *lcyE* can reduce up to 30% the ratio of levels of α- to β-branch. Azmach *et al.* (2013) also reported higher levels of provitamin A in tropical inbreds that possessed favourable alleles at the *crtRB1*-5′TE and 3′TE. Fraser and Bramley (2004) showed that *lcyE* controls the zeaxanthin/lutein ratio and that it is a key gene determining the provitamin A content in maize.

The use of markers in selection for carotenoids has been shown to be effective. Indeed, the use of markers with appropriate breeding scheme have moved the existing variability of β-carotene, 0.24 to 8.80 $\mu g\, g^{-1}$, (Ortiz-Monasterio *et al.*, 2007, p.) to levels matching or exceeding the breeding target in tropical maize inbreds. Up to 13.6 $\mu g\, g^{-1}$ of β-carotene was reported with favourable alleles at *lcyE* (Harjes *et al.*, 2008). Combinations of favourable alleles at both *lcyE* and *crtRB1* allowed increases up to 17.25 $\mu g\, g^{-1}$ (Azmach *et al.*, 2013), from 15 to 20 $\mu g\, g^{-1}$ (Babu *et al.*, 2013) and more recently, up to 22.6 $\mu g\, g^{-1}$ (Menkir *et al.*, 2017) mostly in inbred lines.

Despite efforts that have been made to address the provitamin A deficiency in developing countries through the development of maize cultivars with a significant increase of provitamin A content, the currently released cultivars contain only 6 to 8 $\mu g\, g^{-1}$ of provitamin A (HarvestPlus, 2017).

Furthermore, the predominance of additive genetic effects and high heritability were reported (Egesel *et al.*, 2003) and yet, OPVs with high-level of provitamin A are lacking. On the contrary, Halilu *et al.* (2016) found non-additive genetic effects to be predominant for all carotenoids and their finding supports that of Burt *et al.* (2011) who reported heterosis in carotenoids to be a rare phenomenon. The controversial report on the genetic basis underlying carotenoids accumulation in maize kernels suggest that different gene actions exist depending on the genetic background of the provitamin A materials. Moreover, the cost attached to the use of makers and the High-Performance Liquid Chromatography (HPLC) based carotenoids quantification have led to the extensive use of colour rating in some breeding programmes as an alternative. In fact, maize grain colour, or more precisely, endosperm colour have been reported to be highly correlated with the accumulation of β-carotene (Johnson and Miller 1938; Chander *et al.*, 2008) and more recently, with β-cryptoxanthin and zeaxanthin (Venado *et al.*, 2017). Conversely, Egesel *et al.* (2003); Harjes *et al.* (2008) reported no correlation between endosperm colour and β-carotene accumulation in maize. Even though Chander *et al.* (2008) recommended the use of colour rating along with specific markers for successful breeding, it is clear that its exclusive use could create controversy.

2.6 Molecular characterisation in plant breeding

Genetic variability is a prerequisite for a successful maize breeding to address the increasing demand for resistant genotypes to adverse environmental changes and consumer preferences. To this end, inbred lines are routinely developed from numerous source populations improved through different methods (Hallauer, 1990). These developed inbred lines are used subsequently to form new cultivars or base populations, constitute a sample of the genetic diversity of maize. Thus,

genetic variability assessment among inbred lines has become an unavoidable step in breeding, prior to performing hybrids and maize cultivars development.

Many tools have been used to characterize and assess diversity in maize inbred lines. But nowadays, the availability and accessibility at relatively low cost of Single Nucleotide Polymorphism (SNP) markers has popularized their use in different molecular studies. SNPs are discovered by different platforms among which the most commonly used are genotyping by sequencing (GBS) and diversity array technology sequencing (DArTSeq), which are all sequence-based technologies. Pro and cons of each of the two technologies have been highlighted by Chen *et al.* (2016). GBS generates a very high density of markers (>800,000 SNPs) compared to DArTSeq which yields markers with a number ranging from 50,000 to 350,000 SNPs (Sansaloni *et al.*, 2011). On the contrary, with regard to the coverage of the genotyping, DArTSeq have much higher coverage than GBS (<0.5X) and lower levels of missing data (<20%) in comparison with GBS (> 50%), in the case of maize. Beside these differences, the aforementioned methods have significantly contributed to today's large-scale use of SNP makers in a range of studies for investigating the molecular basis of phenotypic variations in plants such as diversity and association mapping studies, and genomic selection.

Molecular-based diversity study using SNPs has the advantage of exploring the variation between genotypes at the base level and provides a means of differentiating cultivars and classifying inbred lines into heterotic groups (Yang *et al.*, 2011; Dao *et al.*, 2014; Wu *et al.*, 2016); identifying gaps and redundancy in germplasm collections (Semagn *et al.*, 2012; Ertiro *et al.*, 2017). It also allows the understanding of the genetic changes that occur in the process of germplasm conservation, or regeneration, or during breeding. Furthermore, molecular-based diversity study is a means of identifying novel and superior alleles for improvement of agronomic traits.

CHAPTER THREE

3.0 Performance of extra-early maturing maize inbred lines under *Striga*-infested, drought stress and optimal environments

3.1 Introduction

Stress from *Striga* infestation and drought constitutes the most important biotic and abiotic factors limiting maize production and productivity in SSA. Developing cultivars for resistance to these stresses in order to stabilize maize yield production in the sub-region is the major strategy of the Maize Improvement Program (MIP) at the International Institute of Tropical Agriculture (IITA). *Per se* performance evaluation of inbred lines is an important step in plant breeding. It allows the identification of elite lines to be used as parents in planned crosses to develop promising hybrids. But, direct selection for improved performance under stresses, such as *Striga* and drought, based on grain yield alone has been reported inefficient (Edmeades, 2013). Therefore, efficient improvement of maize resistance/tolerance to these stresses has often been based on the use of highly heritable secondary traits for which genetic variability increases under stress conditions (Edmeades *et al.,* 2017). Alternatively, the use of breeding values through the numerator relationship matrix derived either from pedigree (A) or molecular markers (G) has been proposed for predicting the performance of genotypes that would be used as parents for developing new genotypes (Piepho *et al.,* 2008). Several studies have proven the importance of the genetic variance-covariance structure in predicting genotype performance. Bromley *et al.* (2000) showed, for instance, that for inbred lines of maize ignoring pedigree relationships results in a reduction in estimates of genetic variance. The objectives of this study were to (i) determine the performance of extra-early maturing maize inbred lines for resistance to *Striga* and tolerance to drought, and (ii) predict their breeding values using the correlation among their genetic variances.

3.2 Materials and Methods

3.2.1 Genetic materials

The genetic material used in the present study consisted of 152 orange and 28 yellow extra-early (80-85 days to maturity) maturing maize inbred lines. The orange inbreds were selected out of 253 inbreds developed from 2009 TZEE-OR1 STR in IITA-Maize Improvement Programme (MIP) based on their performance under *Striga* infestation and drought stress during 2014 and 2015 field evaluations. The 28 yellow inbreds including five checks (TZdEEI 1, TZdEEI 7, TZdEEI 9, TZdEEI 12, and TZdEEI 13) were also developed in IITA-MIP from different populations.

3.2.2 Experimental design and field trial management

3.2.2.1 Experimental design

Three different field trials with regard to the management were conducted using a 12 x 15 alpha lattice design with two replications. Single-row plots each 3 m long with a spacing of 0.75 m between two adjacent rows and 0.40 m between plants within the row were used. Three seeds were planted per hill, and the seedlings were later thinned to two per hill about 2 weeks after emergence to give a final population density of about 66,667 plants ha^{-1}.

3.2.2.2 Management of drought trials

The drought trials were evaluated at Ikenne (6º53'N, 3º42'E, 60 m altitude, 1200 mm annual rainfall), under managed drought during the January-May dry seasons of 2016/2017 and 2017/2018. The managed drought was achieved using irrigation system. The field was irrigated using sprinkler irrigation system for the first three weeks. Plants were irrigated using a sprinkler irrigation system, which provided 17 mm of water each week. Then, from 22 days after planting (DAP) until maturity, drought was induced by withdrawing irrigation water.

3.2.2.3 Management of trials under Striga infestation

The *Striga*-infested trials were evaluated at Mokwa (9°18' N, 5°4 E, 457 m altitude, 1100 mm rainfall) and Abuja (9°16' N, 7°20' E, 300 m altitude, 1500 mm rainfall) in the southern Guinea Savanna of Nigeria during the rainy season in 2016 and 2017. The artificial infestation of the fields was done following the method developed by IITA's maize programme (Kim, 1991; Kim and Winslow, 1991).

Striga seeds were collected from sorghum fields around the experimental sites at the end of the growing season. The cleaned, sieved and preconditioned *Striga* seeds were mixed with finely sieved sand in the ratio of 1:99 (seed: sand) by weight, at planting. The scoops, which ensure about 5000 germinable *Striga* seeds when filled, were used to infest each planting hole. To avoid the negative effect of fertilizers on the germination ability of the *Striga* seed, fertilizer application was delayed until about 30 days after planting. The fertilizer NPK 15-15- 15 was used at 30 kg N ha^{-1}, 26 kg P ha^{-1}, 50 kg K ha^{-1}. Hand weeding was used to control weeds other than *Striga*.

3.2.2.4 Management of trials under rain-fed conditions

Trials planted under rain-fed conditions were evaluated at Abuja (2016), Mokwa (2016 and 2017), and Ikenne (2016, 2017) during the June-October rainy season of each year. In these trials, nitrogen was applied at 90 kg N ha^{-1}, which is considered as the optimal level of nitrogen under maize cultivation. Therefore, these trials were referred to as "optimal" in the rest of this thesis. The optimal trials received at each location, 60 kg N ha^{-1}, 60 kg P ha^{-1} and 60 kg K ha^{-1} at 2 weeks after planting (WAP) with an additional 30 kg N ha^{-1} top-dressed at 4 WAP.

In all trials except those under *Striga* infestation, Primextra (active ingredient: Atrazine) as pre-emergence and Paraquat (active ingredient: Gramoxone) as post-emergence herbicides were used to control the weeds. The application of these herbicides was done at 5 L/ha each.

3.2.3 Data collection and measured traits

3.2.3.1 Flowering and plant morphological traits

The number of days from planting to when 50% of the plants had emerged silks and had shed pollen was recorded as days to silking (DYS), and days to anthesis (DYA), respectively. The difference between DYS and DYA was calculated as the anthesis-silking interval (ASI).

The plant and the ear heights were measured as the distance from the base of the plant to the height of the first tassel branch (PLHT) and the node of insertion of the upper ear (EHT).

The overall architecture and the appeal to sight of plants in a plot were assess using a scale of 1 to 9 and recorded as plant aspect (PASP) where 1 represents excellent overall phenotypic appeal and 9 represents extremely poor overall phenotypic appeal.

The percentage of plants leaning more than 30° from the vertical, in a plot was recorded as root lodging (RL) while the percentage of plants broken at or below EHT was recorded as stalk lodging (SL).

3.2.3.2 Ear related traits

The extent to which ears are covered by the husk was rated using a scale of 1 to 9 as husk cover (HC) where 1 represents ears with very tight husk extending beyond the tips and 9 represents ears with exposed tips.

The overall appearance of the ears without the husks was assessed as ear aspect (EASP) using a scale of 1 to 9 where 1 represents uniform and fully filled ears with no diseases and 9 represents the case where no ear is produced.

The number of ears per plant (EPP) was calculated by dividing the total number of ears harvested per plot by the number of plants in a plot at harvesting.

3.2.3.3 Grain yield estimation

For the drought trials, the grain yield was adjusted to 15% moisture and computed as follows:

$$GY = Gwt \times \frac{(100-m)}{85} \times \frac{10000}{(rwl \times \Phi)}$$

Where, GY = Grain yield in kg ha^{-1}, Gwt= shelled grain weight per plot (kg), m = grain moisture content at harvest, rwL = row length in m, Φ = inter-plant spacing in a row.

The grain yield in optimal and *Striga*-infested environments was also adjusted to 15% moisture and computed assuming 80% of shelling percentage for the genotypes as follows:

$$GY = fwt \times \frac{(100 - m)}{85} \times \frac{10000}{(rwL \times \Phi)} \times 0.8$$

Where, GY = Grain yield in kg ha^{-1}, fwt= ears weight at harvest per plot, in kg, m = grain moisture content at harvest, rwL = row length in m, Φ = inter-plant spacing in a row.

3.2.3.4 *Striga* resistance and drought tolerance indicator traits

The staygreen characteristic (STGC) was rated under drought stress at 70 days after planting (DAP) using a scale of 1 to 9 where 1 is when dead leaf area represents up to 10% of the total leaf area and 9 is when dead leaf area falls between 90 to 100%.

Under *Striga*-infestation, the damage syndrome on the plants in a plot and the number of emerged *Striga* plants were recorded at 8 and 10 WAP. The *Striga* damage syndrome was rated on plot basis using the scale of 1 to 9 described by Kim (1994).

3.2.4 Data analysis

3.2.4.1 Model description

The data was analysed using the mixed model equation (Equation 3.1) in ASReml-R (Butler *et al.*, 2009; Gilmour *et al.*, 2015) statistical package. All the factors in the model were considered as random.

The mixed model equation is given as follow:

$$Y = X\beta + Z_{Env}u_{Env} + Z_{Env:Rep}u_{Env:Rep} + Z_{Env:Rep:Blk}u_{Env:Rep:Blk} + Z_{Ped}u_{Ped} + Z_{Env:Ped}\,u_{EnvxPed} + e \quad \text{(Equation 3.1)}$$

Where: Y is the vector of unadjusted observations. The X-matrix and Z-matrices are incidence matrices belonging to their respective components, β is a vector of intercepts. u_{Env}, $u_{Env:Rep}$, $u_{Env:Rep:Blk}$ and u_{Ped} are vectors of random effects of environments, replications within environments, blocks within replications and genetic effects, respectively. $u_{EnvxPed}$ and e are respectively the interaction terms of the genetic effect with environments and the random error. The random effects in the model were assumed to follow a multivariate distribution with means and variances defined respectively by the equations 3.2 and 3.3.

$$E\begin{bmatrix} u_{Env} \\ u_{Env:Rep} \\ u_{Env:Rep:Blk} \\ u_{Ped} \\ u_{EnvxPed} \\ e \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix} \quad \text{(Equation 3.2)}$$

$$Var\begin{bmatrix} u_{Env} \\ u_{Env:Rep} \\ u_{Env:Rep:Blk} \\ u_{Ped} \\ u_{EnvxPed} \\ e \end{bmatrix} = \begin{bmatrix} \sigma^2_{Env} & 0 & 0 & 0 & 0 & 0 \\ 0 & \sigma^2_{Rep} & 0 & 0 & 0 & 0 \\ 0 & 0 & \sigma^2_{Blk} & 0 & 0 & 0 \\ 0 & 0 & 0 & \sigma^2_{Ped} & 0 & 0 \\ 0 & 0 & 0 & 0 & I_{Env} \otimes^n_j I_{Ped}\sigma^2_{Ped} & 0 \\ 0 & 0 & 0 & 0 & 0 & R' \end{bmatrix} \quad \text{(Equation 3.3)}$$

Where ⊗ represent the Kronecker direct product (Cullis and Gleeson, 1991).

3.2.4.2 Random error variance structure

A heterogeneous variance structure of the random error was fitted as a direct sum of the identity variance matrices with dimension the number of environments using the "at" variance model function in ASReml-R.

The heterogeneous variance structure is given by the equation 3.4.

$$R' = \bigoplus_j^n I_{Env}\sigma_e^2 = \begin{bmatrix} \sigma_{ej}^2 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & \sigma_{en}^2 \end{bmatrix} \quad \text{(Equation 3.4)}$$

Where $\oplus$ represents the Kronecker direct sum (Cullis and Gleeson, 1991).

3.2.4.3 Estimation of additive genetic variance

The genetic relatedness between inbreds was used in the genetic variance structure through pedigree information. In this case, the numerator relationship matrix (A) was created by ASReml-R based on the pedigree file. The A matrix indicates the additive relationship between the inbreds and allows accurate prediction of breeding values. The diagonals of the matrix are 1+F, where F is the inbreeding coefficient, and off-diagonals are twice the kinship coefficients. Assuming absence of dominance and epistasis, the variance-covariance matrix for genetic effects (breeding values) used is given by the equation 3.5.

$$\sigma_{Ped}^2 = A.\sigma_a^2 = \begin{bmatrix} \varphi_{ii} & \cdots & \varphi_{ij} \\ \vdots & \ddots & \vdots \\ \varphi_{ji} & \cdots & \varphi_{ii} \end{bmatrix} \sigma_a^2 \quad \text{(Equation 3.5)}$$

Where σ_a^2 is the additive genetic variance; $\varphi_{ii} = 1 + F_i$ with F_i = inbreeding coefficient of the i^{th} genotype; $a_{ij} = a_{ji}$.

In the analysis of data from each trial type (*Striga*-infested, drought stress and optimal), the variance-covariance structure in equation 3.5 was used to fit interactions of genetic effects with environment. But, in the combined analysis for all test environments, the loglikelihood of the model fails to converge when the equation 3.5 was used to fit interactions. So, the identity matrix (id (ped)) was rather used assuming independence among individual genotypes.

3.2.4.4 Significance test of random factors

To determine the significance of a random factor in the model, the log-likelihoods of models with and without the appropriate random factor were computed. The p-value of each random factor was then computed using a chi-square test of a statistic equals to twice the difference in likelihoods with degrees of freedom equal to the number of additional parameters in the more complex model.

3.2.4.5 Estimation of narrow sense heritability and breeding values

The narrow-sense heritability was calculated on entry mean basis unbiased by environment, replicates, and blocks variances using the additive genetic variance estimated through the A matrix (section 3.2.4.3). The formula used is given by Equation 3.6 and was implemented in ASReml-R using the "pin" function.

$$h^2 = \frac{\sigma_a^2}{\sigma_P^2} = \frac{\sigma_a^2}{\sigma_a^2 + \frac{\sigma_{Env}^2}{n} + \frac{\sigma_{Rep}^2}{r} + \frac{\sigma_{Blk}^2}{b} + \frac{\sum_j^n \sigma_e^2}{n}}$$ (Equation 3.6)

Where n, r, and b are respectively the number of environments, replicates and blocks; σ_a^2 is the additive genetic variance estimated through the numerator relationship matrix (A).

The Best linear unbiased predictions (BLUPs) based on pedigree information (breeding values) and BLUPs based on multivariate analysis were extracted using the "Predict" function in ASReml-R.

3.2.4.6 Base index for selection and correlation among measured traits

The base indices developed by Menkir and Kling (2007) were used for selection of inbred lines under *Striga* infestation (Equation 3.7) and drought stress (Equation 3.8).

$$I_S = 2 \times GY + EPP - (SDR8 + SDR10) - 0.5 \times (ESP8 + ESP10) \text{ (Equation 3.7)}$$

Where, I_S is the base index under *Striga* infestation, GY is grain yield under *Striga* infestation, EPP is number of ears per plant, SDR8 and SDR10 are *Striga* damage at 8 and 10 WAP, ESP8 and ESP10 are number of emerged *Striga* plants at 8 and 10 WAP, and

$$I_D = 2 \times GY + EPP - ASI - PASP - EASP - STGC \text{ (Equation 3.8)}$$

Where, I_D is the base index under drought stress, GY is grain yield under drought; EPP, is ears per plant; STGC is staygreen characteristic; ASI, is anthesis-silking interval; PASP, is plant aspect; and EASP, is ear aspect.

The genetic correlation coefficients among traits were calculated using META macro in SAS v9.4 (Vargas *et al.*, 2013) in order to validate the use of the traits involved in the base indices.

3.3 Results

3.3.1 Genetic variability among inbreds for the measured traits

Table 3.1 presents the variance components and narrow-sense heritability estimates for measured traits of the inbreds across *Striga*-infested environments. The analysis indicated that the genotype-environment interaction (GEI) variance was not significantly different from zero for most of the traits including grain yield except for the traits with high sensitivity to environmental changes such as anthesis-silking interval, plant, and ear heights. The GEI effects for *Striga* damage at 8 and 10 weeks after planting (WAP), and ear aspect were found to be significantly different from zero at α ($p<0.05$ and $p<0.001$), despite the variance among the two environments was not significant.

On the contrary, the variability among inbreds was significantly different from zero ($p<0.001$) for all traits, indicating a high discrepancy in the response to *Striga* infestation probably due to the observed differences in the correlated additive genetic variances. The additive genetic variance contribution to the total variance ranged from zero for root lodging to 72% for days to silking. It accounted for 70% for grain yield, and 69% and 67% for *Striga* damage at 8 weeks after planting and 10 weeks after planting, respectively. The proportion of genotype x environment interaction varied from zero to 49% for ear height. In general, environmental contribution to the total variation was low with the highest observed for anthesis-silking interval (20%) and root lodging (33%). Narrow-sense heritability estimates varied from zero for ear height and root lodging to 48% for grain yield and days to 50% anthesis. The proportion of variation due to additive genetic variance among inbreds was moderate for *Striga* damage at 8 WAP (42%) and 10 WAP (45%) but low for number of emerged *Striga* plants at 8 and 10 WAP with values of 11% and 14%, respectively.

Table 3.1 Variance components and heritability estimates of measured traits of extra-early maturing maize inbreds under *Striga* infestation at Abuja and Mokwa, 2016-2017

Source of variation	Grain Yield, kgha^{-1}	Anthesis silking Interval	Days to 50% silking	Days to 50% anthesis	Plant height, cm
Environment (Env)	10550.69*** (0.01 ± 0.02)	2.81*** (0.20 ± 0.26)	8.99*** (0.13 ± 0.18)	3.01*** (0.06 ± 0.10)	0.00*** (0.0 ± 0.0)
Replication (Env)	242.04^{ns}	0.21**	2.10***	0.92**	137.95***
Block (Env x Rep)	44337.56***	0.00^{ns}	0.34^{ns}	0.42*	0.00^{ns}
Entry (ped)	1067534.9*** (0.70 ± 0.07)	4.28*** (0.31 ± 0.14)	47.08*** (0.67 ± 0.14)	34.58*** (0.72 ± 0.09)	94.85*** (0.09 ± 0.13)
Env x Entry	117192.6^{ns} (0.08 ± 0.05)	2.62*** (0.19 ± 0.11)	0.00^{ns} (0.0 ± 0.0)	0.00^{ns} (0.0 ± 0.0)	354.18** (0.35 ± 0.14)
Env_1 Residual	307022.29	4.81	15.77	13.32	581.87
Env_2 Residual	248202.67	2.87	8.55	4.69	264.40
h_A^2	0.48±0.07	0.19±0.07	0.46±0.07	0.48±0.06	0.05±0.06
Source of variation	**Ear height, Cm**	***Striga* damage at 8 WAP**	***Striga* damage At 10WAP**	**Number of emerged *Striga* plants 8 WAP**	**Number of emerged *Striga* plants 10 WAP**
Environment (Env)	0.00*** (0.0 ± 0.0)	0.00***(0.0 ± 0.0)	0.03*** (0.01 ± 0.03)	0.00***	0.00*** (0.0 ± 0.0)
Replication (Env)	21.55***	0.00^{ns}	0.00^{ns}	14.52***	21.80***
Block (Env x Rep)	0.00^{ns}	0.08**	0.08***	4.92***	7.61***
Entry (ped)	0.00*** (0.0 ± 0.0)	2.47*** (0.69 ± 0.07)	2.91*** (0.67 ± 0.07)	37.26*** (0.18 ± 0.05)	58.79*** (0.33 ± 0.09)
Env x Entry	124.23*** (0.49 ± 0.09)	0.22***(0.06 ± 0.05)	0.45* (0.10 ± 0.06)	0.00^{ns}	0.00^{ns} (0.0 ± 0.0)
Env_1 Residual	133.36	0.83	0.94	124.91	128.62
Env_2 Residual	81.25	0.83	0.73	21.13	48.81
h_A^2	0.0±0.0	0.42±0.06	0.45±0.06	0.11±0.03	0.14±0.04
Source of variation	**Root logging**	**Stalk logging**	**Husk cover**	**Ear per plant**	**Ear aspect**
Environment (Env)	75.53*** (0.33 ± 0.4)	0.00^{ns} (0.0 ± 0.01)	0.00^{ns} (0.0 ± 0.0)	0.03***(0.11 ± 0.14)	0.00***(0.0 ± 0.02)
Replication (Env)	32.78***	0.00^{ns}	0.00^{ns}	0.00^{ns}	0.00^{ns}
Block (Env x Rep)	1.94^{ns}	5.36^{ns}	0.02^{ns}	0.01**	0.18***
Entry (ped)	0.00^{ns} (0.0 ± 0.0)	35.02^{ns} (0.15 ± 0.10)	1.32*** (0.58 ± 0.08)	0.17*** (0.59 ± 0.11)	2.66*** (0.55 ± 0.08)
Env x Entry	0.75^{ns} (0.0 ± 0.01)	0.00^{ns} (0.0 ± 0.0)	0.17^{ns} (0.08 ± 0.07)	0.00^{ns} (0.0 ± 0.0)	0.18*(0.04 ± 0.04)
Env_1 Residual	195.54	197.42	0.39	0.06	1.09
Env_2 Residual	43.05	193.61	1.14	0.09	2.59
h_A^2	0.0±0.0	0.04±0.03	0.29±0.06	0.34±0.06	0.26±0.06

"*", "**", "***", = significance at p(α =0.05), p(α =0.01), and p(α =0.001), respectively; "ns" = non-significant. Values in parenthesis are contributions of different sources of variance to the total variance with their standard errors; h_A^2 = narrow-sense heritability.

Table 3.2 presents the variance components and narrow-sense heritability estimates for measured traits of the inbreds across drought environments. Results indicated a similarity between the two years, with respect to the management of the drought stress, which resulted in GEI variance being non-significantly different from zero for grain yield, plant aspect, number of ears per plant, and staygreen characteristic. Furthermore, the variance due to environmental effect was low with the highest contribution being 10% for ears per plant and staygreen characteristic and the genotype-environment interaction with a range of zero to 29% for anthesis-silking interval. Similar to *Striga*-infested environments, the inbreds showed different responses to drought stress with the variance of the additive genetic component being significantly ($p<0.001$) different from zero. The contribution of the additive genetic variance to the total variation ranged from 25% for root lodging to 77% for ear height. The contribution of the additive genetic variance to the total variation was high for grain yield (73%), and moderate for ear aspect (52%), staygreen characteristic (41%), ears per plant (41%), plant aspect (33%), and for anthesis-silking interval (26%). Narrow sense heritability estimates ranged from 8% for root lodging to 47% for ear height. Moderate heritability estimates were observed for grain yield (41±7%), staygreen characteristic (25±7%) and ear aspect (25±6%) while low additive genetic effects were found for anthesis-silking interval (12±6%), plant aspect (13±4%), and number of ears per plant (16±5%).

Table 3.2 Variance components and heritability estimates of measured traits of extra-early maturing maize inbreds under drought stress at Ikenne during the 2016/2017 and 2017/2018 dry seasons

Source of variation	Grain Yield, kgha^{-1}	Days to 50% silking	Days to 50% anthesis	Anthesis silking Interval	Plant height, Cm	Ear height, cm
Environment (Env)	0.005**(0.00 ± 0.00)	1.61***(0.05 ± 0.15)	0.29*** (0.01 ± 0.08)	0.00*** (0.0± 0.0)	0.00*** (0.0 ± 0.0)	0.00***(0.0 ± 0.0)
Replication (Env)	2821.9**	2.44***	1.46***	0.16*	28.20***	6.89***
Block (Env x Rep)	0.001^{ns}	0.02^{ns}	0.30^{ns}	0.00^{ns}	7.71^{ns}	0.93^{ns}
Entry (ped)	204064.3***(0.73 ± 0.06)	10.10***(0.35 ± 0.10)	11.35*** (0.46 ± 0.10)	3.15*** (0.26 ± 0.13)	612.96***(0.66 ± 0.08)	267.53***(0.77 ± 0.05)
Env x Entry	0.000^{ns} (0.0 ± 0.0)	1.56*** (0.06 ± 0.05)	1.88*** (0.07 ± 0.06)	3.27* (0.29 ± 0.12)	78.21*(0.08 ± 0.)	0.00^{ns} (0.0 ± 0.0)
Env_1 Residual	80633.5	14.19	9.31	5.23	238.02	92.08
Env_2 Residual	68954.1	12.63	10.50	5.06	198.46	59.65
h_A^2	0.41±0.07	0.15±0.05	0.22±0.06	0.12±0.06	0.41±0.07	0.47±0.06
Source of variation	**Root lodging**	**Husk cover**	**Plant aspect**	**Ear per plant**	**Ear aspect**	**Staygreen characteristic**
Environment (Env)	5.53*** (0.07 ± 0.09)	0.38*** (0.08 ± 0.14)	0.00***(0.0 ± 0.0)	0.00***(0.10 ± 0.19)	0.00***(0.0 ± 0.0)	0.40*** (0.10 ± 0.19)
Replication (Env)	0.00^{ns}	0.02*	0.43***	0.01***	0.44***	0.31***
Block (Env x Rep)	0.30^{ns}	0.00^{ns}	0.05^{ns}	0.00^{ns}	0.06^{ns}	0.05*
Entry (ped)	22.74***(0.25 ± 0.08)	1.75*** (0.34 ± 0.13)	1.01***(0.33 ± 0.09)	0.05***(0.41 ± 0.13)	3.07***(0.52 ± 0.09)	1.79*** (0.41 ± 0.13)
Env x Entry	0.00^{ns} (0.0 ± 0.0)	1.91*** (0.38 ± 0.13)	0.00^{ns} (0.0 ± 0.0)	0.00^{ns} (0.14 ± 0.09)	0 (0.0 ± 0.01)	0.44^{ns} (0.14 ± 0.09)
Env_1 Residual	21.88	1.55	0.79	0.07	2.33	1.14
Env_2 Residual	80.76	0.52	2.18	0.06	2.01	1.05
h_A^2	0.08±0.03	0.25±0.09	0.13±0.04	0.16±0.05	0.25±0.06	0.25±0.07

"*", "**", "***", = significance at p(α=0.05), p(α=0.01), and p(α=0.001), respectively; "ns" = non-significant. Values in parenthesis are contributions of different sources of variance to the total variance with their standard errors; h_A^2 = narrow-sense heritability.

Table 3.3 presents the variance components and narrow-sense heritability estimates for measured traits of the inbreds across optimal environments. There were significant differences among random error variances for grain yield. There were also high and significant variability of the GEI. Despite this variability among environments for grain yield, the narrow sense heritability was moderate with a value of 49±5%. All the measured traits showed high narrow sense heritability estimates except for ASI (18±4%) and husk cover (7±5%). Only the additive genetic component of variance for root lodging was not significantly different from zero.

Table 3.4 presents the variance components and narrow-sense heritability estimates for measured traits of the inbreds across test environments. There was high variability among test environments (*Striga*-infested, drought, and optimal) with the variance due to environment significantly ($p<0.001$) different from zero for all the traits. Consequently, the genotype-environment interaction variance was also found to be significantly ($p<0.001$) different from zero for all the measured traits except for stalk lodging. However, the contribution of the genotype-environment variance to the total variance of measured traits was very low with 17% as the highest for husk cover. This indicated consistency of the genotypic response to test environments despite the high heterogeneity of random error. Thirty (30%) percent of the total variation was due to additive genetic effects among the inbreds for grain yield and 47%, 43% and 29%, respectively, for ear aspect, ears per plant, and anthesis-silking interval. The narrow sense heritability estimates were very low for measured traits used in the combined analysis of variance with the highest value of 26% for plant and ear heights. The observed heritability estimate for grain yield was 8%.

Table 3.3 Variance components and heritability estimates of measured traits of extra-early maturing maize inbreds under five optimal environments at Abuja, Mokwa and Ikenne, 2016-2017

Source of variation	Grain Yield, kgha^{-1}	Days to50% silking	Days to 50% Anthesis	Anthesis-silking Interval	Plant height, Cm	Ear height, cm
Environment	715242*** (0.19±0.16)	0.81***(0.02±0.03)	0.00***(0.0±0.0)	0.50***(0.11±0.12)	107.01***(0.05±0.05)	107.01^{ns} (0.14±0.12)
Replication (Env)	2735^{ns}	0.07^{ns}	0.06^{ns}	0.04^{ns}	7.77^{ns}	7.77^{ns}
Block (Env x Rep)	44942***	0.75***	0.42***	0.08**	41.64***	41.64^{ns}
Entry (ped)	2342034*** (0.62±0.13)	29.94***(0.79±0.04)	25.50***(0.82±0.03)	1.92*** (0.43±0.10)	1748.97***(0.81±0.05)	1748.97***(0.72±0.11)
Env x Entry	347857***(0.09±0.03)	2.70*(0.07±0.03)	2.84**(0.09±0.03)	0.55^{ns} (0.12±0.06)	22.41^{ns} (0.01±0.01)	22.41^{ns} (0.02±0.02)
Env_1 Residual	305857	4.62	2.58	1.32	188.63	188.63
Env_2 Residual	556727	3.07	2.19	1.08	312.48	312.48
Env_3 Residual	153501	2.91	2.35	1.77	153.69	153.69
h_A^2	0.49±0.06	0.57±0.05	0.63±0.04	0.18±0.04	0.56±0.05	0.51±0.05
Source of variation	**Root lodging**	**Stalk lodging**	**Husk cover**	**Ears per plant**	**Plant aspect**	**Ear aspect**
Environment	1.52*** (0.05±0.05)	0.00***(0.00±0.01)	0.97***(0.18±0.18)	0.03***(0.10±0.09)	0.26***(0.05±0.06)	1.13***(0.14±0.13)
Replication (Env)	0.00^{ns}	0.48*	0.02^{ns}	0.00^{ns}	0.00^{ns}	0.01^{ns}
Block (Env x Rep)	0.00^{ns}	0.00^{ns}	0.05*	0.00^{ns}	0.08***	0.07***
Entry (ped)	0.00^{ns} (0.0±0.0)	191.66***(0.78±0.04)	0.56***(0.10±0.08)	0.24***(0.69±0.08)	3.40***(0.69±0.07)	5.18*** (0.65±0.11)
Env x Entry	0.00^{ns} (0.0±0.0)	16.22*(0.07±0.03)	2.99***(0.54±0.14)	0.00^{ns} (0.00±0.00)	0.46**(0.09±0.04)	0.64**(0.08±0.03)
Env_1 Residual	4.52	27.99	0.58	0.03	0.41	0.78
Env_2 Residual	65.02	28.38	0.93	0.09	0.83	1.11
Env_3 Residual	15.91	54.75	1.43	0.10	0.98	0.85
h_A^2	0.00±0.00	0.39±0.05	0.07±0.05	0.34±.005	.046±0.05	0.42±0.05

"*", "**", "***", = significance at p(α =0.05), p(α =0.01), and p(α =0.001), respectively; "ns" = non-significant. Values in parenthesis are contributions of different sources of variance to the total variance with their standard errors; h_A^2 = narrow-sense heritability.

Table 3.4 Variance components and heritability estimates of measured traits of extra-early maturing maize inbreds across test environment

Source of variation	Grain Yield, kgha^{-1}	Days to 50% silking	Anthesis-silking Interval	Plant height, cm	Ear height, cm
Environment	355983.70*** (0.35 ± 0.14)	12.47*** (0.23 ± 0.11)	1.83*** (0.23 ± 0.11)	337.43***(0.18 ± 0.09)	59.22***(0.10 ± 0.06)
Replication (Env)	3919.63***	1.25***	0.10**	58.78***	10.64***
Block (Env x Rep)	9045.00***	0.60***	0.07*	19.41***	4.93***
Entry (ped)	308488.48*** (0.30 ± 0.08)	30.03*** (0.56 ± 0.09)	2.31*** (0.29 ± 0.07)	1211.26***(0.63 ± 0.08)	435.66***(0.71 ± 0.06)
Env x Entry	74581.25*** (0.07 ± 0.02)	1.23*** (0.02 ± 0.01)	0.33*** (0.04 ± 0.01)	55.90***(0.03 ± 0.01)	18.09***(0.03 ± 0.01)
Env_1 Residual	294543.73	15.13	5.21	600.91	160.40
Env_2 Residual	230165.90	7.55	2.85	187.30	51.17
Env_3 Residual	75684.00	14.36	5.39	231.24	83.74
Env_4 Residual	24047.32	9.80	5.56	125.43	38.35
Env_5 Residual	367562.46	3.80	1.11	154.54	81.86
Env_6 Residual	674433.12	2.50	0.93	277.63	165.14
Env_7 Residual	161999.93	2.45	1.54	126.61	43.89
h_A^2	0.08 ±0.02	0.21 ±0.03	0.05 ±0.01	0.26 ±0.03	0.26 ±0.03
Source of variation	**Root lodging**	**Stalk lodging**	**Husk cover**	**Ears per plant**	**Ear aspect**
Environment	18.05***(0.21 ± 0.13)	14.49***(0.06 ± 0.04)	0.71***(0.29 ± 0.12)	0.05*** (0.19 ± 0.10)	0.87*** (0.17 ± 0.09)
Replication (Env)	8.05***	0.92***	0.02*	0.00***	0.19***
Block (Env x Rep)	0.00^{ns}	0.02***	0.02**	0.00***	0.08***
Entry (ped)	1.11^{ns} (0.02 ± 0.01)	137.17***(0.49 ± 0.06)	0.45***(0.19 ± 0.06)	0.11***(0.47 ± 0.08)	2.18*** (0.43 ± 0.07)
Env x Entry	0.01***(0.0 ± 0.0)	10.96^{ns} (0.05 ± 0.01)	0.41***(0.17 ± 0.03)	0.02***(0.07 ± 0.01)	0.43*** (0.08 ± 0.02)
Env_1 Residual	195.89	191.84	0.31	0.05	0.92
Env_2 Residual	45.31	181.10	0.95	0.08	2.29
Env_3 Residual	25.67	57.61	1.53	0.06	2.26
Env_4 Residual	87.82	113.73	0.39	0.04	1.63
Env_5 Residual	4.45	23.89	0.48	0.02	0.63
Env_6 Residual	63.99	24.26	0.89	0.07	0.82
Env_7 Residual	15.94	39.37	1.32	0.09	0.71
h_A^2	0.00 ±0.0	0.08 ±0.02	0.04 ±0.01	0.12 ±0.02	0.10 ±0.02

"*", "**", "***", = significance at p(α =0.05), p(α =0.01), and p(α =0.001) respectively; "ns" = non-significant. Values in parenthesis are contributions of different sources of variance to the total variance with their standard errors; h_A^2 = narrow-sense heritability.

3.3.3 Genetic correlations among traits under stress environments

Genetic correlation coefficients among traits under *Striga*-infested environments are presented in Table 3.5. Grain yield had highly significant positive genetic association with ears per plant (0.98) and plant height (0.91). In addition, negative correlations were detected between grain yield and ear aspect (-0.98), husk cover (-0.68), *Striga* damage at 8 WAP (-0.63) and *Striga* damage at 10 WAP (-0.59). Moderate genetic correlations were observed between grain yield and other traits. A strong positive correlation was observed between *Striga* damage at 8 WAP and 10 WAP (0.98) and between number of emerged *Striga* plants at 8 WAP and 10 WAP (0.96).

Table 3.6 presents the genetic correlation coefficients among traits under drought-infested environments. Low to high genetic associations wer observed between grain yield and other measured traits. The genetic correlation between grain yield and ears per plant was strong and positive (0.84) but grain yield had significant negative genetic correlation with ear aspect (-0.75) and plant aspect (-0.64). The staygreen characteristic and anthesis-silking interval showed moderate correlations with grain yield. Moreover, a highly significant positive genetic correlation was observed between plant aspect and ear aspect (0.86) and between plant aspect and the staygreen characteristic (0.67), all of which are included in the selection index.

Table 3.5 Genetic correlations among grain yield and other traits across *Striga*-infested environments at Abuja and Mokwa, 2016-2017

variable		Trait												
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	**Grain yield**	1												
2	**50% days to silking**	-0.4***	1											
3	**Anthesis-silking interval**	-0.3***	0.22**	1										
4	**Plant height**	0.91***	0.39***	0.98***	1									
5	***Striga* damage 8 WAP**	-0.63***	0.23**	0.28***	-0.82***	1								
6	***Striga* damage 10 WAP**	-0.59***	0.1ns	0.28***	-0.78***	0.96***	1							
7	**Number of Emerged *Striga* plants 8WAP**	-0.39***	-0.03ns	0.24**	0.28***	0.5***	0.67***	1						
8	**Number of Emerged *Striga* plants 10WAP**	-0.58***	0.08ns	0.41***	0.59***	0.38***	0.61***	0.96***	1					
9	**Root lodging**	-0.55***	-0.78***	0.98***	0.58***	0.98***	0.98***	0.98***	0.42***	1				
10	**Stalk lodging**	-0.5***	0.22**	0.08ns	-0.45***	0.73***	0.76***	0.86***	0.94***	0.98***	1			
11	**Husk cover**	-0.68***	0.1ns	0.12ns	-0.68***	0.98***	1	0.7***	0.49***	0.98***	0.7***	1		
12	**Ears per plant**	0.98***	-0.34***	-0.36***	0.84***	-0.76***	-0.84***	-0.58***	-0.64***	-0.58***	-0.98***	-0.98***	1	
13	**Ear aspect**	-0.98***	0.4***	0.34***	-0.41***	0.77***	0.81***	0.44***	0.53***	0.98***	0.46***	0.85***	-0.98***	1

Table 3.6 Genetic correlations among grain yield and other traits across drought stress environments in Ikenne during 2016/2017 and 2017/2018 dry seasons

		Traits												
variable		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	**Grain yield**	1												
2	**50% days to anthesis**	-0.4***	1											
3	**50% days to silking**	-0.55***	0.78**	1										
4	**Anthesis-silking interval**	-0.26**	-0.31**	0.35***	1									
5	**Plant height**	0.16*	-0.04ns	0.01ns	0.09ns	1								
6	**Ear height**	0.18*	0.26**	0.18*	-0.06ns	0.8***	1							
7	**Root lodging**	-0.09ns	-0.17*	-0.36***	-0.31***	0.12ns	-0.04ns	1						
8	**Stalk lodging**	-0.16*	-0.26**	-0.25**	0.02ns	-0.06ns	-0.12ns	0.1ns	1					
9	**Husk cover**	-0.18*	0.41***	0.3***	-0.17*	-0.4***	-0.04ns	-0.7***	-0.04ns	1				
10	**Plant aspect**	-0.64***	0.35***	0.61***	0.41***	-0.39***	-0.25**	-0.05ns	0.21*	0.5***	1			
11	**Ears per plant**	0.84***	-0.3***	-0.6***	-0.51***	0.17*	0.2*	0.07ns	-0.16*	-0.27***	-0.87***	1		
12	**Ear aspect**	-0.75***	0.38***	0.63***	0.39***	-0.21**	-0.19*	-0.17*	0.13ns	0.31***	0.86***	-0.85***	1	
13	**Staygreen characteristic**	-0.27**	-0.23**	-0.1ns	0.21**	-0.39***	-0.31***	-0.05ns	0.43***	0.28***	0.67***	-0.32***	0.42***	1

3.3.3 Performance of inbred lines under stress and across test environments

3.3.3.1 Index-based performance of inbreds

Table 3.7 presents the performance of the top 20% and five worst inbreds under *Striga* infestation. The grain yield across *Striga*-infested environments ranged from zero for TZEEIOR 37 and TZEEIOR 87 to 2946 kg ha^{-1} for TZEEIOR 113 with an average of 931 kg ha^{-1}. *Striga* damage at 8 and 10 WAP ranged from 2 to 7 and 3 to 7 with a mean of 4 and 5, respectively. Similarly, number of emerged *Striga* plants at 8 and 10 WAP ranged from zero to 41 and from zero to 32, respectively. The anthesis-silking interval ranged from zero to 10 days. Number of ears per plant obtained ranged from zero to 2 and ear aspect varied from 3 to 9. Among the selected inbreds under *Striga* infestation, that is high yielding inbreds with few number of emerged *Striga* plants and reduced *Striga* damage, TZEEIOR 113, TZEEIOR 214, TZEEIOR 189, TZEEIOR 221, TZdEEI 7, TZEEIOR 38, TZEEIOR 12, TZEEIOR 76 exceeded the average yield by 216%, 171%, 167%, 138%, 129%, 122%, 109%, and 104%, respectively.

Table 3.8 presents the performance of the top 20% and five worst inbreds under drought stress. The grain yield ranged from zero for TZEEIOR 174 to 1287 kg ha^{-1} for TZEEIOR 109 with a mean of 317 kg ha^{-1}. The plant aspect ranged from 4 to 8 with a mean of 6. The anthesis-silking interval varied from zero to 10 days. Also, on the average, inbreds showed staygreen characteristic of 4 while the range was 2 to 7. The outstanding inbreds exceeding the mean grain yield under drought stress by at least over 140% were TZEEIOR 109 (306%), TZEEIOR 38 (201%), TZEEIOR 11 (180%), TZEEIOR 91 (155%), TZEEIOR 145 (144%), TZEEIOR 195 (143%), and TZEEIOR 42 (140%). The inbreds TZEEIOR 113 and TZEEIOR 76 produced 130% and 138% over the average grain yield under drought stress. These inbreds along with TZEEIOR 221 and TZEEIOR 38 showed outstanding performance under both stress conditions.

Table 3.7 Performance of index-based top 20% and worst 5 inbred lines evaluated across *Striga* environments in Abuja, 2016 and Mokwa, 2017

Inbreds	Grain yield Kg ha^{-1}	*Striga* Damage		Number of emerged *Striga* plants		Ears per plant	Ear aspect	Anthesis Silking Interval	Index
		8 WAP	10 WAP	8 WAP	10 WAP				
TZEEIOR 189	2490	3	4	10	12	2	3	0	12.92
TZEEIOR 221	2220	3	3	0	6	1	6	2	12.42
TZdEEI 7	2135	2	3	8	9	1	4	1	11.73
TZEEIOR 41	1689	2	3	3	5	1	3	3	9.50
TZEEIOR 223	1474	3	3	1	3	1	4	1	9.47
TZEEIOR 113	2946	5	6	6	9	1	4	2	8.89
TZdEEI 12	1763	3	4	7	11	1	4	1	8.54
TZEEIOR 38	2067	3	4	7	14	1	4	1	8.37
TZEEIOR 197	1802	3	4	2	6	1	4	3	8.36
TZEEIOR 243	1570	2	4	3	7	1	4	0	7.96
TZEEIOR 213	1809	3	4	2	5	1	4	1	7.86
TZEEIOR 219	1608	3	4	2	3	1	4	1	7.67
TZEEIOR 195	1760	3	4	4	5	1	5	2	7.59
TZEEIOR 222	1161	2	3	0	0	1	5	2	7.58
TZEEIOR 76	1900	4	5	1	4	1	5	1	7.55
TZEEIOR 214	2519	4	5	10	12	1	4	3	7.35
TZEEIOR 42	1541	3	4	1	6	1	5	3	7.32
TZEEIOR 140	1745	4	4	4	5	1	5	3	6.69
TZEEIOR 244	1229	2	4	7	12	1	4	3	6.31
TZEEIOR 196	1651	4	4	3	4	1	5	4	6.19
TZEEIOR 109	1720	3	5	3	7	1	5	3	6.14
TZEEIOR 252	1646	3	4	4	9	1	4	2	6.00
TZEEIOR 251	1192	2	3	7	12	1	4	1	5.76
TZEEI 79	1225	3	4	4	10	1	5	5	5.28
TZEEIOR 12	1945	4	5	8	9	1	5	4	5.02
TZEEIOR 253	1452	3	5	6	7	1	4	1	4.95
TZEEIOR 145	1535	4	5	2	8	1	5	3	4.94
TZEEIOR 248	1362	4	4	4	5	1	5	2	4.76
TZEEIOR 43	1157	3	4	0	0	1	6	4	4.58
TZEEIOR 217	1301	3	4	3	7	1	5	3	4.56
TZEEIOR 245	1188	2	4	10	13	1	5	3	4.50
TZEEIOR 146	1176	4	4	9	10	1	4	2	4.14
TZEEIOR 154	1816	5	6	9	10	1	5	2	4.06
TZEEIOR 212	1099	3	4	2	5	1	5	0	3.96
TZEEIOR 240	1227	4	5	2	6	1	5	1	3.89
TZEEI 74	935	4	4	0	1	1	6	2	3.72
TZEEIOR 36	0.00	5	6	4	5	0	8	4	-8.58
TZEEIOR 238	224	5	7	17	20	0	8	5	-8.91
TZEEIOR 118	428	7	7	8	8	0	7	7	-9.02
TZEEIOR 86	542	5	6	26	32	0	7	0	-9.70
TZEEIOR 87	0.00	6	6	8	19	0	9	3	-10.41
Mean	931	4	5	7	10	1	6	3	
Minimum	0.00	2	3	0	0	0	3	0	
Maximum	2946	7	7	41	32	2	9	7	
S.E	405	1	1	6	7	0	1	2	

S.E = standard error

Table 3.8 Performance of index-based top 20% and worst 5 inbred lines evaluated across drought stress environments at Ikenne, during 2016/2017 and 2017/2018 dry seasons

Inbreds	Grain Yield, kgha^{-1}	Plant Aspect (1-9)	Ears per plant	Ear Aspect (1-9)	Stay green (1-9)	Anthesis-Silking interval	Index
TZEEIOR 109	1288	4	1	4	3	3	16.85
TZEEIOR 38	954	4	1	4	3	3	13.16
TZEEIOR 91	808	5	1	5	3	1	11.84
TZEEIOR 161	705	4	1	5	4	1	11.01
TZEEIOR 29	682	4	1	5	3	2	10.55
TZEEIOR 11	888	5	1	5	4	4	9.54
TZEEIOR 126	421	4	1	4	3	2	9.20
TZEEIOR 35	542	5	1	5	4	1	9.17
TZdEEI 13	490	4	1	4	4	2	8.99
TZEEIOR 145	773	6	1	4	5	2	8.46
TZEEIOR 32	495	4	1	5	3	3	8.33
TZEEIOR 42	762	5	1	6	4	3	8.30
TZEEIOR 53	513	5	1	5	5	1	8.25
TZEEI 64	511	5	0	5	3	2	8.14
TZEEIOR 113	687	5	0	5	4	3	7.92
TZdEEI 9	641	6	1	6	3	2	7.72
TZEEIOR 76	755	5	1	6	4	3	7.42
TZEEIOR 5	675	5	1	6	4	3	7.20
TZEEIOR 100	674	6	1	6	3	3	7.15
TZEEIOR 195	770	5	1	4	5	6	6.84
TZEEIOR 123	280	4	1	6	3	2	6.77
TZEEIOR 24	418	5	1	5	4	3	6.69
TZEEIOR 203	574	4	0	5	3	5	6.37
TZEEIOR 102	477	5	1	5	3	5	6.27
TZEEIOR 31	541	5	1	6	4	4	5.83
TZEEIOR 10	474	6	0	5	4	1	5.53
TZEEIOR 85	375	5	0	5	3	2	5.41
TZEEIOR 197	513	5	1	6	4	4	5.36
TZEEIOR 45	475	6	0	5	3	2	5.30
TZEEIOR 232	181	5	1	7	3	0	5.05
TZEEIOR 167	402	5	1	5	4	3	4.87
TZEEIOR 108	528	5	0	6	3	5	4.80
TZEEIOR 104	625	6	1	6	4	4	4.77
TZEEIOR 221	730	5	0	6	5	3	4.76
TZEEIOR 125	371	5	0	5	3	4	4.63
TZEEIOR 97	462	5	0	4	4	4	4.63
TZEEIOR 191	49	6	0	8	5	7	-9.30
TZEEIOR 87	46	7	0	8	6	6	-9.91
TZEEIOR 135	58	7	0	8	7	6	-10.82
TZEEI 96	110	8	0	8	5	10	-11.66
TZEEIOR 174	0.00	7	0	9	5	9	-13.48
Mean	317	6	0	6	4	4	
Minimum	0.00	4	0	4	2	0	
Maximum	1288	8	1	9	7	10	
S.E	196	1	0	1	1	2	

S.E = standard error

3.3.3.2 BLUPs-based performance of inbreds

Table 3.9 presents the pedigree-based BLUPs (breeding values) and multivariate BLUPs of grain yield under *Striga*-infested, drought stress and across test environments. The top 10 outstanding inbreds based on the estimated breeding values for grain yield under *Striga* infestation were: TZdEEI 7 (1910±322 kg ha^{-1}), TZdEEI 12 (1712±322 kg ha^{-1}), TZEEIOR 214 (1574±168 kg ha^{-1}), TZEEIOR 221 (1522±188 kg ha^{-1}), TZEEIOR 109 (1453±211 kg ha^{-1}), TZEEIOR 213 (1432±163 kg ha^{-1}), TZEEIOR 189 (1421±180 kg ha^{-1}), TZEEIOR 113 (1418±162 kg ha^{-1}), TZEEIOR 38 (1372±209 kg ha^{-1}), and TZEEIOR 215 (1371±168 kg ha^{-1}). Under drought stress, the best 10 inbreds in the pedigree based on the predicted additive genetic effects of grain yield were as follows: TZEEIOR 109 (744±96 kg ha^{-1}), TZdEEI 12 (727±133 kg ha^{-1}), TZEEIOR 38 (674±95 kg ha^{-1}), TZdEEI 7 (612±133 kg ha^{-1}), TZEEIOR 130 (529±99 kg ha^{-1}), TZdEEI 9 (517±133 kg ha^{-1}), TZdEEI 13 (502±134 kg ha^{-1}), TZEEIOR 5 (488±103 kg ha^{-1}), TZEEIOR 76 (488±83 kg ha^{-1}), and TZEEIOR 37 (487±83 kg ha^{-1}). Selection based on the BLUPs from test environments, including the optimal, revealed inbreds TZdEEI 7, TZEEIOR 109, TZdEEI 12, TZEEIOR 221, TZEEIOR 195, TZEEIOR 189, TZEEIOR 12, TZEEIOR 236, TZEEI 73, and TZEEIOR 196 to be the best 10 performers. Results also showed a general tendency of low breeding values of the inbreds under drought stress compared to those predicted under *Striga* infestation. Moreover, ranking of inbreds based on BLUPs of grain yield from multivariate analysis identified 7 out of 10 best-selected inbreds based on breeding values across test environments.

Table 3.9 Pedigree BLUPs-based and multivariate BLUPs-based performances of the top 12% of the inbreds across pedigree under *Striga*-infested, drought and across test environments, 2016-2017

Inbreds	Breeding values for Grain yield, Kgha^{-1})								Multivariate blups ranking		
	Striga	S.E	Inbreds	Drought	S.E	Inbreds	Across	S.E	Inbreds	MVBlups	S.E
TZdEEI7	1910.4	322	TZEEIOR109	744	96	TZdEEI7	1630	271	TZdEEI7	0.81	0.27
TZdEEI12	1711.8	322	TZdEEI12	727	133	TZEEIOR109	1262	250	TZEEI73	0.39	0.27
TZEEIOR214	1574.1	168	TZEEIOR38	674	95	TZdEEI12	1261	271	TZdEEI12	0.32	0.27
TZEEIOR221	1522.1	188	TZdEEI7	612	133	TZEEIOR221	1197	245	TZEEI81	0.31	0.27
TZEEIOR109	1452.7	211	TZEEIOR130	529	99	TZEEIOR195	1195	244	TZEEIOR189	0.24	0.20
TZEEIOR213	1431.7	163	TZdEEI9	517	133	TZEEIOR189	1187	243	TZEEIOR195	0.24	0.20
TZEEIOR189	1420.5	180	TZdEEI13	502	134	TZEEIOR12	1173	237	TZEEIOR221	0.24	0.20
TZEEIOR113	1418.0	162	TZEEIOR5	488	103	TZEEIOR236	1143	254	TZEEIOR197	0.23	0.19
TZEEIOR38	1371.5	209	TZEEIOR76	488	83	TZEEI73	1139	271	TZEEIOR196	0.22	0.19
TZEEIOR215	1370.7	168	TZEEIOR37	487	83	TZEEIOR196	1139	239	TZEEIOR194	0.21	0.19
TZEEIOR154	1351.0	225	TZEEIOR100	478	86	TZEEIOR10	1138	237	TZEEIOR243	0.20	0.20
TZEEIOR223	1333.3	178	TZEEI64	477	133	TZEEIOR197	1137	239	TZEEIOR12	0.20	0.18
TZEEIOR252	1324.6	180	TZEEIOR75	476	83	TZEEIOR14	1132	236	TZEEIOR244	0.19	0.20
TZEEIOR243	1321.5	174	TZEEIOR195	470	83	TZEEIOR11	1127	237	TZEEIOR10	0.19	0.18
TZEEIOR222	1315.3	178	TZEEIOR4	469	113	TZEEIOR13	1122	236	TZEEIOR192	0.19	0.20
TZEEIOR217	1314.3	157	TZEEIOR36	468	83	TZEEIOR8	1121	236	TZEEIOR191	0.19	0.19
TZEEIOR244	1306.8	174	TZEEIOR101	464	90	TZEEIOR192	1117	242	TZEEI63	0.19	0.27
TZEEIOR112	1304.4	162	TZEEIOR11	462	66	TZEEIOR193	1111	242	TZEEIOR193	0.18	0.20
TZEEIOR195	1301.2	184	TZEEIOR35	459	86	TZEEIOR240	1108	252	TZEEIOR14	0.18	0.18
TZEEIOR140	1289.4	182	TZEEIOR113	457	74	TZEEIOR9	1105	236	TZEEIOR222	0.18	0.20
TZEEIOR111	1286.5	162	TZEEIOR12	452	66	TZEEIOR7	1103	238	TZEEIOR13	0.18	0.18
TZEEIOR216	1286.2	157	TZEEIOR112	450	74	TZEEIOR194	1103	239	TZEEI64	0.17	0.27
TZEEIOR253	1271.9	180	TZEEIOR114	448	87	TZdEEI13	1103	271	TZEEIOR8	0.17	0.18
TZEEIOR219	1270.9	184	TZEEIOR32	443	75	TZEEIOR6	1092	237	TZEEIOR223	0.17	0.20
TZEEIOR114	1269.3	194	TZEEIOR31	441	75	TZEEIOR191	1090	239	TZEEIOR252	0.17	0.20
TZEEIOR197	1268.1	159	TZEEIOR91	441	74	TZEEIOR38	1088	250	TZEEIOR253	0.17	0.20
TZEEIOR145	1251.3	186	TZEEIOR111	439	74	TZEEIOR244	1087	242	TZEEIOR240	0.17	0.22
TZEEIOR249	1238.1	200	TZEEIOR34	438	86	TZEEIOR243	1087	242	TZEEIOR11	0.17	0.18
TZEEIOR250	1238.1	200	TZEEIOR33	438	78	TZEEIOR187	1085	256	TZEEIOR245	0.16	0.20
TZEEIOR212	1237.5	163	TZEEIOR29	437	75	TZEEI64	1072	271	TZEEIOR109	0.16	0.21
TZEEIOR251	1236.9	190	TZEEIOR108	426	103	TZEEIOR241	1071	252	TZEEIOR7	0.16	0.18
TZEEIOR245	1232.8	191	TZEEIOR82	425	83	TZEEI81	1069	271	TZEEIOR9	0.16	0.18
TZEEIOR196	1232.6	158	TZEEIOR42	423	68	TZEEIOR219	1066	244	TZEEIOR236	0.16	0.22

S.E, standard error of estimation; MVBlups, multivariate Best Linear Unbiased predictions

4.5 Discussion and Conclusions

Differences in the response to *Striga* infestation and drought stress observed among the inbreds for most of the traits including grain yield is an indication of the existence of genetic variability needed for a successful selection for improvements of the traits. This finding is supported by previous reports of Badu-Apraku *et al.* (2011) and Badu-Apraku and Oyekunle (2012).

In each of the trials, the non-significant genotype-environment interaction (GEI) observed for grain yield and most of the traits indicated similarity in the management and showed that individual inbreds responded similarly in each of the environments. However, the highly significant GEI observed, in the combined analysis, for all the traits except stalk lodging depicted the heterogeneity among environments and indicated a differential response of the inbreds to the different test environments. This result supports the findings of Badu-Apraku *et al.* (2012a) and highlights the importance of considering stress and non-stress environments in determining tolerant genotypes.

Moreover, in the ranking of inbred lines and prediction of response to selection, genetic effects are needed and their estimation in plant breeding is often done ignoring relationships among lines. To ensure accurate and precise estimates of genetic effects and reduce biases in selecting superior lines, in the present study, genetic variance-covariance relationships among the 180 inbred lines were included in the analysis. Comparable narrow sense heritabilities were observed for grain yield under *Striga* infestation (48%) and drought stress (41%) suggesting possible improvement for grain yield under these stresses. However, across test environments, low narrow sense heritabilities were observed for all traits including grain yield (8%), probably due to the heterogeneity in error variances associated with the differences in the management of the environments.

Narrow-sense heritabilities obtained for *Striga* damage rating at 8 and 10 weeks after planting were respectively, 0.42 and 0.45. In addition, high and negative genetic correlation between each of

these traits and grain yield were obtained. A similar correlation between grain yield and number of emerged *Striga* plants at 8 and 10 weeks after planting were also observed. This finding supports that of Badu-Apraku *et al.* (2013) and justifies the use of these traits in indirect selection for increased grain yield under *Striga* environments. Nevertheless, the high and positive genetic correlation between *Striga* damage at 8 and 10 weeks after planting (0.96^{**}), on one hand, and between number of emerged *Striga* plants at 8 and 10 weeks after planting (1), on the other hand, indicated that the use of both (8 and 10 WAP) might over-parametrise the base index actually in use with same effects. The use of only one rating (8 or 10 WAP) could allow other important secondary traits to be considered in the index.

A resistant genotype to *Striga* is a genotype which when grown under conditions of *Striga* infestation, supports significantly fewer *Striga* plants and has a higher yield than a susceptible cultivar (Menkir, 2006). The same author defined tolerant genotype as the one that shows smaller yield reductions than susceptible cultivars under the same level of infestation. This definition might not suite that of pure pathologists but the base index fulfills both definitions and, thus, its use can allow identification of resistant/tolerant genotypes. Eighty-two (82) out of 180 inbreds and 78 out of 152 orange lines were selected as combining high grain yield, low *Striga* damage, low number of emerged *Striga* plants and high number of ears per plant. Under drought stress, 79 out of 180 inbreds (44%) and 72 out of 152 (47%) orange lines showed levels of tolerance to drought by combining high yield, reduced interval of days to flowering and reduced and prolonged stay green characteristic. The orange inbred lines were extracted from a *Striga* resistant genetic broad base variety and the identification of resistant inbreds among the evaluated lines is not surprising. Also, 47% of the orange lines with some level of drought tolerance were identified. This finding is a proof that the population from which these inbreds were extracted contained some alleles for

tolerance to drought. Indeed, Badu-Apraku *et al.* (2007) included some sources of drought tolerance germplasm during the formation of the population that served as base for extraction of the orange inbreds involved in the present study. The selection using pedigree BLUPs in this study confirmed that resistance/tolerance to *Striga* and tolerance to drought stress in the inbreds identified by the base indices is genetically controlled and highly heritable. TZEEIOR 189, TZEEIOR 221, TZdEEI 7, TZEEIOR 113, TZdEEI 12, and TZEEIOR 38 were selected both by the index and the pedigree BLUPs among the top ten performers under *Striga* infestation. TZEEIOR 109, TZEEIOR 38 and TZEEIOR 29 were the three out of the top ten performers which were consistently selected by these methods under drought stress. In addition, four inbreds namely, TZEEIOR 7, TZEEIOR 187, TZEEIOR 249, and TZEEIOR 250 with predicted breeding values for grain yield of 1103 kg ha^{-1}, 1085 kg ha^{-1}, 1028 kg ha^{-1}, and 1028 kg ha^{-1} respectively, were not among the selected inbreds evaluated in this study. Inbred TZEEIOR 114 was selected as combining *Striga* resistance and drought stress using the multivariate BLUPs across the pedigree with predicted breeding values of 1269 kg ha^{-1} and 447 kg ha^{-1} under *Striga* infestation and drought stress, respectively. At the same time, inbred TZEEIOR 33 was selected among the best under drought stress with a breeding value of 438 kg ha^{-1}. The six inbreds: TZEEIOR 7, TZEEIOR 187, TZEEIOR 249, TZEEIOR 250, TZEEIOR 114, and TZEEIOR 33 described above, initially not selected from the pedigree, should be tested to confirm their performance and eventually used in population improvement.

In conclusion, there was significant genetic variation among the inbred lines evaluated for different traits under different environments. Selection of superior inbred parents for use in hybrid and/or population development is possible. Eighty-two inbreds including 78 orange lines were selected

as drought tolerant. Seventy-nine inbreds including seventy 72 orange lines were selected as *Striga* resistant. About 19% (34 out of 180) of the inbreds combined *Striga* resistance/tolerance with drought tolerance. Genetic values of the inbreds across pedigrees for tolerance to drought were lower than the genetic values for resistance/tolerance to *Striga*. Seventy percent (70%) of the selected inbreds combining resistance to *Striga* and tolerance to drought were also selected by the multivariate BLUPS, thus confirming that their performance was due to genetic effects.

CHAPTER FOUR

4.0 Genetic diversity and homogeneity of extra-early maturing orange maize inbred lines

4.1 Introduction

The success of maize breeding in addressing the increasing demand for resistant genotypes to adverse environmental changes, a myriad of consumer preferences, and some hidden nutrition-related issues is based on availability of genetic variability. Inbred lines derived from numerous source populations serve as source of new cultivars and parental inbred lines that constitute a sample of the genetic diversity available in maize. Hence, genetic variability assessment of inbred lines has become a crucial step in breeding, prior to the development of outstanding hybrids and open-pollinated maize cultivars. Many tools have been used to characterise and assess diversity in maize inbred lines. Presently, the availability and accessibility at the relatively low cost of Single Nucleotide Polymorphism (SNP) markers has made their use popular.

Molecular-based diversity study using SNPs has the advantage of exploring the variation between genotypes at the base level and provides a means of differentiating cultivars and classifying inbred lines into heterotic groups (Fan *et al.,* 2008; Lu *et al.,* 2009; Dao *et al.,* 2014); identifying gaps and redundancy in germplasm collections (Ertiro *et al.,* 2017; Semagn *et al.,* 2012) ; monitoring genetic shifts that occur during germplasm conservation, regeneration, domestication, and breeding; identifying novel and superior alleles for improvement of agronomic traits; and constructing a representative subset or core collection. The objectives of the present study were to: (i) determine the genetic purity of selected extra-early orange inbred lines and, (ii) classify the selected inbred lines based on the molecular diversity within the population.

4.2 Materials and Methods

4.2.1 Plant materials

One hundred and fifty-two extra-early maturing orange inbred lines developed by IITA-MIP constituted the inbred lines used in the study. The set of inbred lines were selected from the inbreds described in chapter three, section 3.2.1 based on their performance under both *Striga* infestation and drought stress during the 2014 and 2015 field evaluations.

4.2.2 Leaf samples collection and DNA extraction

Leaf samples were collected from seedlings at 2-3 leaf-stage grown in the breeding nursery, in Ibadan. For each inbred line, one maize leaf per plant was collected from 10 representative plants, placed in paper envelopes and immediately freeze-dried. Genomic DNA samples were isolated from freeze-dried leaf tissues of each inbred line following the DArT DNA extraction protocol (https://www.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 concentration of 30 ng μl^{-1} were used.

4.2.3 Diversity Array Technology sequencing (DArTseq) genotyping and analysis

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. 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)

4.2.4 Filtering of the SNPs

The SNP single row file (mapping format) containing DArTseq markers was first converted to a "genlight object" using "dartR" package in "R" for SNP filtering. The initial DArTseq data comprised of 44,391 SNPs for the 152 genotyped orange maize inbred lines. A series of filters was used to select the SNPs for the genetic analysis, in this order: filtering loci on callrate with a threshold of 95%; filtering individuals on call rate with a threshold of 90%; filtering on average repeatability of each locus with a threshold of 100%; filtering out monomorphic loci; filtering out loci with trimmed sequence tags that are too similar (possible paralogues); filtering out all SNPs that did not align with the reference genome.

4.2.5 Summary statistics and cluster analyses

The retained markers were subjected to PowerMarker, version 3.25 (Liu and Muse, 2005) for descriptive statistics including number of markers, heterogeneity, gene diversity, minor allele frequency (MAF) and polymorphism information content (PIC) per chromosome and also for Roger's genetic (Roger, 1972) distance matrix generation. The PIC was calculated according to the following formula:

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

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

A phylogenetic tree was constructed from the genetic distance matrix using the neighbour-joining algorithm with 1000 nonparametric bootstrapping across different loci in PowerMarker. The R-package "Ape" was then used to visualize the genetic relationships among the inbreds. The relative kinship between each pair of lines was calculated using TASSEL software version 5.2.12 (Bradbury *et al.,* 2007).

4.2.6 Population structure and principal component analyses

The admixture model-based clustering method was used to infer the population structure of the 152 inbreds using the software package STRUCTURE, version 2.3.4 (Pritchard *et al.,* 2000). To determine the genetic structure of the 152 lines, shared allele frequencies and unlinked loci were assumed. STRUCTURE was run by setting the number of clusters (K) from 1 to 10. Each K was run 10 times with a burn-in period of 10,000 and 100,000 Markov Chain Monte Carlo (MCMC) replications after burn-in. The optimal value of K was estimated using the *ad hoc* statistic ΔK through online computation, in the Structure Harvester (Evanno *et al.,* 2005). The ΔK is based on the second order rate of change of P(X|K), the posterior probability of the data with respect to a given K and considers that the rate of change of LnP(D) increases as K increases and tends to be maximum at the true value of K. It was computed as follows:

$$\Delta K = \frac{M[|L(K-1) - 2L(K) + L(K+1)|]}{S[L(K)]}$$

where: L(K) is the K^{th} LnP(D), M is the mean of 10 runs, and S their standard deviation.

The probability of membership obtained for the retained K value was used to assign individuals into the different clusters. Individuals with a probability of membership ≥ 70% were assigned to the same group while those with < 70% probability memberships in any single group were assigned to a “mixed” group (Lu *et al.,* 2009; Yang *et al.,* 2011). To confirm the clustering based on the Bayesian statistics, a principal coordinate analysis (PCoA) of the same set of DArTseq markers

was performed using GenALEx version 6.5 (Peakall and Smouse, 2006; 2012). The results of the STRUCTURE analysis were used as *a priori* information for the phylogeny tree and the PCoA.

4.3 Results

4.3.1 Description of DArTseq SNP markers in the Population

Four thousand six hundred and twenty (4,620) SNPs (10.41% of the original data) were obtained from the filtering and used in the genetic diversity study.

Figure 4.1 presents the summary statistics of the retained 4,620 SNP markers for genetic analysis. The SNPs comprised 26.32% of A, 24.97% of G, 25.54% of T and 23.17% of C. The total transitions among the SNPs occurred at a rate of 59.61% while the transversions occurred at a rate of 40.39%, giving a rate transition/ transversion of 1.48 (Table 4.1). On the average, 10% of the retained markers were distributed on each of the 10 chromosomes, indicating an even distribution of the markers on the chromosomes. The percentage markers on each chromosome varied from 6.1% on chromosome 10 to 15.2% on chromosome 1. The major allele frequency varied from 0.85 to 0.88 while the gene diversity ranged from 0.18 to 0.21 (on chromosomes 2 and 9). The average proportion of the heterozygous inbreds per marker ranged from 0 to 0.87. This proportion varied from 0.03 to 0.04 on average loci per chromosome. The mean polymorphism information content (PIC) across loci per chromosome ranged from 0.15 to 0.18 while, for all the SNPs, it ranged from 0.007 to 0.375 with an average of 0.18.

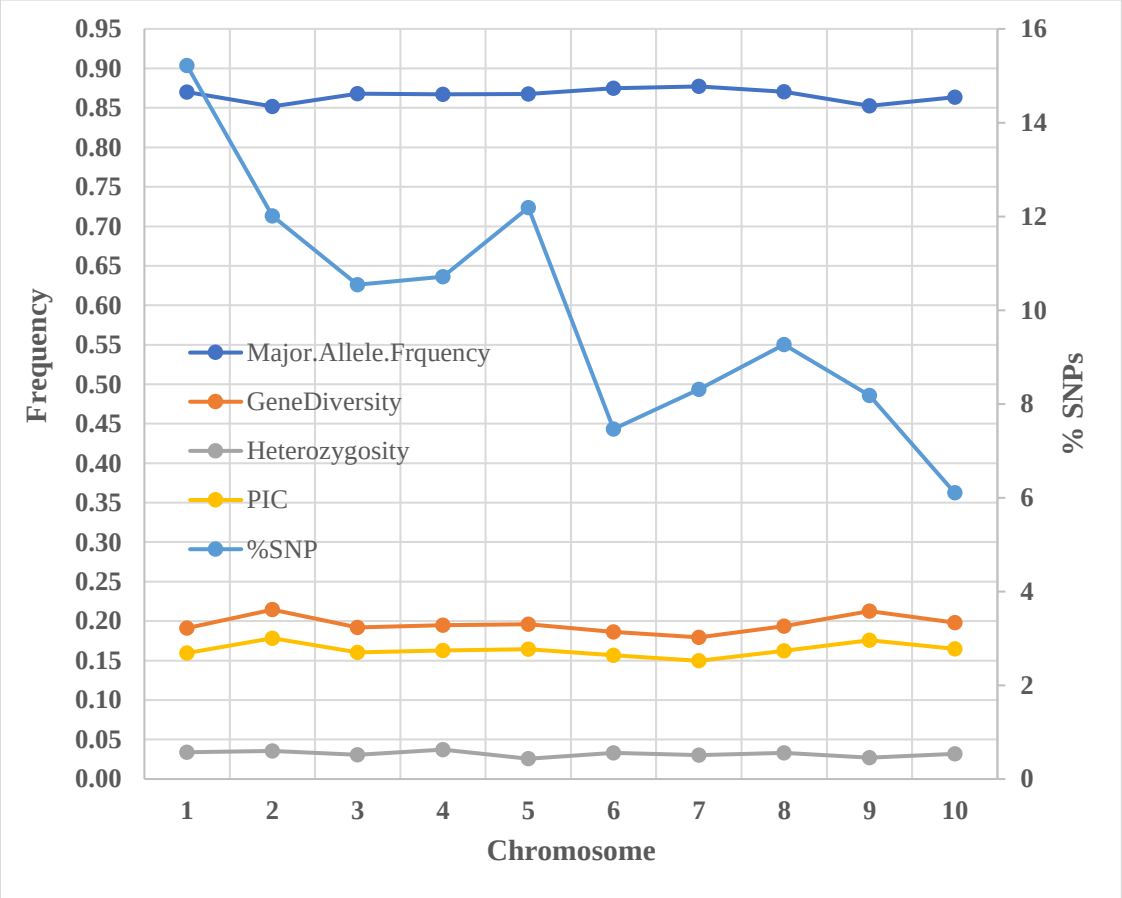


Figure 4.1 Summary statistics of the 4620 SNPs used for characterising the 152 orange maize inbred lines from IITA-MIP breeding programs

4.3.2 Genetic purity of the inbred lines

The summary of the heterogeneity of the 152 inbred lines using 4620 SNPs evenly distributed on the 10 chromosomes is presented in Figure 4.2. Four inbreds out of 152 (3%) had heterogeneity less than 1%, which represents the expected heterogeneity at six generations of inbreeding. The majority (89%) of the inbred lines showed heterogeneity between 1.1% and 5%. Thirteen inbreds had heterogeneity greater than 5% among which the revealed heterogeneity fell between 5.1% and 12.5% for 9 inbreds while for 4 of them heterogeneity was greater than 12.5%.

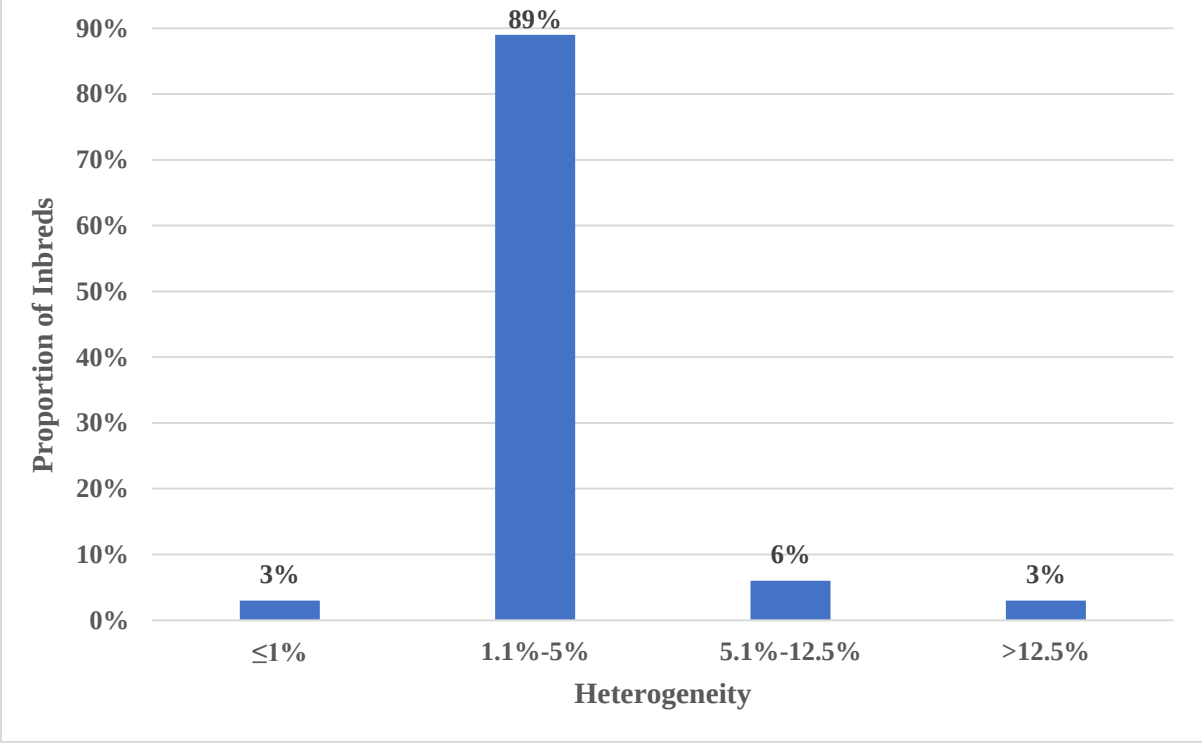
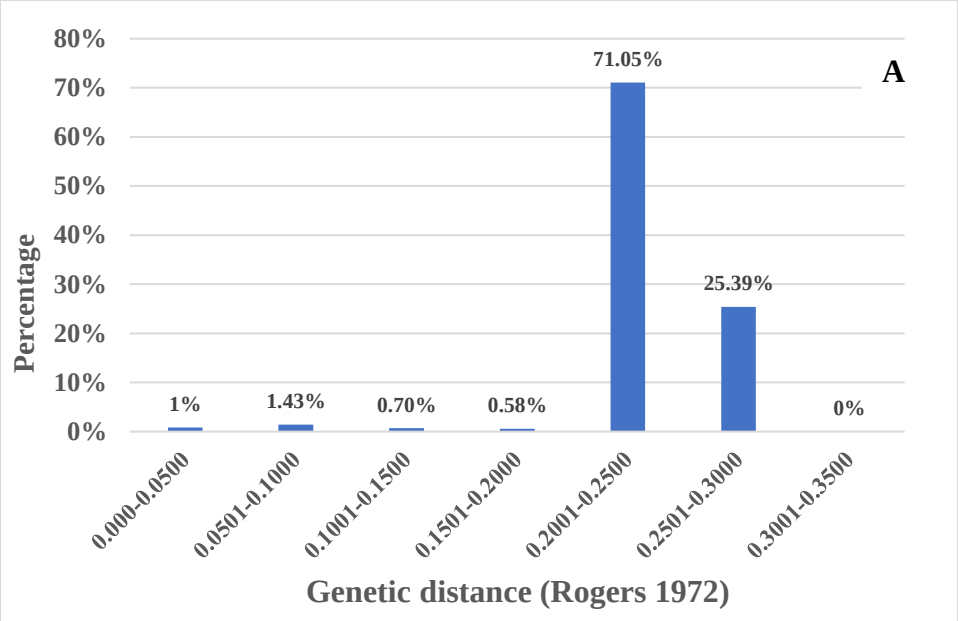


Figure 4.2 Summary of the heterogeneity of 152 inbred lines based on 4,620 polymorphic SNPs

4.3.3 Genetic distance and relatedness among the inbreds

Figure 4.3 presents the distribution of pairwise genetic distance of the inbred lines. Rogers genetic distance between pairwise comparisons of all the 152 inbreds ranged from 0.009 to 0.276 with an average distance of 0.237. Majority of the distances between each pair of inbreds (71.05%) fell between 0.2001 and 0.2500. Distances between 0.2501 and 0.3000 represented 25.39%, giving 96.44% of the pairwise genetic distances falling between 0.2001 and 0.3000.

The distribution of the relative kinship coefficients between pairs of inbreds are presented in Figure 4.3 (B). Results show that the relatedness coefficient varied from 0.01 to 0.59 with an overall average of 0.374. However, 55.18 and 37.09% of the values fell in the range of 0.3001 to 0.4000 and 0.4001 to 0.5000 resulting in about 92% of the kinship coefficients falling within 0.3001 and 0.5000.



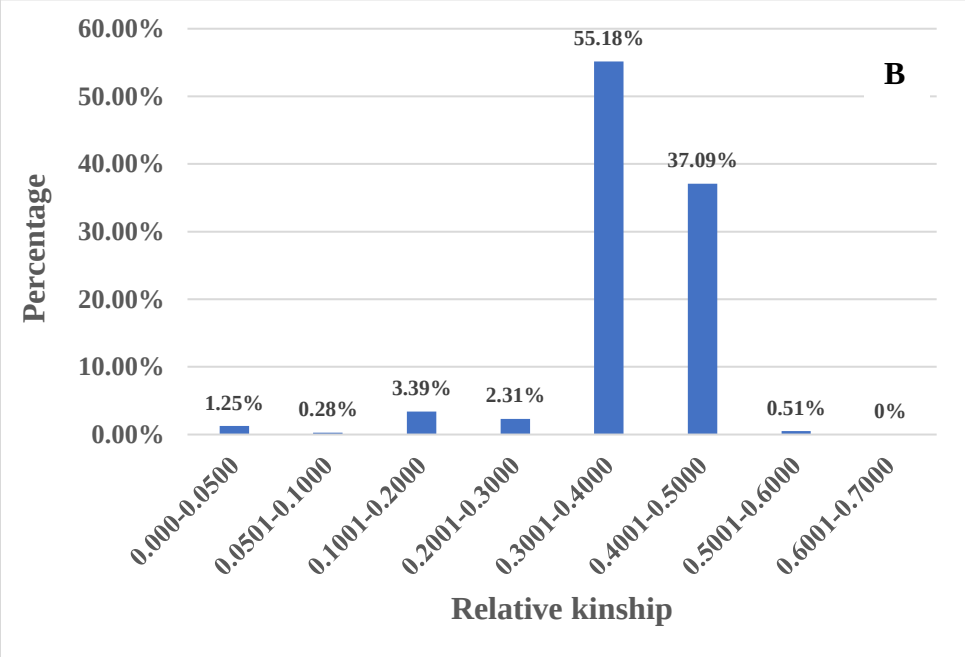


Figure 4.3 Distribution of pairwise (A) Rogers genetic distance and (B) relative kinship among 152 orange maize inbred lines based on 4,620 SNPs

4.3.4 Population structure analysis

Figure 4.4 presents results of the second order of likelihood, ΔK, and the clustering of individual inbreds into different groups from K =2 to K =4. Results indicated that there was higher likelihood at K= 4 and sharp decrease of the likelihood at K =5. At K= 6, the likelihood increased again. But the likelihood at K = 4 was higher than that observed at K =6. Therefore, K= 4 was used to partition the inbreds into the respective groups.

Each individual is represented by a single vertical line that is partitioned into K coloured segments on the x-axis, with lengths proportional to the estimated probability of membership (y-axis) to each of the K inferred clusters. Thus, a cut-off point of 70% of estimated probability of membership was used to decide on the individuals of group 1 (red colour in the Figure) and group 4 (yellow colour) that showed a shared probability among clusters.

All the inbreds with a probability of membership less than 0.7 were assigned arbitrarily to a "mixed" group. A total of 71% of the inbreds were assigned to a group. Group 1 is the largest with 47 inbreds out of 152 followed by group 4 with 30 inbreds. Group 3 (blue, in Figure 4.4) is composed of 21 inbreds and Group 2 (green) is constituted of 10 inbreds.

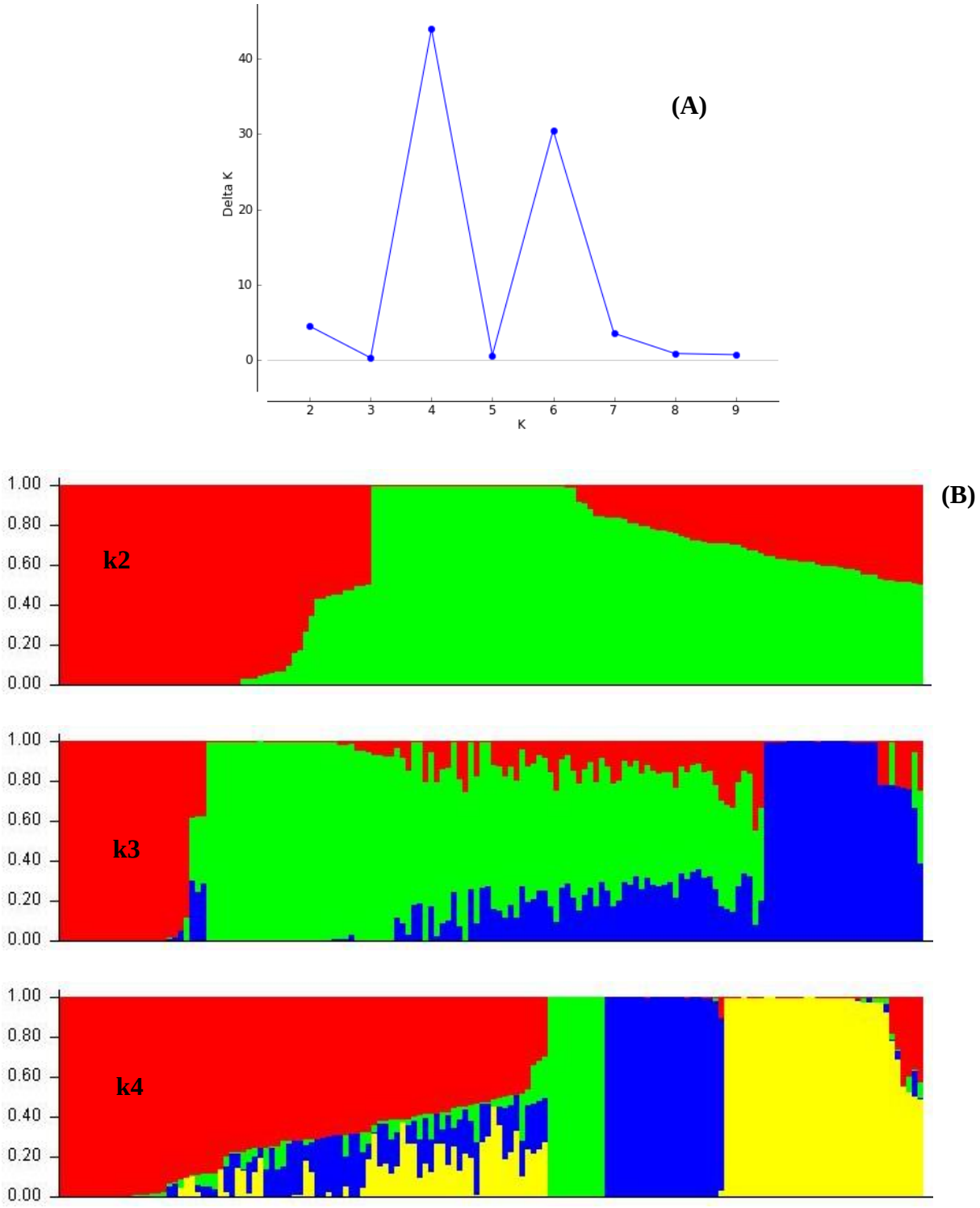


Figure 4.4 Analysis of the population structure of 152 orange maize inbred lines, (A) Estimated Δk over ten repeats of STRUCTURE analysis; (B) Population structure assessed by STRUCTURE for k =2, k =3, and k = 4

4.3.5 Principal coordinate analysis and Distance-based grouping

Figure 4.5 shows the percentage variation in the data explained by each of the new axes in the Principal coordinate analysis (PCoA). The results showed that the loci that loaded high on axis 1 and 2 were influential in discrimination among inbreds with a total contribution of 11.5% of the total variation. Results presented in Figure 4.6 indicated a clear disagreement between clusters obtained using the STRUCTURE and the PCoA. Figure 4.6 showed that individuals in groups 1, 3 and "mixed" were clustered together while group 2, which was highly correlated with loci loaded on axis 1, still stands alone as a different group. Group 4 was subdivided into 3 with a portion sharing similar properties with group 3, 1 and "mixed". On the contrary, genetic distance-based phylogenetic tree revealed almost the same pattern in the population of inbreds compared to the results of the STRUCTURE analysis (Figure 4.7). Members in each of the *a priori* group were consistently identified by the neighbour-joining method as belonging to the group. In this analysis, two sub-groups were identified in Group 1 with the sub-group (b) containing the majority of members of Group 2. Group 3 while containing a few members of Group 2, has few of its members in Group 3. The "mixed" group obtained from STRUCTURE analysis appeared as a group of members sharing properties with members of other groups and, was singled out as a genetically distant group (Figure 4.7). From the two-dimension plot of PCoA (Figure 4.6), it could be seen that the group 1 obtained from STRUCTURE analysis was completely mixed with the "mixed" group. Furthermore, there were also some subdivisions in Groups 3 and 4. Group 3 could be subdivided into two to obtain subgroup a (3a) and subgroup b (3b) with the subgroup b being mixed with the "mixed" group. Also, the PCoA revealed that Group 4 could be further subdivided into 3 to obtain subgroup a (4a) , b (4b), and c (4c) with subgroup b grouped together with the "mixed" group.

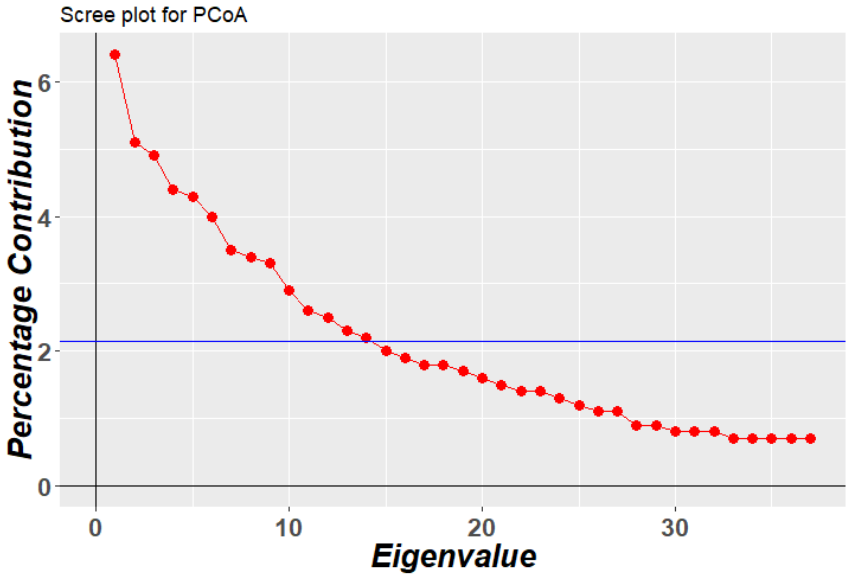


Figure 4.5 Contribution of the axis to the total variation explained in the genotypic data set

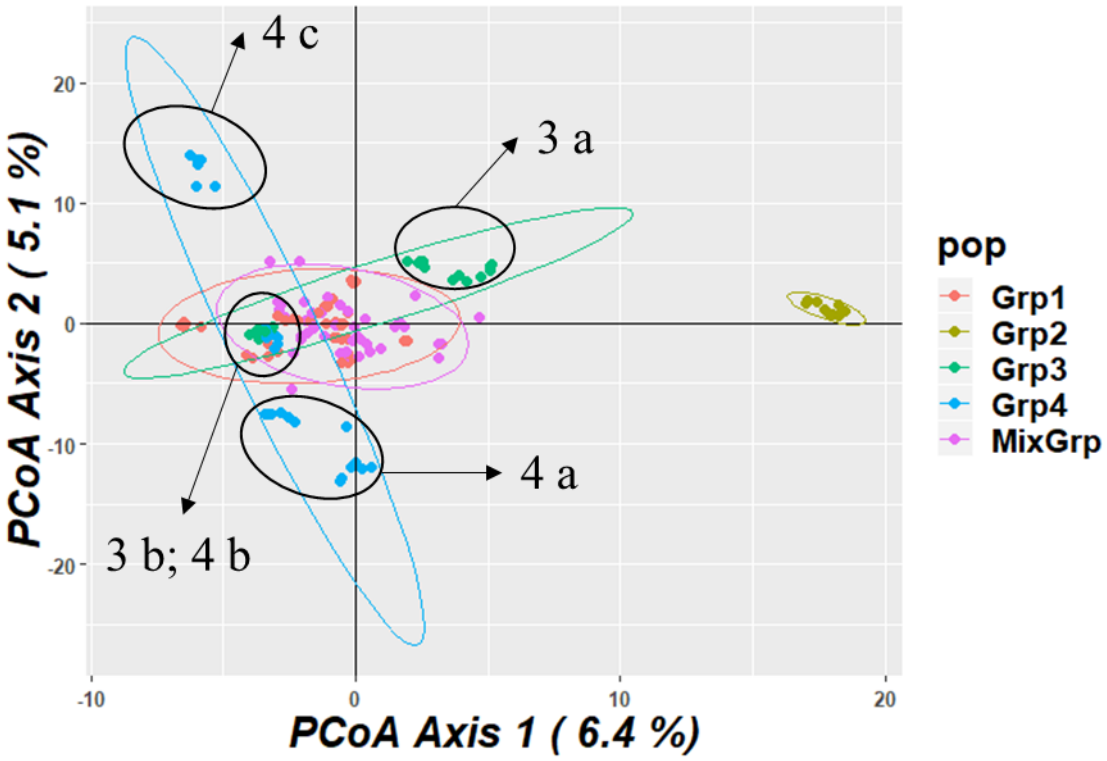


Figure 4.6 Population structure of 152 orange maize inbred lines using 4,620 SNP markers and the *a priori* information from STRUCTURE analysis in Principal Coordinate Analysis (PCoA)

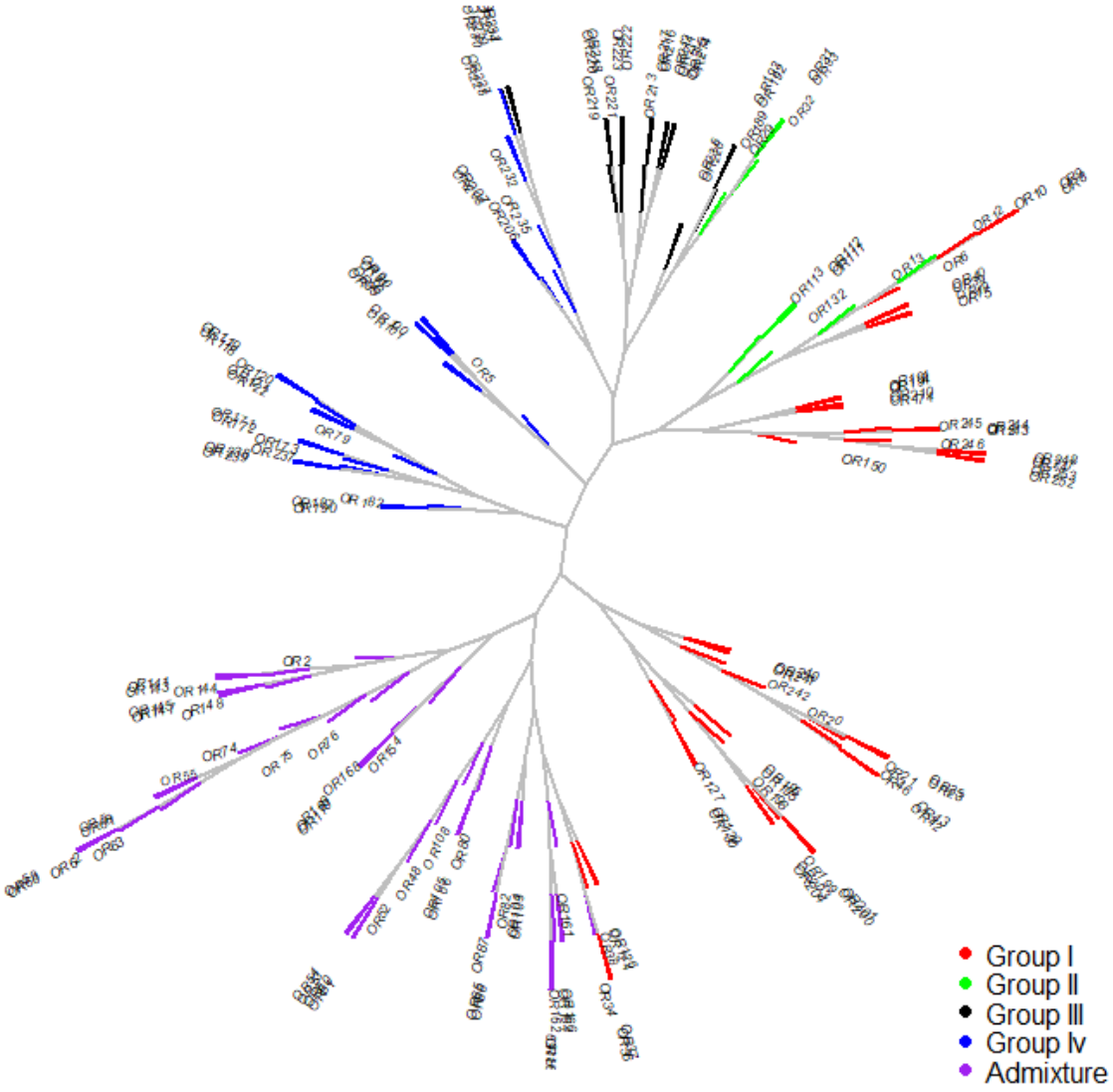


Figure 4.7 Neighbour-joining grouping of 152 orange maize inbred lines based on Rogers genetic distance calculated from 4,620 SNP markers

4.4 Discussions

Genetic purity of an inbred line ensures that it possesses genetic properties for which it has been selected. However, during seed regeneration and maintenance (especially, at different places), minor changes in allelic frequencies may occur at different stages of the process (Warburton *et al.*, 2010) without noticeable adverse impact. However, significant changes in the genetic constitution of an inbred line may affect its performance and even lead to the production and distribution of wrong hybrids or varieties (Semagn *et al.*, 2012). The inbreds used in the present study were at S_6 generation and therefore, were considered almost pure or fixed. The study clearly showed the presence of some level of genetic heterogeneity in about 8% of the total inbred lines (Heterogeneity >5%) but more importantly, highlighted the homogeneity of the majority, 92%, of the inbreds (Heterogeneity <5%) according to Semagn *et al.* (2012). Among the homogeneous inbreds, TZEEIOR 54, TZEEIOR 121, TZEEIOR 122 and TZEEIOR 148 reached the expected heterozygosity (< 1%) of S_6 lines. On the contrary, Ertiro *et al.* (2017) reported 30% heterogeneity among 30 inbreds from IITA maize programme and justified it by a probable pollen contamination and seed admixture during inbred line maintenance, as those inbreds were commonly used. The high homogeneity revealed in this study among IITA inbred lines could therefore be explained by the fact that these inbreds are newly developed and the seeds used were from the same source. The study also identified TZEEIOR 75, TZEEIOR 76, TZEEIOR 161 and TZEEIOR 38 as highly heterogeneous inbreds with heterogeneity level greater than 12.5%. This could be explained by seed admixture during processing or lower levels of inbreeding than expected. In the latter, these inbreds might need to go through another three or four generations of inbreeding to be fixed while two generations of selfing may be sufficient to fix those with heterozygosity between 5.1 and 12.5%, assuming the heterogeneity observed was not due to seed admixture.

The results of the pairwise genetic distance among the 152 orange maize inbreds revealed that about 96% of the pairs fell within 0.2 and 0.28 suggesting a narrow genetic variation among the lines. This finding supports that of Ertiro *et al.* (2017) who reported a range of 0.200 and 0.300 for 55% of the pairs among inbreds from IITA maize programme. This could be attributed to the background of the inbreds as all of them were selected for the same main traits. Furthermore, 92% of the inbreds involved in the present study showed relative kinship coefficients ranging from 0.3 to 0.5. These results indicate a moderate relatedness among the inbreds and could be attributed to a shared pedigree. Similarly, Semagn *et al.* (2012) reported a range of 0.05 to 0.5 for 79% of the kinship coefficients among 450 maize inbred lines from CIMMYT's eastern and southern Africa maize breeding programmes. The similarity arose from the narrow genetic base of the collection used in the studies. Moreover, even though inbreds in this study were extracted from a genetically broad base population, the selection imposed for the same traits might have led to the isolation of similar genetic constitutions. On the other hand, this finding contradicts those of Ertiro *et al.* (2017) who reported 59% of the kinship coefficients close to zero for 265 inbred lines; Dao *et al.* (2014), who reported 61% of the kinship coefficients close to zero for 100 inbred lines, and Wen *et al.* (2011), who reported that about 60 % of the pairwise kinship coefficients among 359 inbred maize lines were close to zero. It should be emphasized that these authors used in their studies, larger panels of inbreds lines from different sources, thus confirming the argument that the moderate relatedness found among the inbreds in the present study may be attributed to their genetic backgrounds.

Variability is needed among individuals subjected to selection for any given trait to ensure high performance of new varieties in plant breeding. It is also well known that crosses between genetically distant parents, in the presence of linkage disequilibrium, increase progeny variance

and, thus ensures successful selection of potential parents. Measures such as pairwise genetic distance and relative kinship coefficients are, therefore, often used by plant breeders to assess genotypes before their use in planned crosses. The present study confirms the relatedness and the identity of the inbreds while highlighting the narrow genetic distance among them. But how much is the genetic distance between two parents determining the variability needed in the progeny? In fact, controversial reports about the relationship between genetic distance and progeny genetic variance have been published (Benchimol *et al.*, 2000; Reif *et al.*, 2003; Bertan *et al.*, 2007; Flint-Garcia *et al.*, 2009; Hung *et al.*, 2012). Also, the performance of the inbreds roots in an extra-early broad base population improved both for *Striga* resistance and elevated levels of carotenoids. Thus, genetic differences could have been expected, assuming a sharp difference among genes for earliness, *Striga* resistance, and carotenoid accumulation. But the markers used in this study were randomly selected and their relation with the aforementioned traits is unknown and could explain the genetic close relationships among the inbreds. Alternatively, it could be that similar group of genes with similar effects or different group of genes with similar effects were present among the inbreds. The results suggest that specific markers may be needed to explore the true genetic relationship among these inbreds.

The maize inbred lines involved in the present study were selected among the top-performers of a population of 253 extra-early inbreds newly developed for *Striga* resistance and assumed elevated levels of carotenoids due to the colour of their endosperms. They also showed some levels of drought tolerance for two seasons' evaluations during which high variability was detected for the two stresses. Therefore, one of the major objectives of the current study was to explore whether there could be genetically distinct groups among the selected and if yes, identify quantitative evidence supporting their field performance. In this regard, 4,620 DArTseq SNP

markers evenly distributed on the 10 chromosomes were used as genetic variables that were subjected to STRUCTURE, PCoA, and Neighbour-Joining cluster analyses to determine genetic differentiation, population structure and the pattern of relationship among the inbreds. The model-based population structure assigned 71% of the inbreds into four genetically distinct groups with 39% sharing properties of at least two of the defined groups. Using the *a priori* subdivision of the population in the PCoA and tree-based analysis, results indicated concordance in population partition between STRUCTURE and phylogenetic tree analysis. The PCoA showed a more complex structure with subdivisions within groups 3 and 4 and overlaps of subgroups ("mixed", group1, group 3 a, and group 4 b). This result is in disagreement with the reports of Yang *et al.* (2011), Dao *et al.* (2014), and Wu *et al.* (2016) that indicated rather a high concordance between model-based structure and the PCoA. However, Yang *et al.*(2011) highlighted the spurious inference of population structure that often occurs for populations which do not fulfill the algorithm assumptions among which is the assumption of Hardy–Weinberg equilibrium (Falush *et al.*, 2003). The inbreds in this study did not conform to Hardy–Weinberg equilibrium because they were highly selected and thus, the shared pedigree might have caused the low genetic differentiation among some individuals. Many reasons have been given to justify the unreliability of tree-based population structure (Dao *et al.*, 2014). Partly, because a tree-based analysis only classifies individuals to a fixed position while PCoA and STRUCTURE provide the probability for an individual to belong to a group. Yang *et al.* (2011) also cautioned on the use of phylogenetic trees to explore the genetic relationship among individuals with complex genetic relatedness. Hence, the clustering inferred by the PCoA provided more details about the groups obtained from STRUCTURE and revealed the complexity in grouping the majority of the inbreds due to their relatedness.

In conclusion, the study revealed that majority (92%) of the inbreds were genetically pure and fixed. The inbreds were closely related with moderate relatedness. However, 71% of the inbreds were assigned to four different groups while 39% showed admixture using STRUCTURE software. Only 10 inbreds (TZEEIOR6, TZEEIOR8, TZEEIOR9, TZEEIOR10, TZEEIOR12, TZEEIOR13, TZEEIOR15, TZEEIOR19, TZEEIOR39, and TZEEIOR40) were consistently assigned to a distinct genetic group both by STRUCTURE and PCoA.

CHAPTER FIVE

5.0 Estimates of combining abilities and heterosis of extra-early maturing orange inbred lines and performance of their hybrids

5.1 Introduction

Drought and *Striga* constitute the prominent constraints to maize production in savanna agro-ecologies of SSA. Breeding high yielding extra-early maturing hybrids, which combine resistance to *Striga* and tolerance to drought, will increase the productivity and production of maize in the sub-region. Appropriate breeding strategy in such context, needs to identify parental inbred lines that form superior hybrids under these stresses and also understand the genetic basis for hybrid performance. Therefore, information regarding the nature of combining ability of the parents available as well as the nature of gene action involved in the expression of the desirable traits are of great importance. General combining ability (GCA) and specific combining ability (SCA) effects are indicators of the potential value of inbred lines in hybrid combinations and in grouping materials into heterotic groups. The use of heterotic groups, when aided with good testers in a breeding program can result in the production of high yielding hybrids. However, when there are no appropriate testers to be used, a diallel mating design can be used to identify inbred lines with superior combining ability that may be used as testers in a breeding program, and for identifying superior crosses that may be candidates for single cross hybrids. The study was designed to:

i. determine the combining ability of extra-early orange parental lines and the gene action for resistance to *Striga* and tolerance to drought;
ii. identify potential testers for grouping inbred lines into heterotic groups;
iii. determine the performance of hybrids and examine the stability of performance in tolerance to *Striga* and drought.

5.2 Materials and methods

5.2.1 Genetic materials

Fifteen promising inbreds, each with at least one positive selection index either under *Striga*-infested or drought stress environments selected among the 253 inbreds described in Chapter 3 plus two well-known inbred testers (TZdEEI 7 and TZdEEI 12) constituted the parents of the plant material used in this study (Table 5.1). The seventeen extra-early inbred lines were crossed in all possible crosses to generate crosses and reciprocals employing the complete diallel mating design in the IITA breeding nursery, Ibadan in 2016. However, crosses and reciprocals were mixed assuming no maternal effect, giving 136 single cross hybrids generated. The 136 single cross hybrids (Half Diallel) plus 4 experimental hybrid checks were used in the study.

5.2.2 Field Evaluation and data collection

A 10 x 14 (10 entries x 14 blocks) alpha lattice design with two replications was used in the present study. Single-row plots each of 3 m long with a spacing of 0.75 m between two adjacent rows and 0.40 m between plants within the row were used. Three seeds were planted per hill, and the seedlings were later thinned to two per hill about 2 weeks after emergence to give a final population density of about 66,667 plants ha^{-1}. Similar to the inbreds trial described in Chapter 3, the hybrid trial was evaluated at three locations (Abuja, Mokwa, and Ikenne), under *Striga* infestation, induced drought stress, and optimal environments for two years. Evaluation under *Striga* infestation was carried out in Abuja and Mokwa while those under optimal conditions were conducted at Abuja, Mokwa, and Ikenne, in 2016 and 2017 during June-October rainy seasons. In addition, the same trial was evaluated under managed drought stress during January-May of 2016/2017 and 2017/2018 dry seasons at Ikenne. The management of the trials and data collection

under the three conditions (managed drought stress, *Striga* infestation, and optimal) were done according to the methods described in Chapter 3, section 3.2.2.

Table 5.1 Characteristic of the selected 17 extra-early maturing maize inbred lines used in the diallel crosses with their reaction to drought stress and *Striga* infestation in 2015

Entry	INBRED	Grain colour	Pedigree	Reaction to	
				Drought	*Striga*
1	TZEEIOR 12	orange	2009 TZEE-ORI STR S5 3-1/1-1/3-5/6-1/1-1/1	T	T
2	TZEEIOR 42	deep orange	2009 TZEE-ORI STR S5 8-3/3-2/3-3/5-2/3-1/1	T	T
3	TZEEIOR 53	light orange	2009 TZEE-ORI STR S6 9-1/1-2/2-3/3-1/2-1/1	T	S
4	TZEEIOR 76	deep yellow	2009 TZEE-ORI STR S6 12-2/2-1/2-1/1-2/2-2/2	T	T
5	TZEEIOR 100	deep orange	2009 TZEE-ORI STR S6 15-2/2-2/2-3/3-1/3-1/3	T	T
6	TZEEIOR 113	orange	2009 TZEE-ORI STR S6 20-2/2-1/2-1/2-1/1-1/1	T	T
7	TZEEIOR 130	orange	2009 TZEE-ORI STR S6 35-1/2-3/3-1/1-1/3-1/1	T	T
8	TZEEIOR 141	orange	2009 TZEE-ORI STR S6 46-1/2-1/3-2/2-2/3-1/2	T	T
9	TZEEIOR 145	orange	2009 TZEE-ORI STR S6 46-2/2-1/3-2/2-1/2-1/1	T	S
10	TZEEIOR 161	orange	2009 TZEE-ORI STR S6 53-1/2-2/3-2/2-4/4-1/1	T	S
11	TZEEIOR 196	deep orange	2009 TZEE-ORI STR S6 68-1/3-2/3-1/2-1/3-1/1	S	T
12	TZEEIOR 218	orange	2009 TZEE-ORI STR S5 80-2/3-3/3-1/2-1/6-1/1	T	T
13	TZEEIOR 219	orange	2009 TZEE-ORI STR S6 80-2/3-1/1-1/1-3/3-1/1	T	T
14	TZEEIOR 222	orange	2009 TZEE-ORI STR S5 80-3/3-3/3-1/2-1/3-1/1	T	T
15	TZEEIOR 223	orange	2009 TZEE-ORI STR S6 80-3/3-3/3-2/2-1/6-1/1	T	T
16	TZdEEI 7	pale yellow	TZEE-Y POP STR 106 S6 189/194-1/1-1/2-4/5-7/9	T	T
17	TZdEEI 12	light orange	TZEE-Y POP STR 106 S5 2/194-1/1-1/2-1/2-4/4	T	S

T = tolerant; S = susceptible.

5.2.3 Data Analysis and genetic parameter estimate

Before the statistical analysis, the distribution of the data on each measured trait was assessed using the UNIVARIATE procedure. Traits deviating from the normal distribution according to the Shapiro-Wilk test were transformed with either a natural logarithm or square root to obtain a normal distribution.

5.2.3.1 Model description

The block model (B) was described as: Environment /Replication /Block /Plot and the genotype model (G) as: genotype (Gen) = GCA_1 +GCA_2 +SCA. The environment in the model is defined as year and location interaction. The main effects of years and locations were assumed to be negligible and not accounted for. Data analysis was performed using the following general linear model: Y = B + G + B*G + error, where Y is the measured parameter. The linear mixed model was implemented in SAS V9.4 by modifying the macro written by Isik (2009). Data from each trial condition were pooled together and analysed by fitting environments, replicates within environments, and checks as fixed factors while blocks within replicates, GCAs and SCAs and their interactions with the environments were fitted as random effects. The detailed model described in equation 5.1 was used with MIXED procedure.

$$Y = X\beta + Z_{GCA}u_{GCA} + Z_{SCA}v_{SCA} + Z_{GCA\ x\ Env}u_{GCA\ x\ Env} + Z_{SCA\ x\ Env}v_{SCA\ x\ Env} + e$$

(Equation 5.1)

Where: Y is the vector of unadjusted observations. The X-matrix and Z-matrices are incidence matrices belonging to their respective components; β is a vector of fixed effects of environment (Env), replicates within environments, and check; u_{GCA} and v_{SCA} are vectors of general combining ability (GCA) effects and specific combining ability (SCA) effects across the environments.

$u_{GCAxEnv}$, $v_{SCAxEnv}$, and e are the interaction terms of combining abilities with environments and pooled error.

The random effects in the model were assumed to follow a multivariate distribution with means and variances defined by the equations 5.2 and 5.3, respectively.

$$E\begin{bmatrix} u_{GCA} \\ v_{SCA} \\ u_{GCA\ x\ Env} \\ v_{SCA\ x\ Env} \\ e \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix} \text{ (Equation 5.2)}$$

$$\text{Var}\begin{bmatrix} u_{GCA} \\ v_{SCA} \\ u_{GCA\ x\ Env} \\ v_{SCA\ x\ Env} \\ e \end{bmatrix} = \begin{bmatrix} \oplus_j^n \sigma^2_{GCA} & 0 & 0 & 0 & 0 \\ 0 & \oplus_j^n \sigma^2_{SCA} & 0 & 0 & 0 \\ 0 & 0 & I_{Env} \otimes I_{Geno} \sigma^2_{GCA\ x\ Env} & 0 & 0 \\ 0 & 0 & 0 & I_{Env} \otimes I_{Geno} \sigma^2_{SCA\ x\ Env} & 0 \\ 0 & 0 & 0 & 0 & R \end{bmatrix}$$

(Equation 5.3)

Where $\oplus$ and $\otimes$ represent the Kronecker direct sum and direct product, respectively (Cullis and Gleeson, 1991).

Where 0 is a null matrix; I_{Env} and I_{Geno} are identity matrices with order equal to the number of environments (Env) and number of genotypes (Geno).

5.2.3.2 Genetic components variance structures

Different covariance structures in different parts of the genetic variance were fitted by using multiple RANDOM statements with different options. Thus, banded Toeplitz covariance structure option was used to group parents together and estimate a single variance component across all levels of parents. In addition, GCA and SCA variances were considered heterogeneous across all the environments and, therefore, were fitted using the diagonal matrix as a direct sum of individual matrices with order equals to the number of environments (Equation 5.3). The heterogeneous variance structure was implemented using SUBJECT option in the random statement.

The SUBJECT option was removed for the combined analysis for test environments because the loglikelihood failed to converge.

Restricted maximum likelihood (REML) in MIXED procedure was used to estimate variances of all random effects.

5.2.3.3 Significance tests

The Z probability was used to test the significance of random effects variances while the fixed effects were tested by F probability. Kenward-Roger method was used to approximate the degree of freedom of fixed effects in the analysis of data from each trial. But in the combined analysis of data from all test environments, the residual method was used.

5.2.3.4 Genetic value and heritability estimates

The computed best linear unbiased predictions (BLUPs) of the GCA and SCA effects were extracted and used to calculate breeding values of hybrids as the sum of the GCA effects of both parents plus the SCA effects which is the deviation from mean of the estimated GCA values.

Broad-sense (H^2) and narrow-sense (h^2) heritability estimates were calculated on full-sib family mean basis unbiased by variances of all factors in random statement as follow:

$$H^2 = \frac{2x\sigma^2_{GCA} + \sigma^2_{SCA}}{\sigma^2_P} = \frac{2x\sigma^2_{GCA} + \sigma^2_{SCA}}{2x\sigma^2_{GCA} + \sigma^2_{SCA} + \frac{2x\sigma^2_{GCAxEnv}}{t} + \frac{\sigma^2_{SCAxEnv}}{t} + \frac{\sigma^2_{Blk}}{b} + \frac{\sigma^2_{error}}{rxt}}$$

$$h^2 = \frac{2x\sigma^2_{GCA}}{\sigma^2_P} = \frac{2x\sigma^2_{GCA}}{2x\sigma^2_{GCA} + \sigma^2_{SCA} + \frac{2x\sigma^2_{GCAxEnv}}{t} + \frac{\sigma^2_{SCAxEnv}}{t} + \frac{\sigma^2_{Blk}}{b} + \frac{\sigma^2_{error}}{rxt}}$$

Where, t, b, and r are respectively, number of environments, blocks, and replications and σ^2_P, the phenotypic variance.

The option ASYCOV of the MIXED procedure was used to produce the variances of variance components (diagonal elements) and the covariances (off-diagonal elements) between them. The

standard error of heritabilities were calculated using Dickerson formulae (Isik, 2009), assuming constant variance of heritability as follow:

$$\boldsymbol{S.E}(h^2) = \sqrt{\frac{2x\,Var(\sigma^2_{GCA})}{(\sigma^2_P)^2}};$$

$$\boldsymbol{S.E}(H^2) = \sqrt{\frac{2x\,Var(\sigma^2_{GCA})+Var(\sigma^2_{SCA})+2Cov(2x\sigma^2_{GCA},\sigma^2_{GCA})}{(\sigma^2_P)^2}}.$$

Where $Var(\sigma^2_{GCA})$, is the variance of GCA component of variance, $Var(\sigma^2_{SCA})$, is the variance of SCA component of variance, $Cov(\sigma^2_{GCA},\sigma^2_{GCA})$, is the covariance between GCA and SCA variances as given by ASYCOV option.

Trial conditions were assumed to represent the different growing conditions in West and Central Africa, which is the target zone of most of the materials developed by IITA-Ibadan. Hence, in each trial type and across growing conditions, the environments were set fixed to account for their effects on genotypic performance prediction. The alternative which could have considered the environments as a random sample of a population of agroecological zones encountered in the sub-region was not dealt with in this research.

5.2.3.5 Relative importance of GCA and SCA

The relative importance of GCA and SCA was investigated using two criteria:

- the Baker's (1978) ratio modified by Hung and Holland (2012) and given as follows:

$$\mathrm{R_B} = \frac{2\hat{\sigma}^2_{\mathrm{GCA}}}{2\hat{\sigma}^2_{\mathrm{GCA}} + \hat{\sigma}^2_{\mathrm{SCA}}}$$

Where, $\mathrm{R_B}$ is the value of the ratio; $\hat{\sigma}^2_{\mathrm{GCA}}$ is the variance of general combining ability; $\hat{\sigma}^2_{\mathrm{SCA}}$ is variance of specific combining ability. When $R \approx 1$, Additive gene effects are predominant and predictions can be done based on GCA estimates alone.

- The GCA/SCA ratio defined as follows:

$$R = \frac{\hat{\sigma}^2_{GCA}}{\hat{\sigma}^2_{GCA}}$$

Where, R is the value of the ratio; $\hat{\sigma}^2_{GCA}$ is the variance of general combining ability; $\hat{\sigma}^2_{SCA}$ is variance of specific combining ability. When $R > 1$, Additive gene effects are primary type of gene action; when $R < 1$, non-additive gene effects are primary type of gene action.

5.2.3.6 Heterotic Groupings of parental inbreds

Parental inbred lines were assigned to heterotic groups under *Striga* infestation, drought stress, and across test environments based on GCA of multiple traits (HGCAMT) method proposed by Badu-Apraku *et al.*(2013, 2015). The GCA effects of the traits with significant GCA variance were scaled (mean of zero and standard deviation of 1) to make variables comparable and the standardised data set were used to compute Euclidean-based distance matrix. The HGCAMT statistical model is defined as follow:

$$Y = \sum_{i=1}^{n} \left(\frac{(Y_i - \bar{Y}_i)}{s} \right) + \varepsilon_{ij}$$

where Y is HGCAMT, which is the genetic value that measures relationship among genotypes based on the GCA of multiple traits *i* to n; Y_i is the individual GCA effect of genotypes for trait *i*, $\bar{Y}_i$ is the mean of GCA effects across genotypes for trait *i*, s is the standard deviation of the GCA effects of trait *i* and ε_{ij} is the residual of the model associated with the combination of inbred *i* and trait *j*.

Prior to the grouping, clustering tendency or validity of the clustering was assessed using Hopkins statistic which measures the probability that a given data set is generated by a uniform data distribution and a value less than 0.5 indicates that the data contain meaningful clusters. The optimal number of clusters (K) in the data set was determined through Gap statistics method,

silhouette approach, and Elbow method. The K suggested by at least two of the three methods was used to perform the hierarchical grouping.

Pairs of inbreds were grouped using Ward's minimum variance ("ward. D2", implemented in R) linkage method. Ward's minimum variance method processes by steps in each of which the pair of clusters with minimum between-cluster distance are merged, thus minimising the total within-cluster variance.

To assess whether the cophenetic distances (heights in the dendrogram) reflect the original distances accurately, a correlation between the two was calculated and values ≥ 0.75 are considered to reflect good clustering solution. Finally, Baker Gamma correlation coefficient among the three dendrograms generated were computed and used to check if individuals were clustered the same way under different stress environments with the across test grouping.

5.2.3.7 Inbred testers identification

The selection of testers was based on the three criteria proposed by Pswarayi and Vivek (2008). According to the authors, an inbred line is considered as a tester if it:

i. belongs to a known heterotic group,
ii. has a highly significant positive GCA of grain yield across the test environments,
iii. has high yield *per se*.

The assessment of the third criteria was done with reference to the results presented in Chapter 3.

5.2.3.8 Heterosis estimates and analysis

Gardner & Eberhart (1966) analysis II was used to examine the heterosis for grain yield of the hybrids across test environments and for measured traits involved in the base index in each of the stress environments. The statistical model of the method is given as follows:

$$Y_{ij} = \mu_v + \frac{1}{2}(v_i + v_j) + \gamma\bar{h} + \gamma(h_i + h_j) + \gamma S_{ij}$$

$$\text{With } \gamma = \begin{bmatrix} 0 \text{ where i} = \text{j} \\ 1 \text{ where i} \neq \text{j} \end{bmatrix} \text{ and } \bar{h} + h_i + h_j + S_{ij} = h_{ij}$$

Where: Y_{ij}: observed value of the cross between parents i and j; μ_v.: mean of all parental inbreds; $v_i \; and \; v_j$: variety effects; $\bar{h}$: average heterosis contributed by the particular set of genotypes used in crosses; h_i: the average heterosis contributed by variety I in its crosses measured as a deviation from $\bar{h}$ ($\sum_i h_i = 0$); S_{ij} : specific heterosis that occurs when variety i is mated to variety j; h_{ij}: is the overall heterosis due to differences in gene frequencies in parental inbreds, i and j and to dominance in crosses.

The software GENES v.38 (Cruz, 2016) was used to execute the model through two-stage analysis where the error variances and means generated in the analysis of variance (stage I) were used in the model described above (stage II).

Mid-parent heterosis (MPH), heterobeltiosis (HPH) for each of the traits were estimated as follows:

MPH $= \frac{F_1 - MP}{MP}$; and HPH $= \frac{F_1 - HP}{HP}$.

Where F_1 is the mean performance of the F_1 hybrid, MP is the mean of the two inbred parents and HP is the mean of the better parent.

5.2.3.9 Genetic correlation among test environments and stability analysis of hybrid performance

META-SAS v4 (Vargas *et al.,* 2013) was used to calculate the heritabilities of the environments and also check the genetic correlation among them before the stability analysis.

A graphical assessment of the stability of hybrids performance through GxE interaction analysis was performed in PBTools v1.3 following the GGE Biplot model (Yan *et al.*, 2000; Yan and Kang, 2002):

$$Y_{ij} - \overline{Y}_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij}$$

Where: Y_{ij}, is the average yield of genotype *i* in environment *j*; $\overline{Y}_j$, is the average yield over all genotypes in environment *j*; $\lambda_1$and λ_2, are the singular values decompositions for the first and second principal components, PC1 and PC2, respectively; $\xi_{i1}$and ξ_{i2}, are vector scores of genotype *i* on PC1 and PC2, respectively; $\eta_{j1}$and η_{j2}, are vector scores of environment *j* on PC1 and PC2, respectively; and ε_{ij}, is the residual of the associated model.

5.3 Results

5.3.1 Genetic analysis of performance of extra-early maturing maize inbred lines under contrasting environments

Table 5.2 presents variance components and heritability estimates for grain yield and other measured traits under *Striga*-infested environments. There were significant ($p < 0.01$) differences among the 17 inbred parents for GCA component of genetic variance for grain yield, *Striga* damage at 10 weeks after planting (WAP), number of emerged *Striga* plants at 8 WAP, husk cover, and ear aspect. Highly significant ($p < 0.001$) differences were observed for *Striga* damage at 10 WAP and number of emerged *Striga* plants at 10 WAP while GCA variances for anthesis-silking interval (ASI) and ears per plant (EPP) were not significantly different from zero. The SCA component of genetic variance for grain yield, anthesis-silking interval, ears per plant and ear aspect were significantly different from zero ($p < 0.001$). Also, no significant differences were detected among the inbreds for number of emerged *Striga* plants, *Striga* damage at 8 and 10 WAP, and husk cover.

The GCA-environment and SCA-environment interactions effects were highly significant ($p < 0.001$) for all the measured traits indicating high differential response among hybrids due to the genetic constitution of inbred parents. Generally, the contribution of GCA variance to the total

explained variance were low compared to that of SCA variance for the reported traits. GCA variance contribution ranged from 0.9% for ASI to 3.79% for *Striga* damage at 8 WAP (SDR8) while the SCA variance contribution ranged from 0.26% for number of emerged *Striga* plants at 8 WAP (ESP8) to 20.14% for grain yield.

Table 5.2 Variance components, heritabilities, and combining ability ratios for grain yield and other traits of extra-early maturing diallel crosses among 17 selected inbreds under four *Striga*-infested environments in Nigeria, 2016-2017

Parameters	Num. DF	Grain Yield, kg ha^{-1}	Anthesis-Silking interval	*Striga* damage (1-9)		Number of Emerged *Striga* plants		Husk Cover (1-9)	Ears per plant	Ear Aspect (1-9)
				8 WAP	10WAP	8WAP	10WAP			
Environment	3	*	***	**	*	***	***	***	***	NS
Replicate (Env)	4	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Subject				Random effect variances					
Block (Env x Rep)		412513***	0.64***	0.38***	0.31***	0.04***	0.03***	0.22***	0.01***	0.26***
GCA	Env	42187**	0.04^{ns}	0.05***	0.05**	0.01**	0.00***	0.04**	0.00^{ns}	0.03**
SCA	Env	340054***	0.59***	0.02^{ns}	0.07^{ns}	0.01*	0.00^{ns}	0.03^{ns}	0.01***	0.19***
Env x GCA		974.38***	0.00***	0.00***	0.00***	0.00***	0.00***	0.00***	-	0.00***
Env x SCA		17095***	0.00***	0.01***	0.01***	0.00***	0.00***	0.00***	0.00***	-
Residual		875595	2.68	0.94	0.92	0.09	0.07	0.71	0.03	0.63
Narrow sense heritability		0.15	0.07	0.39	0.32	0.15	0.18	0.34	0.08	0.18
S.E.		0.04	0.04	0.08	0.07	0.08	0.07	0.08	0.04	0.05
Broad sense heritability		0.74	0.63	0.46	0.56	0.16	0.22	0.50	0.71	0.72
S.E.		0.11	0.23	0.18	0.17	0.29	0.26	0.15	0.13	0.12
GCA/total variance (%)		2.50	0.90	3.79	3.72	0.98	1.30	3.59	1.31	2.77
SCA/total variance (%)		20.14	14.90	1.44	5.48	0.26	0.59	3.23	19.70	17.36
Baker ratio		0.20	0.11	0.84	0.58	0.88	0.81	0.69	0.12	0.24
GCA/SCA		0.12	0.06	2.63	0.68	3.74	2.20	1.11	0.07	0.16

"*", "**", "***" = significance at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively, "NS" = non-significant. GCA = general combining ability, SCA = specific combining ability, S.E = standard error, Num. DF= numerator degree of freedom, Env = environment, Rep = replication. All "0.00" are not absolute values. All "0" = estimated variance equals zero.

The observed relative importance of GCA over SCA was very low for traits such as ASI (0.06) and EPP (0.07) and high for traits such as ESP8 (3.74) and SDR8 (2.63). In addition, Baker ratio for the latter traits as well as the number of emerged *Striga* plants at 10 WAP (ESP10) were close to 1 with values respectively of 0.88, 0.84, and 0.81. Broad-sense heritability ranged from 0.16 for ESP8 to 0.74 for grain yield. Low (0.07 for ASI) to moderate (0.39 for SDR8) narrow sense heritabilities were observed for all traits. The proportion of additive genetic variance to the phenotypic variance for grain yield was estimated at 0.15 ± 0.04.

Table 5.3 presents variance components and heritability estimates for grain yield and other measured traits under drought environments. Genetic variance components were significantly ($p < 0.01$) different from zero for most of the reported traits. For grain yield as well as anthesis-silking interval and ear aspect, non-significant differences were found among environments, yet GCA and SCA components of genetic variance for grain yield were significantly ($p < 0.001$ and $p < 0.01$, respectively) different from zero. Differences among inbred parents as explained by GCA and SCA components did not follow similar trends for all the traits. The GCA effects were not significantly different from zero for days to 50% anthesis (DYA) and EPP. On the other hand, SCA variance was not significantly different from zero for ASI, days to 50% silking (DYS), ear aspect, and staygreen characteristic (STGC). But, the contribution of GCA component to the total variance, like under *Striga* infestation, was lower than that of SCA component. The contribution of GCA ranged from 0.24% for EPP to 7.5% for STGC while SCA contribution ranged from 1.96% for STGC to 30.26% for grain yield.

Table 5.3 Variance components, heritabilities, and combining ability ratios for grain yield and other traits of extra-early maturing diallel crosses among 17 selected inbreds across two drought environments in Nigeria, 2016-2017

Parameters	Num. DF	Grain Yield, kg ha^{-1}	Anthesis-Silking interval	Days to anthesis	Days to silking	Plant aspect (1-9)	Husk cover (1-9)	Ears per plant	Ear Aspect (1-9)	Leaf Death (1-9)
Environment	1	NS	NS	***	***	*	***	***	NS	*
Replicate (Env)	2	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Subject				Random effect variances					
Block (Env x Rep)		3868^{ns}	0	0.21^{ns}	0.74*	0.001^{ns}	0	0.001^{ns}	0	0.04^{ns}
GCA	Env	12478*	0.17*	0.23^{ns}	0.56*	0.027*	0.04*	0.000^{ns}	0.04*	0.08**
SCA	Env	120139***	0.30^{ns}	0.88*	1.17^{ns}	0.137***	0.28***	0.009**	0.11^{ns}	0.02^{ns}
Env x GCA		0	0.00***	0.01***	0.01***	0	0.00***	0.000***	0.00***	0.00***
Env x SCA		1706***	0.03***	0.01***	0	0.001***	0.02***	0	0.01***	0
Residual		258836	5.00	7.47	12.76	0.411	0.68	0.036	1.10	0.89
Narrow-sense heritability		0.12	0.18	0.14	0.20	0.18	0.16	0.01	0.15	0.39
S.E.		0.05	0.07	0.06	0.07	0.06	0.06	0.04	0.06	0.10
Broad-sense heritability		0.69	0.34	0.41	0.41	0.65	0.67	0.50	0.39	0.44
S.E.		0.13	0.19	0.15	0.16	0.13	0.13	0.15	0.17	0.16
GCA/total variance (%)		3.14	3.14	2.63	3.65	4.64	4.18	0.24	2.84	7.50
SCA/total variance (%)		30.26	5.38	9.94	7.70	23.77	27.30	19.68	8.75	1.96
Baker ratio		0.17	0.54	0.35	0.49	0.28	0.23	0.02	0.39	0.88
GCA/SCA		0.10	0.58	0.26	0.47	0.20	0.15	0.01	0.32	3.83

"*", "**", "***" = significance at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively, "NS" = non-significant. GCA = general combining ability, SCA = specific combining ability, S.E = standard error, Num. DF= numerator degree of freedom, Env = environment, Rep = replication.

All "0.00" are not absolute values. All "0" = estimated variance equals zero.

Moreover, Table 5.3 indicated that GCA was predominant for STGC with the GCA/SCA ratio equal to 3.83 and Baker's ratio of 0.88, indicating that additive gene effects were the primary type of gene action for STGC. The relative importance of GCA over SCA was 0.10 for grain yield. Almost equal contribution of GCA and SCA to the genetic variance was observed for DYS (0.47) and for ASI (0.58).

Broad-sense heritability ranged from moderate to high with the highest values observed for grain yield (0.69), husk cover (0.67), and for plant aspect (0.65). The proportion of additive genetic variance to the total phenotypic variance varied from 0.01 for EPP to 0.39 for STGC. Narrow sense heritability for grain yield and ASI were low under drought stress with values of 12$\pm$5% and 18$\pm$7%, respectively. Baker's ratio was 0.88 for STGC.

Table 5.4 presents variance components, heritability estimates and combining ability ratios for grain yield and other measured traits under optimal environments. Significant differences were observed among the five optimal environments for all the measured traits. Despite the very high variability among the environments, the contribution of components of genetic variance to the total variance was quite important under optimal conditions than under *Striga*-infested and managed drought stress environments. The GCA component of variance ranged from 1.87% for EPP to 16.04% for days to 50% anthesis while SCA component of variance varied from 9.08% for husk cover to 31.75% for grain yield. In addition, interactions between environments and components of genetic variance were highly significant for all traits.

The relative importance of GCA over SCA as given by the GCA/SCA ratio varied from 0.09 for ears per plant to 0.85 for days to 50% anthesis. In addition to DYA, the predominance of SCA variance was observed in traits such as grain yield (0.22), husk cover (0.26), plant aspect (0.29), and ear aspect (0.39).

Table 5.4 Variance components, heritabilities, and combining ability ratios for grain yield and other traits of extra-early maturing diallel crosses among 17 selected inbreds across five optimal environments in Nigeria, 2016-2017

Parameters	Num. DF	Grain Yield, kg ha^{-1}	Anthesis-Silking interval	Days to anthesis	Days to silking	Plant aspect (1-9)	Husk cover (1-9)	Ears per plant	Ear Aspect (1-9)	Root lodging	Stalk lodging
Environment	4	***	***	***	***	***	***	***	***	***	**
Replicate (Env)	5	*	NS	**	**	***	*	NS	NS	NS	*
	Subject				Random effect variances						
Block (Env x Rep)		111878***	0.02*	0.38***	0.35***	0.04***	0.03^{ns}	0.00^{ns}	0.04*	2.8*	8.5**
GCA	Env	102138***	0.06***	0.43***	0.51***	0.03***	0.06*	0.00*	0.06***	10.4***	19.0***
SCA	Env	462770***	0.07**	0.51***	0.66***	0.10***	0.24*	0.01***	0.15***	14.7***	23.8***
Env x GCA		6168.21***	0.00***	0.00***	0.00***	0	0.00***	0	0.00***	0	0.5***
Env x SCA		64136***	0.00***	0.01***	0.00***	0.01***	0.02***	0.00***	0.02***	1.0***	4.0***
Residual		710255	0.48	1.34	1.69	0.37	2.26	0.02	0.50	62.0	93.3
Narrow sense heritability		0.27	0.49	0.56	0.55	0.30	0.21	0.12	0.36	0.49	0.52
S.E.		0.05	0.08	0.07	0.07	0.06	0.06	0.04	0.06	0.07	0.08
Broad-sense heritability		0.88	0.78	0.89	0.90	0.80	0.61	0.75	0.82	0.84	0.85
S.E.		0.09	0.12	0.09	0.09	0.11	0.18	0.13	0.11	0.10	0.10
GCA/total variance (%)		7.01	8.93	16.04	15.96	5.53	2.35	1.87	7.63	11.48	12.75
SCA/total variance (%)		31.75	10.47	19.18	20.52	18.99	9.08	20.13	19.62	16.22	15.98
Baker ratio		0.31	0.63	0.63	0.61	0.37	0.34	0.16	0.44	0.59	0.61
GCA/SCA		0.22	0.85	0.84	0.78	0.29	0.26	0.09	0.39	0.71	0.80

"*", "**", "***" = significance at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively, "NS" = non-significant. GCA = general combining ability, SCA = specific combining ability, S.E = standard error, Num. DF= numerator degree of freedom, Env = environment, Rep = replication. All "0.00" are not absolute values. All "0" = estimated variance equals zero.

For ASI, DYA, DYS, root and stalk lodging, the values of GCA/SCA ratio was close to one, indicating equal proportions of GCA and SCA variances in determining genetic variability for these traits.

Broad-sense heritability under rain-fed conditions was higher compared to the stress environments. It ranged from 0.61 for husk cover to 0.9 for days to silking. But the proportion of the phenotypic variance due to additive genetic effects varied from 0.12 for EPP to 0.56 for DYA. For grain yield, ASI and ear aspect, this proportion was 0.27, 0.49, and 0.36, respectively.

Table 5.5 presents variance components, heritability estimates and combining ability ratios for grain yield and other measured traits across test environments. There were significant ($p < 0.001$) differences among environments and blocks within replicates nested in environments with the latter justifying the need to recover inter-blocks information. Highly significant ($p < 0.001$) differences were also found among inbred parents for interactions between environments and components (GCA and SCA) of genetic variance for all the measured traits. There were significant ($p < 0.05$) differences in GCA component of genetic variance for most of the measured traits except for husk cover, ears per plant, and ear aspect. The results indicated, in addition, that there were highly significant ($p < 0.001$) differences in SCA estimates among hybrids for grain yield, days to 50% anthesis, and for ear aspect. However, there were not significant differences among crosses for anthesis-silking interval and ear height with regard to SCA estimates.

The GCA/SCA ratio, was very low for husk cover (0.04) and ears per plant (0.09), thus indicating the predominance of SCA effect for these traits among this set of inbreds. The highest value of this ratio was observed for stalk lodging, 3.03, indicating the predominance of GCA effect for the trait. Moreover, similar relative importance of GCA over SCA were observed for days to 50% anthesis (1.29), days to 50% silking (1.05), and also for plant and ear height with values of 1.18

and 2.55, respectively. The contribution of the additive genetic variance to the phenotypic variance varied from 2% for husk cover to 56% for stalk lodging. This contribution of the additive gene effects to phenotypic variance was 20% for grain yield, 21% for anthesis-silking interval, 11% for ear aspect, and 0.07 for ears per plant with total genetic variance contributing 65, 34, 56 and 43%, respectively, to the total phenotypic variance.

Table 5.5 Variance components, heritabilities, and combining ability ratios for grain yield and other traits of extra-early maturing diallel crosses among 17 selected inbreds across 11 test environments in Nigeria, 2016-2017

Parameters	Num. DF	Grain Yield, kg ha^{-1}	Anthesis-Silking interval	Days to anthesis	Days to Silking	Plant height, cm	Ear height, cm	Husk cover (1-9)	Ears per plant	Ear Aspect (1-9)	Root lodging	Stalk lodging
Den DF (Residual)		3054	3051	3054	3051	3051	3051	3054	3054	3054	3054	3054
Environment	10	***	***	***	***	***	***	***	***	***	***	***
Replicate (Env)	11	NS	NS	NS	NS	***	***	*	NS	NS	*	NS
Block (Env x Rep)		198804***	0.24***	0.78***	1.69***	50.75***	27.74***	0.09***	0.00***	0.11***	4.7***	13.0***
GCA		22296*	0.02*	0.20*	0.28*	4.60*	2.60*	0.00^{ns}	0.00^{ns}	0.01^{ns}	1.4*	6.7*
SCA		101296***	0.03^{ns}	0.15***	0.27**	3.89*	1.02^{ns}	0.03*	0.00**	0.06***	0	2.2*
Env x GCA		45213***	0.05**	0.21***	0.33***	10.88***	5.57***	0.05***	0.00*	0.04***	6.2***	8.8***
Env x SCA		290244***	0.31***	0.84***	1.28***	28.17***	13.38***	0.15**	0.01***	0.11***	10.7***	14.8***
Residual		689121	2.07	3.00	5.26	214.27	101.24	1.42	0.03	0.67	59.2	96.3
Narrow sense heritability		0.20	0.21	0.46	0.54	0.30	0.35	0.02	0.07	0.11	0.35	0.56
S.E.		0.08	0.09	0.14	0.17	0.11	0.12	0.05	0.05	0.07	0.14	0.17
Broad-sense heritability		0.65	0.34	0.64	0.80	0.42	0.41	0.25	0.43	0.56	0.35	0.66
S.E.		0.12	0.12	0.15	0.19	0.13	0.13	0.12	0.12	0.12	0.14	0.17
GCA/total variance (%)		1.66	0.86	3.80	3.08	1.47	1.72	0.06	0.32	0.72	1.69	4.75
SCA/total variance (%)		7.52	1.04	2.94	2.93	1.25	0.67	1.65	3.51	5.75	0.00	1.56
Baker ratio		0.31	0.62	0.72	0.68	0.70	0.84	0.07	0.15	0.20	1.00	0.86
GCA/SCA		0.22	0.83	1.29	1.05	1.18	2.55	0.04	0.09	0.13	-	3.03

"*", "**", "***" = significance at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively, "NS" = non-significant. GCA = general combining ability, SCA = specific combining ability, S.E = standard error, Den. DF = denominator degree of freedom, Num. DF= numerator degree of freedom, Env = environment, Rep = replication. All "0.00" are not absolute values. All "0" = estimated variance equals zero.

5.3.2 Performance in crosses of the 17 inbred parents

5.3.2.1 GCA effects and GCA-based heterotic grouping of the inbred parents

Table 5.6 presents the GCA effects of the inbred parents, Hopkins statistic, and the correlation coefficient between the cophenetic distances and original distances. The GCA effects for grain yield ranged from -91 kg ha^{-1} for TZEEIOR 113 to 142 kg ha^{-1} for TZdEEI 7. Other inbreds with positive GCA effect for grain yield under drought stress include, in decreasing order of effect, TZEEIOR 42, TZEEIOR 218, TZEEIOR 76, TZEEIOR 141, TZEEIOR 161, TZEEIOR 219, TZdEEI 12, and TZEEIOR 53 with GCA effect of 65, 61, 41, 36, 26,15, 7, and 2 kg ha^{-1}, respectively. Genotypes TZEEIOR 42, TZEEIOR 76, and TZdEEI 7 had negative GCA effects for both STGC and ASI. On the contrary, genotypes TZEEIOR 100 and TZEEIOR 222 expressed significant negative effects for both STGC and ASI, in addition to negative GCA effects for grain yield.

The optimal number of heterotic groups among inbred parents under drought environments using HGCAMT method is shown in Figure 5.1. Results indicated that there were two heterotic groups among the inbred parents. The Hopkins value of the clustered data was 0.32 with a validity of 0.74. All the inbreds with positive GCA effect for grain yield plus TZEEIOR 223 were clustered together in group I while others with negative GCA effects were assigned to group II (Figure 5.2). Based on their performance in crosses, TZEEIOR 42 was found closely related to TZdEEI 7 than TZdEEI 7 to TZdEEI 12. Similarly, TZEEIOR 53 was closer related to TZEEIOR 223 than TZEEIOR 223 to TZEEIOR 222. The prediction of GCA effect of TZEEIOR 12 were zero for all the traits. Moreover, it was closely related to TZEEIOR 196 and were clustered together in group II, characterised by negative GCA effects for grain yield.

Table 5.6 General combining ability effects of inbred parents for grain yield and other traits across drought stress environments in Nigeria, 2016-2017

Inbred	Grain Yield, kg ha^{-1}	Anthesis-Silking interval	Days to 50% silking	Plant aspect (1-9)	Husk cover (1-9)	Ear aspect (1-9)	Leaf death (1-9)
TZEEIOR 12	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TZEEIOR 42	64.596	-0.115	-0.457	-0.183	-0.041	-0.066	-0.043
TZEEIOR 53	2.046	-0.019	-0.035	0.024	0.025	0.001	0.137
TZEEIOR 76	41.249	-0.163	0.136	-0.029	-0.024	0.060	-0.458
TZEEIOR 100	-33.363	-0.029	0.350	0.132	0.125	0.163	-0.007
TZEEIOR 113	-90.926	0.013	-0.023	0.078	0.208	-0.043	0.085
TZEEIOR 130	-48.969	0.092	0.240	-0.057	0.125	0.097	0.203
TZEEIOR 141	36.035	0.084	0.120	-0.048	0.108	-0.058	0.172
TZEEIOR 145	-35.232	0.250	-0.054	0.114	0.125	0.104	0.047
TZEEIOR 161	25.555	0.005	0.217	-0.003	0.025	-0.006	0.155
TZEEIOR 196	-69.196	0.562	0.863	0.158	0.000	0.163	-0.130
TZEEIOR 218	61.432	-0.019	-0.873	0.024	-0.083	-0.132	0.027
TZEEIOR 219	15.281	-0.458	-0.379	-0.048	-0.224	-0.073	0.123
TZEEIOR 222	-28.291	-0.043	-0.027	0.051	-0.058	0.016	-0.043
TZEEIOR 223	-50.639	-0.203	-0.247	0.006	-0.008	-0.051	0.105
TZdEEI 7	142.100	-0.362	-0.583	-0.254	-0.182	-0.117	-0.259
TZdEEI 12	6.996	0.060	-0.177	-0.038	-0.074	-0.073	0.029
S.E	88	0.31	0.51	0.12	0.15	0.15	0.17
H				**0.3201**			
R				**0.7427**			

S.E = standard error; **H** = Hopkins statistic; **r** = Correlation coefficient between cophenetic distance and the original distance.

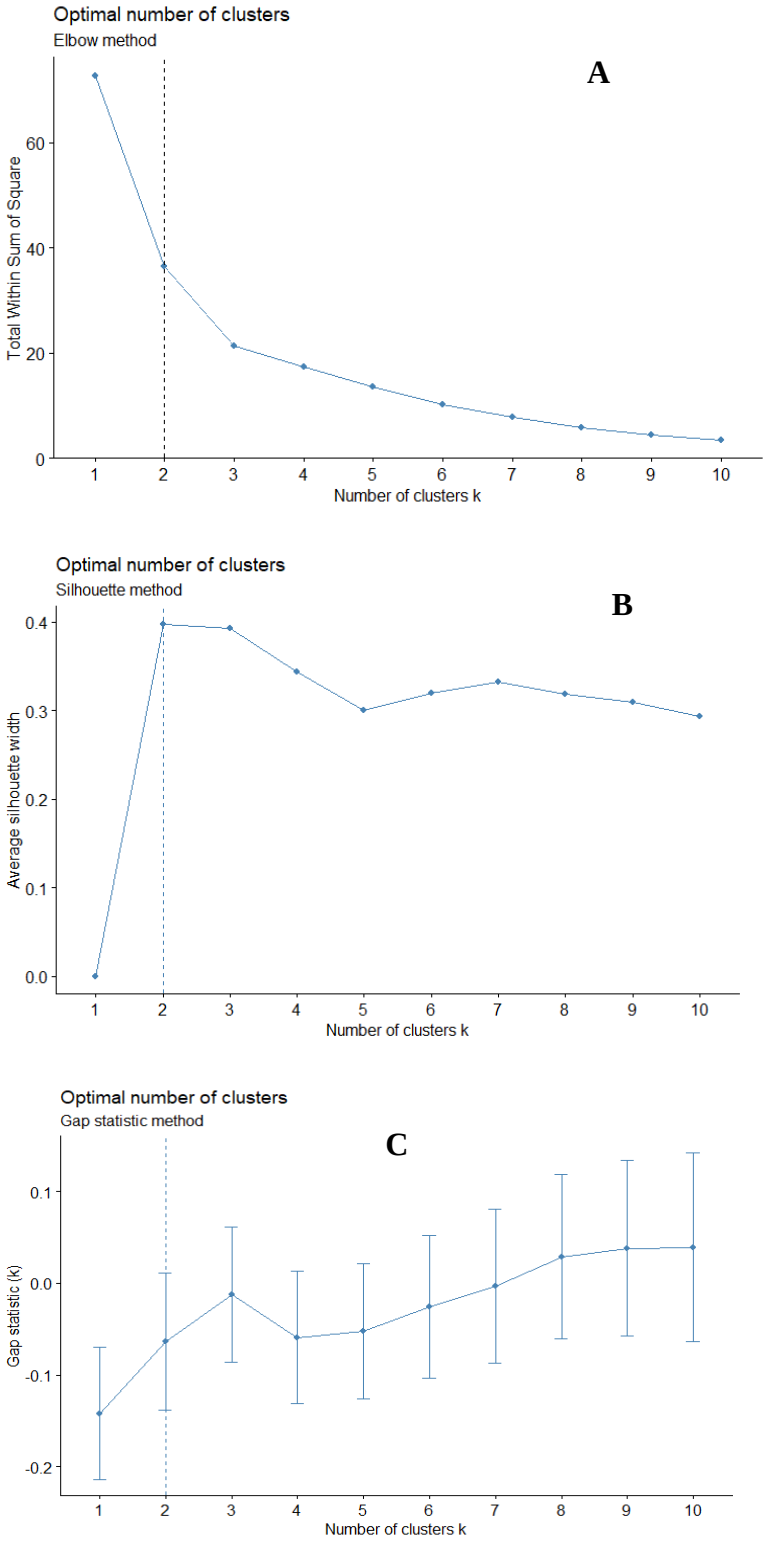


Figure 5.1 Optimal number of heterotic groups suggested by Elbow method (A), Silhouette method (B), and Gap statistic method (C) under drought environments

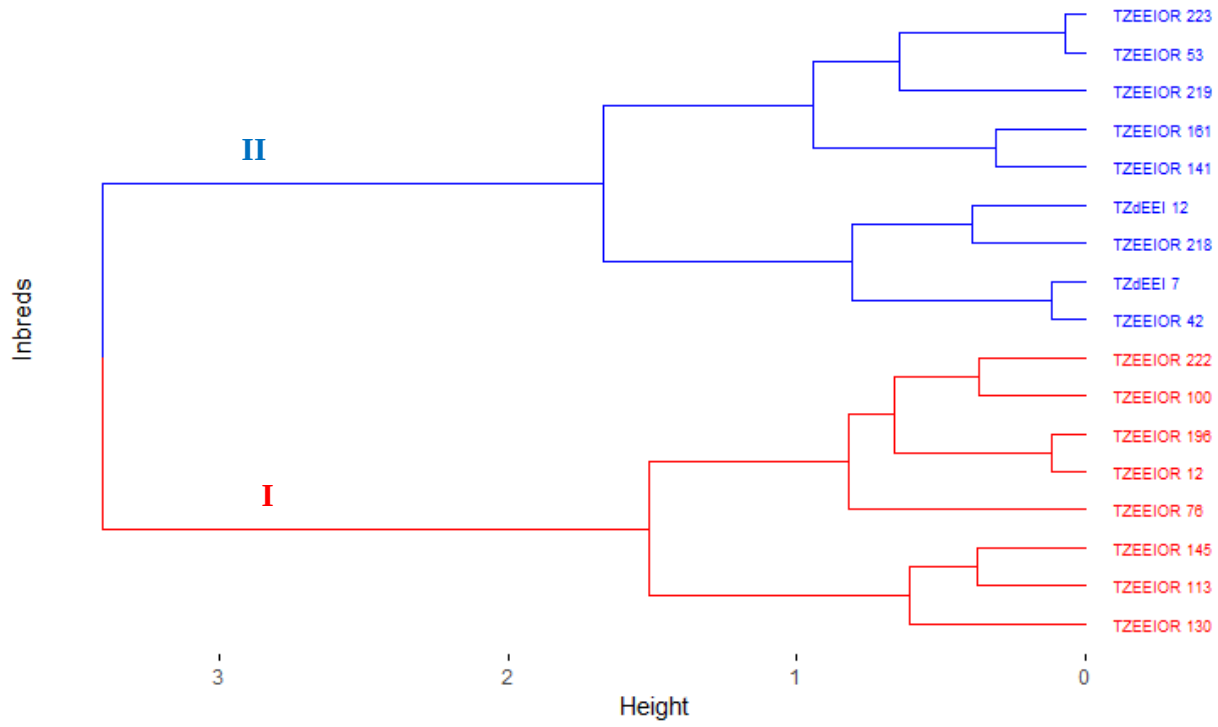


Figure 5.2 Heterotic grouping of inbred parents under drought environments using HGCAMT-Ward.D2

Table 5.7 presents the GCA effects of inbred parents for grain yield, anthesis-silking interval, *Striga* damage at 8 and 10 WAP, number of emerged *Striga* plants at 8 and 10 WAP, husk cover, and ear aspect across *Striga*-infested environments. The inbred TZEEIOR 141 showed the highest GCA effect for grain yield (152) while TZEEIOR 42 had the lowest value of GCA effect (-119) for the trait. Inbreds with positive GCA effects for grain yield ranked in decreasing order of importance were as follow: TZEEIOR 53(140), TZdEEI 7(134), TZEEIOR 145(85), TZEEIOR 12(44), TZEEIOR 219(26), TZEEIOR 113(20), and TZEEIOR 130(16). However, TZdEEI 7 and TZEEIOR 130 showed consistent negative GCA effects for *Striga* damage and number of emerged *Striga* plants at 8 and 10 WAP. For the inbred TZEEIOR 12, positive GCA effect (44.8) was associated with consistent positive GCA effects for the other traits. On the contrary, TZEEIOR 196 showed negative GCA effect (-103.2) for grain yield with positive GCA effects for the other traits.

Figure 5.3 presents the results of optimal number of heterotic groups in the GCA of multiple traits data under *Striga* environments. There were three different heterotic groups among the inbred parents (Elbow and Gap statistic methods).

Figure 5.4 presents the inbred parents in the three different heterotic groups. The group I was composed of eight inbreds including three inbreds with positive GCA effects for grain yield, TZEEIOR 141, TZEEIOR 53, and TZEEIOR 12. Group III was constituted of the remaining five inbreds with positive GCA effects plus TZEEIOR 76, which was clustered closer to TZEEIOR 113 and TZEEIOR 130 with a cophenetic distance less than 0.5. Inbreds TZEEIOR 222, TZEEIOR 100, and TZdEEI 12 were grouped in the cluster II, which is characterised by negative GCA effects for grain yield and anthesis-silking interval and positive GCA effects for majority of the other traits. The correlation between the cophenetic distances and the GCA-based distances was 0.807.

Table 5.7 General combining ability effects of inbred parents for grain yield and other traits across *Striga*-infested environments in Nigeria, 2016-2017

Inbred	Grain Yield, Kgha^{-1}	Anthesis-Silking Interval	*Striga* damage (1-9)		Number of emerged *Striga* plants		Husk Cover (1-9)	Ear Aspect (1-9)
			8 WAP	10 WAP	8 WAP	10 WAP		
TZEEIOR 12	44.77	0.04	0.05	0.02	0.34	0.49	0.03	0.04
TZEEIOR 42	-119.44	0.04	-0.03	0.01	0.32	0.57	-0.03	0.09
TZEEIOR 53	139.58	0.10	-0.15	-0.18	0.25	0.42	-0.17	-0.11
TZEEIOR 76	-32.85	-0.03	-0.05	-0.03	-0.54	-0.68	-0.01	-0.01
TZEEIOR 100	-92.98	-0.01	0.12	0.09	-0.50	-0.43	0.10	0.14
TZEEIOR 113	20.33	-0.01	0.00	-0.01	-0.15	-0.15	-0.02	0.04
TZEEIOR 130	16.15	0.05	-0.04	-0.06	-0.37	-0.70	-0.03	0.03
TZEEIOR 141	152.21	-0.09	-0.16	-0.18	0.20	0.20	-0.09	-0.12
TZEEIOR 145	85.41	-0.05	0.02	0.03	-0.46	-0.47	-0.01	-0.02
TZEEIOR 161	-13.06	-0.07	-0.01	0.00	0.71	0.64	0.02	-0.07
TZEEIOR 196	-103.22	0.03	0.05	0.06	0.75	1.13	0.06	0.05
TZEEIOR 218	-29.79	-0.01	-0.01	0.02	0.63	0.69	-0.03	-0.03
TZEEIOR 219	25.63	-0.02	-0.04	0.01	-0.57	-0.75	-0.01	-0.01
TZEEIOR 222	-107.95	-0.05	0.19	0.19	0.10	0.00	0.16	0.02
TZEEIOR 223	-28.82	-0.04	0.03	0.01	0.10	0.27	0.00	-0.05
TZdEEI 7	133.92	0.07	-0.10	-0.08	-0.58	-0.93	-0.05	-0.08
TZdEEI 12	-89.88	0.08	0.12	0.10	-0.26	-0.30	0.09	0.08
S.E	159	0.17	0.15	0.15	1.31	1.39	0.13	0.13
H				**0.228**				
r				**0.807**				

S.E = standard error; **H** = Hopkins statistic; **r** = Correlation coefficient between cophenetic distance and the original distance.

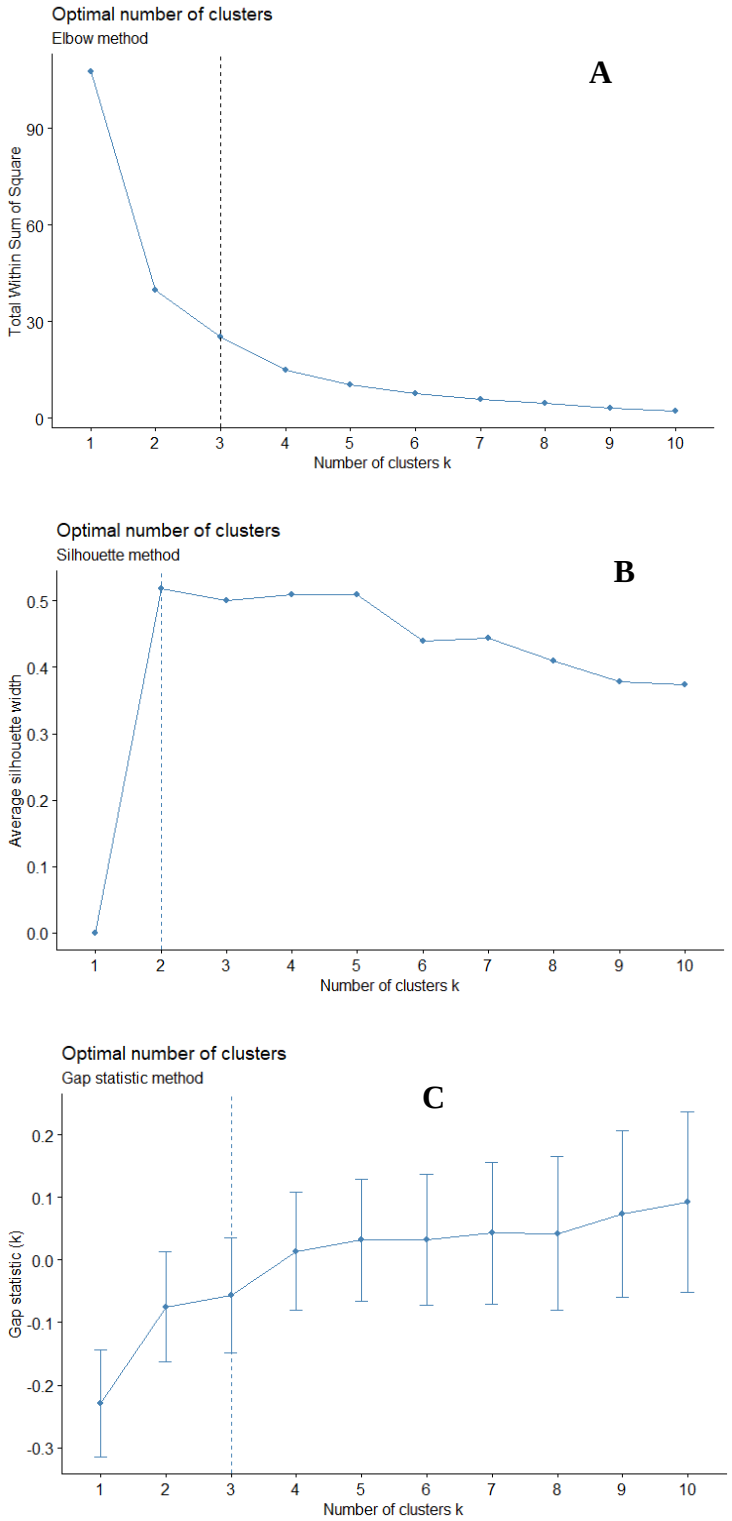


Figure 5.3 Optimal number of heterotic groups suggested by Elbow method (A), Silhouette method (B), and Gap statistic method (C) under *Striga* environments

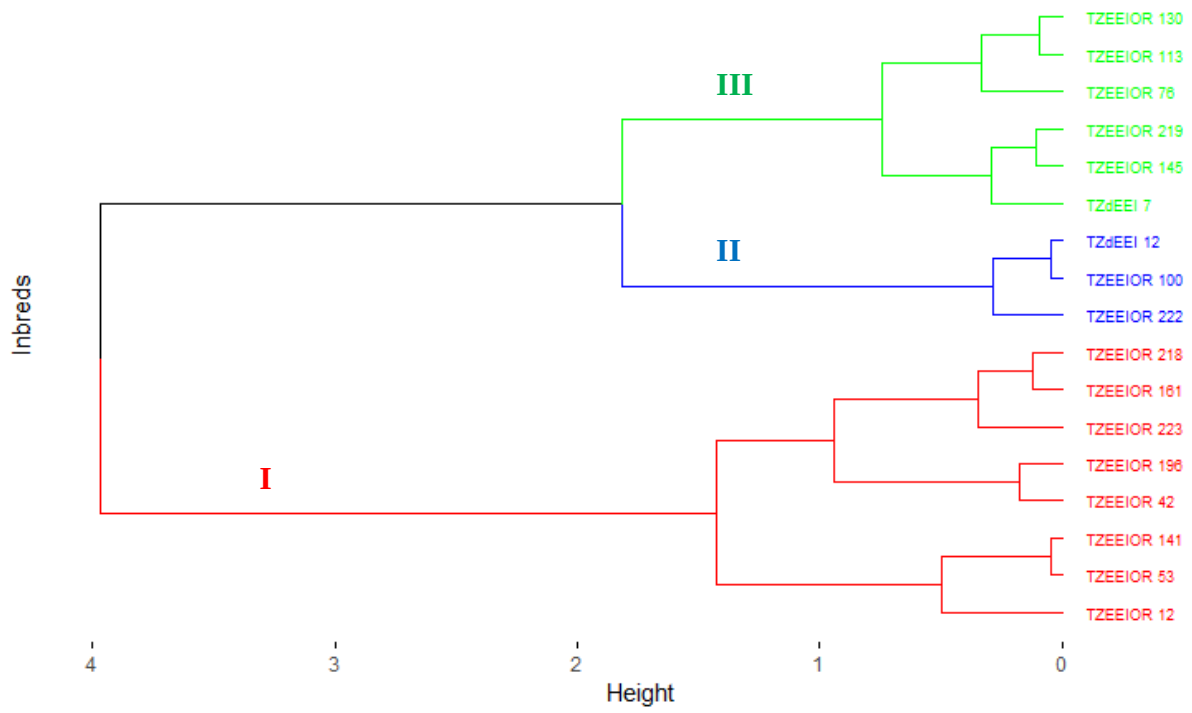


Figure 5.4 Heterotic grouping of inbred parents under *Striga*-infested environments using HGCAMT-Ward.D2

Table 5.8 presents the GCA effects of grain yield and other measured traits across test environments. The GCA effects of grain yield ranged from -130 for TZEEIOR 100 to 258 for TZEEIOR 141. Inbreds TZEEIOR 141 and TZEEIOR 53 (239) had high positive and significant ($p < 0.01$) GCA effects for grain yield. Moderate positive but non-significant GCA effects were found in TZEEIOR 12 (91) and TZdEEI 7 (89). The GCA effect of grain yield in TZEEIOR 141 was associated with significant ($p < 0.001$) GCA effects of days to 50% anthesis and silking, respectively. Highly significant ($p < 0.01$) GCA effects were observed in TZEEIOR 42 and TZEEIOR 145 for stalk lodging. Also, inbreds TZEEIOR 222 showed significant ($p < 0.05$) GCA effect for stalk lodging.

Figure 5.5 presents the optimal number of heterotic groups among inbred parents as suggested by the three methods across test environments. There were different heterotic groups suggested by the three methods. Therefore, five heterotic groups suggested by the Elbow method was used.

Figure 5.6 shows the clustering of the inbred parents into the five different groups. Genotypes TZEEIOR 141 and TZEEIOR 53, the top best based on GCA effects for grain yield, were singled out in one group (Group II) while the two-top worst, TZEEIOR 100 and TZEEIOR 76, were clustered together with TZdEEI 7 and TZdEEI 12 in the Group III. Group I was composed of six inbreds including TZEEIOR 12, TZEEIOR 42, and TZEEIOR 161, which expressed moderate positive and non-significant GCA effects for grain yield. Members in Group IV were characterised by an average of -47.35 GCA effect for grain yield, negative GCA effects for plant and ear height and for the traits related to *Striga* resistance. Inbreds TZEEIOR 222 and TZEEIOR 145 composed the Group V, which was characterised by an average of -78.2 GCA effect for grain yield, negative GCA effects for flowering traits and plant and ear heights, but with positive GCA effects for *Striga* damage rating at 8 and 10WAP.

Table 5.8 General combining ability effects of inbred parents for grain yield and other traits across test environments in Nigeria, 2016-2017

Inbred	Grain Yield, Kg ha^{-1}	Anthesis-Silking interval	Days to anthesis	Days to silking	Plant height, cm	Ear height, cm	Husk cover (1-9)	Ear per plant	Ear Aspect (1-9)	Root lodging	Stalk lodging
TZEEIOR 12	90.90	0.07	0.00	0.08	-0.64	1.65	0.01	0.00	-0.04	-1.02	-1.63
TZEEIOR 42	19.90	0.09	0.04	0.14	3.23*	3.56***	-0.01	0.01	-0.02	0.38	5.94***
TZEEIOR 53	239.39*	0.21*	-0.29	-0.02	1.94	-0.20	-0.02	0.01	-0.10	-1.21	-2.05
TZEEIOR 76	-115.33	-0.09	0.05	-0.07	-1.76	-0.74	0.00	-0.01	0.09	0.68	-2.31
TZEEIOR 100	-129.66	-0.05	0.35	0.28	-2.78*	-1.50	0.01	-0.01	0.07	-0.18	0.38
TZEEIOR 113	-50.28	0.05	0.20	0.27	-1.08	-0.12	0.01	-0.01	0.08	-1.20	-2.01
TZEEIOR 130	-73.82	0.10	0.37	0.49	-0.16	-0.23	0.00	-0.01	0.06	-0.70	-1.65
TZEEIOR 141	258.39*	-0.15	-0.72**	-0.91**	-1.37	-2.07*	0.00	0.01	-0.08	-0.58	-1.17
TZEEIOR 145	-5.12	-0.09	-0.77	-0.85**	-2.78*	-0.71	0.00	0.00	0.04	0.27	4.20**
TZEEIOR 161	23.57	0.01	-0.45*	-0.42	1.77	0.91	0.01	0.00	-0.05	-0.87	-1.75
TZEEIOR 196	-56.03	0.03	0.08	0.12	-0.43	1.27	0.00	0.01	-0.02	0.52	0.88
TZEEIOR 218	-74.61	-0.13	-0.17	-0.33	0.54	-0.89	-0.02	0.00	-0.01	-0.33	-0.73
TZEEIOR 219	-17.95	-0.07	0.50*	0.40	-0.33	-0.07	0.00	0.00	0.00	0.00	-1.00
TZEEIOR 222	-151.13	-0.11	-0.30	-0.43	-0.85	-1.09	0.01	-0.01	0.00	2.09*	2.88*
TZEEIOR 223	-51.82	-0.19*	0.15	-0.09	1.49	1.39	-0.01	0.00	-0.02	-0.20	-0.53
TZdEEI 7	88.86	0.13	0.55*	0.71*	0.52	-0.73	0.00	0.00	-0.03	1.43	-0.79
TZdEEI 12	4.75	0.18	0.41*	0.64*	2.68*	-0.44	0.01	0.00	0.02	0.92	1.33
S.E	**99**	**0.10**	**0.21**	**0.26**	**1.27**	**0.91**	**0.03**	**0.01**	**0.07**	**0.75**	**1.21**
H						**0.373**					
r						**0.698**					

S.E = standard error; **H** = Hopkins statistic; **r** = Correlation coefficient between cophenetic distance and the original distance.

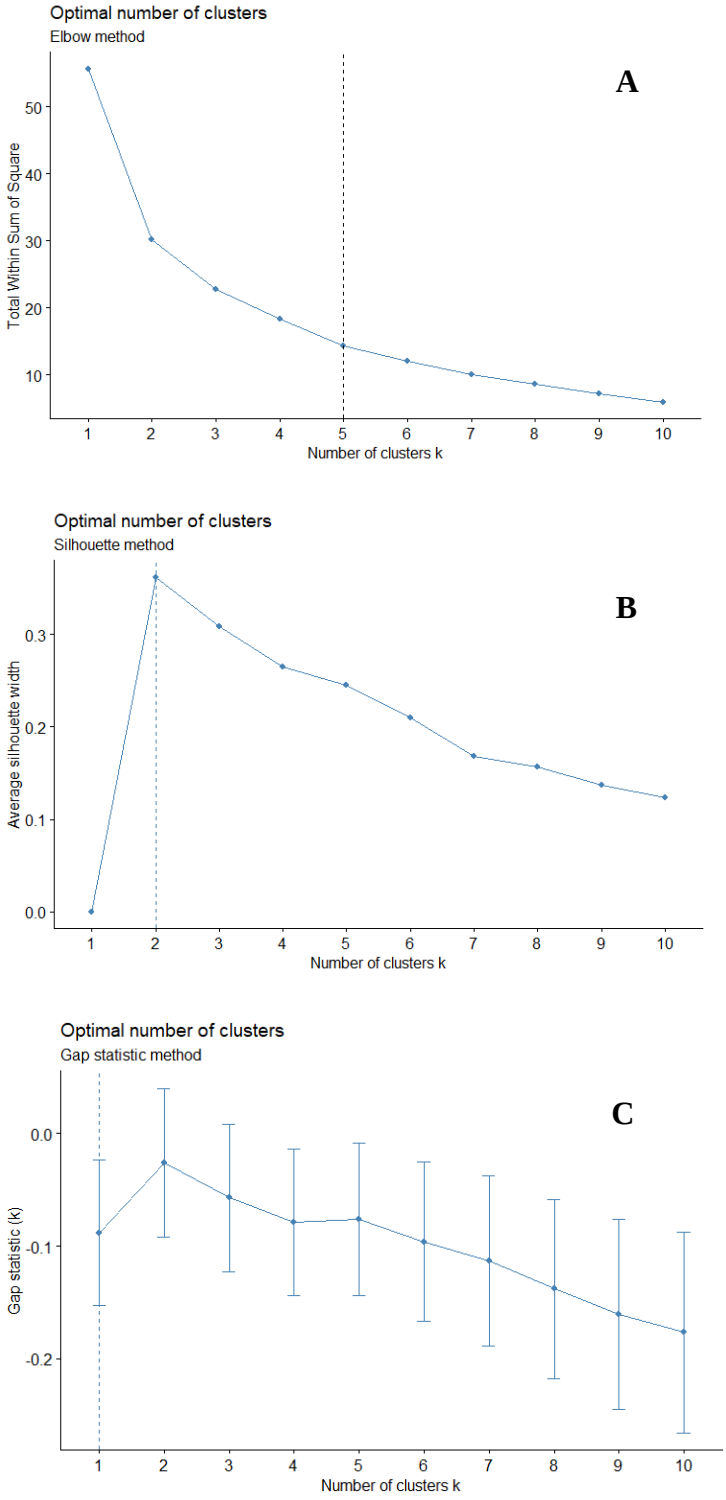


Figure 5.5 Optimal number of heterotic groups suggested by Elbow method (A), Silhouette method (B), and Gap statistic method (C) across test environments

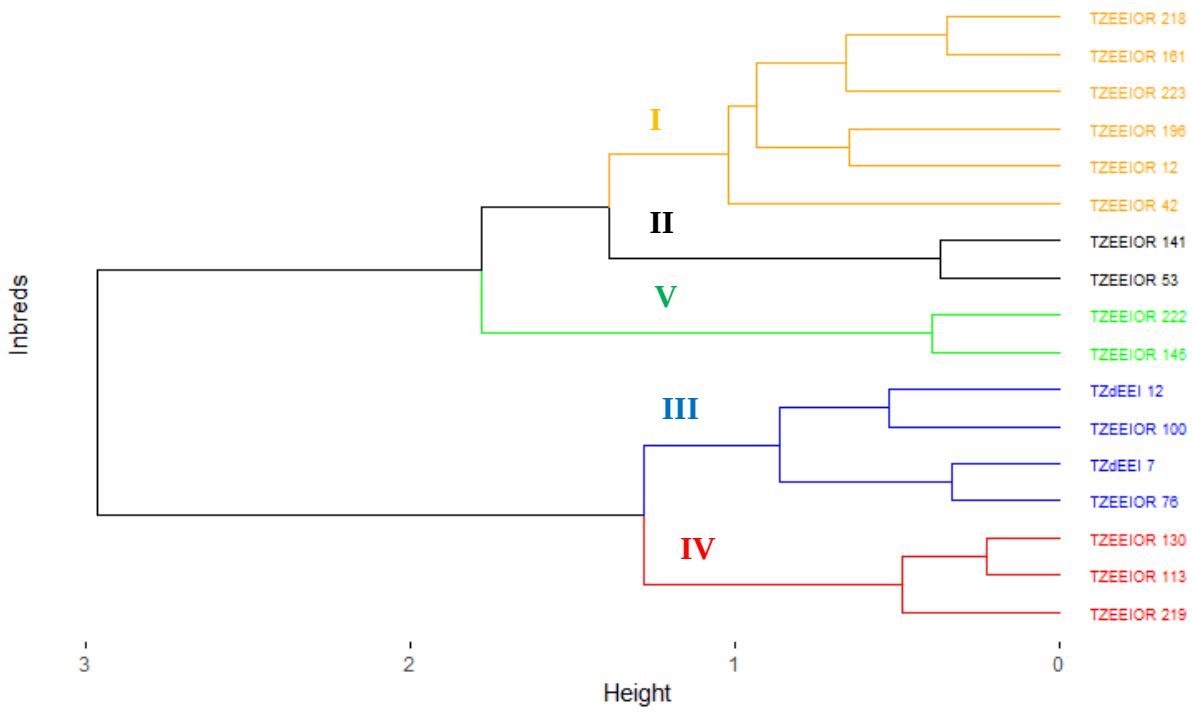


Figure 5.6 Heterotic grouping of inbred parents across test environments using HGCAMT Ward.D2

5.3.2.2 Identification of Testers

The GCA effect of grain yield across test environments was positive and significant ($p < 0.01$) for TZEEIOR 53 and TZEEIOR 141, thus, making them potential inbred testers. However, the multiple traits GCA-based grouping across test environments clustered TZEEIOR 53 and TZEEIOR 141 in the same group. Inbred TZEEIOR 53 and TZEEIOR 141 yielded 859±243 kg ha^{-1} and 959±239 kg ha^{-1}, respectively, which represent -8% and 3% yield over the average yield (933±250 kg ha^{-1}) across the pedigree.

In summary, inbred TZEEIOR 141 was identified as tester for Group II, because it belongs to a heterotic group, has positive and significant GCA effect for grain yield, has higher than average grain yield *per se*. In addition, TZEEIOR 53 x TZEEIOR 141 was identified as a single cross tester for group II. However, no inbred lines no single-cross hybrids in the other groups satisfied the criteria for identification of testers.

5.3.2.3 Heterosis and SCA effects of inbred parents for grain yield

Table 5.9 presents results of Gardner & Eberhart analysis II (1966) for grain yield under *Striga* infestation, drought stress, and optimal conditions. The mean squares of heterosis were highly significant ($p < 0.001$). However, the inbred heterosis, that is the contribution of heterosis by inbred *i* in its crosses were not significant under drought but were highly significant ($p < 0.001$) under *Striga*, and also significant ($p < 0.05$) under optimal conditions. The contribution of the specific heterosis under the different trials managements was also highly significant.

Table 5.10 presents the specific heterosis effects, the heterosis, and the heterobeltiosis of the top 20% and 3 worst hybrids obtained under *Striga* environments. The specific heterosis under *Striga* environments ranged from -2555 kg ha^{-1} for the hybrid TZEEIOR 219 x TZEEIOR 222 with significant mid-parent heterosis of -134% to 2869 kg ha^{-1} for hybrid TZEEIOR 141 x TZEEIOR

222 with mid-parent heterosis of 489% (Table 5.11). Also, the mid-parent heterosis ranged from -134 to 538 % for TZEEIOR 130 x TZEEIOR 218 while the better parent heterosis varied from -127 to 483%. The ranking of the genotypes based on the specific heterosis was not consistent with the ranking based on either mid-parent or better-parent heterosis indicating high differences among the *per se* performances of the inbred parents.

Table 5.9 Means squares of 17 extra-early maturing inbred parents with their 136 diallel hybrids for grain yield across environments under *Striga* infestation, drought stress, and optimal conditions in Nigeria, 2016-2017

Variance components		Grain yield, Kgha^{-1} across environments		
	DF	***Striga***	**Drought**	**Optimal**
Genotypes	152	2254499.1***	606845.7***	8785056.0***
Inbreds	16	3546360.1***	915732.9***	7756395.7***
Heterosis	**136**	**2102515.5*****	**570506.0*****	**8906074.9*****
Mean Heterosis ($\bar{h}$)	1	50001001.6***	17277684.5***	738145831.8***
Inbreds Heterosis (h_i)	16	1683728.1***	333185.3^{ns}	1643855.2*
Specific Heterosis (S_{ij})	119	1756314.7***	462018.2***	3754442.6***
Error	305	221295.5	221295.5	810435.4
Mean		2497	1331	4506
CV, %		19	35	20

Table 5.10 Specific heterosis effect (S_{ij}), heterosis (H), and heterobeltiosis (Hb) for grain yield across two *Striga*-infested environments of top 20% and 3 worst extra-early maturing single cross hybrids selected based on the specific heterosis in Abuja in 2016 and Mokwa in 2017

Hybrid	Grain yield, kg ha^{-1}				
	S_{ij}	H	H%	Hb	Hb%
TZEEIOR 141 x TZEEIOR 222	2869.47	5074.45**	488.90	4920.37**	412.78
TZEEIOR 223 x TZdEEI 7	2264.01	3876.30**	271.37	3689.11**	228.34
TZEEIOR 12 x TZEEIOR 222	2144.21	3509.94**	265.00	3069.29**	173.88
TZEEIOR 100 x TZEEIOR 196	2111.38	4771.19**	420.95	4445.13**	304.56
TZEEIOR 196 x TZEEIOR 223	1871.22	4004.03**	260.41	3925.97**	243.00
TZEEIOR 113 x TZEEIOR 219	1550.86	1233.23**	60.21	639.79ns	24.22
TZEEIOR 12 x TZEEIOR 76	1426.78	2162.49**	122.71	2159.57**	122.34
TZEEIOR 130 x TZEEIOR 218	1415.01	3219.42**	537.77	3162.72**	482.60
TZEEIOR 100 x TZEEIOR 113	1395.64	2373.63**	137.64	1456.48**	55.13
TZEEIOR 76 x TZdEEI 7	1331.88	2475.27**	164.99	2216.23**	125.97
TZEEIOR 12 x TZdEEI 12	1307.20	2609.51**	178.43	2306.81**	130.68
TZEEIOR 222 x TZdEEI 7	1223.24	2996.65**	282.03	2817.96**	227.03
TZEEIOR 53 x TZEEIOR 196	1199.41	2459.83**	149.60	2275.10**	124.39
TZEEIOR 76 x TZEEIOR 130	1075.33	2129.05**	176.34	1577.07**	89.64
TZEEIOR 161 x TZEEIOR 219	1026.02	1188.62**	90.33	1049.71*	72.15
TZEEIOR 145 x TZEEIOR 223	1016.90	2096.31**	140.04	1977.59**	122.40
TZEEIOR 42 x TZdEEI 12	1009.73	2904.12**	222.23	2757.06**	189.64
TZEEIOR 53 x TZEEIOR 113	954.50	533.10ns	23.85	126.75ns	4.80
TZEEIOR 53 x TZEEIOR 219	951.93	909.00*	55.36	721.91ns	39.47
TZEEIOR 12 x TZEEIOR 145	937.04	1623.00**	103.26	1429.51**	80.98
TZEEIOR 161 x TZEEIOR 218	934.75	2147.74**	249.89	1830.22**	155.50
TZEEIOR 113 x TZEEIOR 145	828.75	761.06ns	37.86	129.32ns	4.90
TZEEIOR 161 x TZEEIOR 196	827.30	2293.25**	173.96	2151.99**	147.45
TZEEIOR 42 x TZEEIOR 218	827.21	2905.68**	291.17	2449.73**	168.50
TZEEIOR 218 x TZdEEI 12	807.98	2785.53**	327.38	2476.63**	213.55
TZEEIOR 196 x TZEEIOR 219	761.79	2125.98**	145.90	2123.63**	145.50
TZEEIOR 76 x TZEEIOR 141	743.46	2318.41**	157.11	2034.75**	115.66
TZEEIOR 76 x TZEEIOR 223	-1778.28	-649.10ns	-38.47	-720.95ns	-40.98
TZEEIOR 222 x TZEEIOR 223	-1942.57	-183.37ns	-14.67	-549.26ns	-34.00
TZEEIOR 219 x TZEEIOR 222	-2554.71	-1564.13**	-133.76	-1849.60**	-127.14
Minimum	-2554.71	-1564.13	-133.76	-1849.60	-127.14
Maximum	2869.47	5074.45	537.77	4920.37	482.60
Mean	0	1286.25	110.34	1002.82	75.72
S.E.	311.15				

Table 5.11 presents the specific heterosis effects, the heterosis, and the heterobeltiosis of the top best 20% and 3 worst hybrids obtained under drought environments. The specific heterosis ranged from -1121 kg ha^{-1} for TZEEIOR 12 x TZEEIOR 113 to 1190 kg ha^{-1} in the cross between TZEEIOR 42 and TZdEEI 7. Also, heterobeltiosis varied from -81.91% in the cross TZEEIOR 76 x TZEEIOR 161 to 666.71% for TZEEIOR 141 x TZEEIOR 222 (hybrid not among the top 20%), which was the best hybrid under *Striga* infestation based on specific heterosis.

Table 5.12 presents the specific heterosis effects, the heterosis, and the heterobeltiosis of the top best 20% and 3 worst hybrids obtained under optimal environments. The specific heterosis ranged from -3414.96 kg ha^{-1} for TZEEIOR 218 x TZEEIOR 219 to 1977 kg ha^{-1} for TZEEIOR 113 x TZEEIOR 222. The highest heterosis and heterobeltiosis obtained were 9467.65 kg ha^{-1} and 3658.11 kg ha^{-1}, respectively and were observed for the hybrid TZEEIOR 113 x TZEEIOR 130 with a specific heterosis of 1363 kg ha^{-1}.

Table 5.11 Specific heterosis effect (S_{ij}), heterosis (H), and heterobeltiosis (Hb) for grain yield across two drought stress environments of top 20% and 3 worst extra-early maturing single cross hybrids selected based on the specific heterosis at Ikenne during 2016/2017 and 2017/2018 dry seasons

Hybrid	Grain yield, kg ha^{-1}				
	S_{ij}	H	H%	Hb	Hb%
TZEEIOR 42 x TZdEEI 7	1189.99	1442.87**	177.59	1437.36**	175.72
TZEEIOR 196 x TZEEIOR 222	1136.72	2114.39**	587.54	1725.47**	230.44
TZEEIOR 100 x TZEEIOR 130	1100.82	2205.82**	348.78	2161.67**	319.50
TZEEIOR 53 x TZdEEI 12	1056.94	2160.07**	291.28	2072.70**	250.04
TZEEIOR 12 x TZEEIOR 42	995.38	1535.84**	188.73	1529.00**	186.32
TZEEIOR 130 x TZEEIOR 196	946.30	2113.23**	296.52	2077.13**	277.40
TZEEIOR 12 x TZEEIOR 145	904.88	1734.09**	188.62	1635.37**	160.63
TZEEIOR 145 x TZEEIOR 218	715.83	1234.94**	151.49	1032.07*	101.37
TZEEIOR 113 x TZEEIOR 161	698.60	1101.16**	127.35	927.74*	89.37
TZEEIOR 161 x TZEEIOR 222	672.59	1326.77**	262.97	793.18^{ns}	76.41
TZEEIOR 113 x TZEEIOR 145	606.93	1218.57**	142.58	1055.17*	103.64
TZEEIOR 218 x TZdEEI 12	596.39	1375.13**	190.82	1266.82**	152.82
TZEEIOR 219 x TZdEEI 7	557.87	982.09*	120.42	979.70*	119.77
TZEEIOR 12 x TZEEIOR 161	496.21	1116.34**	120.12	1007.60*	97.06
TZEEIOR 12 x TZEEIOR 141	475.74	1589.43**	315.33	1272.85**	155.10
TZEEIOR 42 x TZEEIOR 141	470.02	996.00*	200.32	686.26^{ns}	85.04
TZEEIOR 113 x TZEEIOR 219	465.12	959.34*	127.53	898.38^{ns}	110.48
TZEEIOR 42 x TZEEIOR 219	461.04	585.11^{ns}	72.23	581.99^{ns}	71.57
TZEEIOR 76 x TZEEIOR 130	458.61	1384.03**	172.80	1259.67**	136.14
TZEEIOR 12 x TZEEIOR 218	458.26	1276.33**	178.14	1172.17*	142.84
TZEEIOR 161 x TZEEIOR 223	453.61	1176.75**	227.17	656.64^{ns}	63.25
TZEEIOR 12 x TZEEIOR 219	442.88	1154.67**	141.34	1150.95*	140.25
TZEEIOR 100 x TZEEIOR 113	422.02	1086.13**	169.77	1034.63*	149.67
TZEEIOR 53 x TZEEIOR 223	388.47	1633.94**	501.13	1305.80**	199.60
TZEEIOR 76 x TZEEIOR 113	388.04	872.57*	107.95	755.56^{ns}	81.66
TZEEIOR 76 x TZdEEI 7	366.43	780.96^{ns}	89.60	727.31^{ns}	78.60
TZEEIOR 12 x TZEEIOR 223	355.88	1587.07**	387.78	1175.70*	143.27
TZEEIOR 145 x TZEEIOR 222	-950.04	-86.78^{ns}	-17.55	-610.33^{ns}	-59.95
TZEEIOR 76 x TZEEIOR 161	-987.91	-793.86*	-80.87	-850.27^{ns}	-81.91
TZEEIOR 12 x TZEEIOR 113	-1121.40	-210.79^{ns}	-27.88	-275.47^{ns}	-33.57
Minimum	-1121.40	-793.86	-9494.16	-850.27	-81.91
Maximum	1189.99	2205.82	1714.56	2161.67	666.71
Mean	0	756.10	81.68	576.05	69.64
S.E.	311.15				

Table 5.12 Specific heterosis effect (S_{ij}), heterosis (H), and heterobeltiosis (Hb) for grain yield across three optimal environments of top 20% and 3 worst extra-early maturing single cross hybrids selected based on the specific heterosis at Ikenne in 2016 and 2017 and at Mokwa in 2017

Hybrid	Grain yield, kg ha^{-1}				
	S_{ij}	H	H%	Hb	Hb%
TZEEIOR 113 x TZEEIOR 222	1976.63	5570.51**	985.78	5175.40**	538.99
TZEEIOR 42 x TZEEIOR 218	1861.68	5719.93**	822.80	5690.39**	785.19
TZEEIOR 100 x TZEEIOR 223	1669.83	5581.16**	777.05	5320.58**	543.57
TZEEIOR 130 x TZEEIOR 223	1639.97	6165.03**	2927.19	5917.97**	1293.07
TZEEIOR 223 x TZdEEI 7	1623.75	5317.64**	410.87	4481.06**	210.30
TZEEIOR 145 x TZEEIOR 219	1583.88	5777.33**	881.12	5685.63**	760.74
TZEEIOR 161 x TZEEIOR 218	1560.72	5030.76**	558.68	4795.93**	422.44
TZEEIOR 218 x TZdEEI 7	1540.05	4802.85**	343.49	4070.26**	191.02
TZEEIOR 141 x TZEEIOR 161	1436.51	5541.43**	518.33	5475.23**	482.28
TZEEIOR 76 x TZEEIOR 222	1393.16	4577.10**	477.38	4575.70**	476.53
TZEEIOR 113 x TZEEIOR 130	1362.52	6321.04**	9467.65	6217.83**	3658.11
TZEEIOR 196 x TZEEIOR 223	1320.41	5654.20**	518.95	5022.32**	291.75
TZEEIOR 145 x TZEEIOR 161	1291.90	5870.59**	690.95	5584.94**	491.94
TZEEIOR 161 x TZEEIOR 219	1285.02	4989.69**	530.07	4795.74**	422.42
TZEEIOR 145 x TZEEIOR 218	1278.42	5237.23**	851.84	5186.40**	779.16
TZEEIOR 12 x TZEEIOR 42	1277.79	5178.97**	366.11	4489.09**	213.31
TZEEIOR 196 x TZEEIOR 219	1248.14	5385.48**	436.28	4898.46**	284.56
TZEEIOR 12 x TZEEIOR 141	1223.45	4751.40**	305.81	4200.61**	199.60
TZEEIOR 141 x TZEEIOR 219	1197.48	4917.15**	561.87	4789.39**	477.55
TZEEIOR 76 x TZEEIOR 141	1178.71	5118.35**	522.20	5095.60**	508.08
TZEEIOR 53 x TZEEIOR 196	1120.25	6081.73**	465.95	5665.54**	329.12
TZEEIOR 145 x TZEEIOR 223	1106.44	5496.35**	1075.97	5443.19**	965.13
TZEEIOR 76 x TZdEEI 12	1099.23	4800.27**	678.80	4550.04**	475.25
TZEEIOR 100 x TZEEIOR 218	1096.62	4576.85**	556.64	4420.26**	451.59
TZEEIOR 12 x TZdEEI 12	1042.44	4331.80**	338.23	3508.04**	166.69
TZEEIOR 53 x TZEEIOR 219	1006.64	5150.21**	629.46	5079.39**	571.35
TZEEIOR 141 x TZEEIOR 218	1001.53	4486.57**	537.78	4317.93**	430.54
TZEEIOR 218 x TZEEIOR 223	-2539.25	741.99^{ns}	132.11	638.01^{ns}	95.85
TZEEIOR 222 x TZEEIOR 223	-2900.15	260.28^{ns}	36.71	9.01^{ns}	0.94
TZEEIOR 218 x TZEEIOR 219	-3414.96	-330.18^{ns}	-46.73	-371.05^{ns}	-49.65
Minimum	-3414.96	-330.18	-46.73	-371.05	-49.65
Maximum	1976.63	6321.04	9467.65	6217.83	3658.11
Average	0.00	4035.17	619.60	3702.59	389.42
S.E.	486.19				

5.3.3 Performance of hybrids and stability analysis

5.3.3.1 Index-based performance under *Striga* environments

Table 5.13 presents grain yield and other measured traits of the top 20% and 3 worst hybrids using the base index for selection under *Striga*-infested environments. Grain yield under *Striga* environments varied from 1134 kg ha^{-1} for TZEEIOR 53 x TZEEIOR 161 to 4035 kg ha^{-1} for TZEEIOR 222 x TZdEEI 7 with a mean of 1427 kg ha^{-1}. *Striga* damage on the plants ranged from mild leaf blotching with some purplish-brown necrotic spots (3) to extensive leaf scorching with mostly grey necrotic spots (6) at eight weeks after planting (WAP). The disease symptoms did not vary much from 8 to 10 WAP (Table 5.14). A similar trend was observed between 8 and 10 WAP for the number of emerged *Striga* plants which ranged from 4 to 40 at 8 WAP and from 4 to 33 at 10 WAP.

Seventy-one out of 140 hybrids evaluated were selected as resistant/tolerant to *Striga* (positive index value). None of the four experimental hybrid checks used in this study was selected among the top 20% performing hybrids presented in Table 5.14. Hybrids TZEEIOR 222 x TZdEEI 7 (4035 kg ha^{-1}), TZEEIOR 141 x TZEEIOR 218 (3595 kg ha^{-1}), TZEEIOR 76 x TZEEIOR 196 (3326 kg ha^{-1}), TZEEIOR 100 x TZEEIOR 196 (4035 kg ha^{-1}), and TZEEIOR 130 x TZEEIOR 141 (4035 kg ha^{-1}) were the top five performers under *Striga*. Among the top performing hybrids, TZEEIOR 100 x TZEEIOR 141 and TZEEIOR 100 x TZdEEI 7 supported very few *Striga* plants with an average of four plants each. They were followed by hybrid TZEEIOR 141 x TZEEIOR 218 which supported on the average 6 *Striga* plants. Also, these hybrids expressed similar symptoms with an average *Striga* damage of 4, which was not significantly different from that of the top five performers. Under these conditions, the anthesis-silking interval and the ear aspect ranged from 1 to 5 days and 4 to 7 with an average of 2-5 days, respectively.

Table 5.13 Performance of index-based top 20% and 3 worst extra-early maturing single cross hybrids across *Striga*-infested environments at Abuja and Mokwa in 2016 and 2017

Hybrid	Grain Yield, Kg ha^{-1}	*Striga* damage (1-9)		Number of emerged *Striga* plants		Anthesis-silking interval	Ear Aspect (1-9)	INDEX
		8 WAP	10 WAP	8 WAP	10 WAP			
TZEEIOR 222 x TZdEEI 7	4035	4	4	13	16	2	4	11.60
TZEEIOR 141 x TZEEIOR 218	3595	3	4	6	6	1	4	11.46
TZEEIOR 76 x TZEEIOR 196	3326	3	4	10	12	1	4	10.68
TZEEIOR 100 x TZEEIOR 196	3606	4	4	15	18	2	5	9.11
TZEEIOR 130 x TZEEIOR 141	3674	4	4	16	15	2	4	8.78
TZEEIOR 76 x TZEEIOR 161	3133	4	4	16	13	2	5	7.49
TZEEIOR 223 x TZdEEI 7	3611	4	5	20	18	1	5	6.83
TZEEIOR 145 x TZdEEI 7	3295	4	4	16	17	2	4	6.62
TZEEIOR 42 x TZEEIOR 196	3499	4	4	22	24	2	4	6.10
TZEEIOR 100 x TZEEIOR 141	3161	4	5	4	4	2	5	5.98
TZEEIOR 196 x TZdEEI 7	3596	4	4	16	22	2	5	5.78
TZEEIOR 141 x TZdEEI 12	3688	4	5	12	14	1	5	5.74
TZEEIOR 219 x TZdEEI 7	3198	4	5	14	13	1	5	5.59
TZEEIOR 222 x TZdEEI 12	3075	4	5	14	17	1	5	5.54
TZEEIOR 196 x TZdEEI 12	2914	4	4	11	15	4	5	5.38
TZEEIOR 113 x TZEEIOR 219	3138	4	5	9	10	2	5	5.33
TZEEIOR 76 x TZdEEI 12	3502	4	5	17	18	2	5	5.13
TZEEIOR 113 x TZEEIOR 141	3403	4	5	12	15	2	4	5.11
TZEEIOR 130 x TZEEIOR 196	2853	4	5	10	13	2	4	4.90
TZEEIOR 100 x TZdEEI 7	2725	4	5	5	4	2	5	4.88
TZEEIOR 100 x TZdEEI 12	3561	5	5	11	14	1	5	4.87
TZEEIOR 12 x TZEEIOR 222	3677	4	5	24	25	3	4	4.62
TZEEIOR 196 x TZEEIOR 222	3415	4	5	14	16	3	5	4.58
TZEEIOR 42 x TZdEEI 7	3138	4	5	17	18	2	5	4.41
TZEEIOR 42 x TZEEIOR 222	2885	4	5	10	10	2	5	4.31
TZEEIOR 53 x TZEEIOR 196	3110	4	5	21	23	2	4	3.96
TZEEIOR 100 x TZEEIOR 222	2903	4	5	19	18	2	5	3.85
TZEEIOR 12 x TZEEIOR 130	3397	4	5	12	14	2	5	3.75
TZEEIOR 218 x TZEEIOR 219	1279	5	6	14	16	3	7	-11.29
TZEEIOR 219 x TZEEIOR 222	1134	6	6	17	16	3	7	-12.05
TZEEIOR 53 x TZEEIOR 161	1157	5	6	30	33	4	6	-12.15
Minimum	1134	3	4	4	4	1	4	
Maximum	4035	6	6	40	33	5	7	
Mean	2621	5	5	17	17	2	5	
S.E	458	0	0	1	1	1	0	

S.E = standard error.

5.3.3.2 Index-based performance under drought environments

Table 5.14 presents grain yield and other measured traits of the top 20% and three worst performing hybrids using the base index for selection under drought environments. There were more days to 50% silking under drought stress than those in *Striga* infestation with anthesis-silking interval ranging from 1 to 9 days. Ear aspect ranged from 4 to 8 with a mean of 5. Hybrid TZEEIOR 130 x TZEEIOR 196 was the highest yielding with 2881 kg ha^{-1} while the lowest yielding hybrid was TZEEIOR 42 x TZEEIOR 161 with 505 kg ha^{-1}. On the average, 50-60% of dead leaf area (rate 5) were observed with individual genotype having dead leaf area falling within a range of 20-30% to 60-70%.

Seventy-two out of 140 hybrids evaluated had positive base index values, and were therefore considered as tolerant to drought. The index-based ranking of the top five hybrids is as follows: TZEEIOR 100 x TZEEIOR 130 (2496 kg ha^{-1}), TZEEIOR 130 x TZEEIOR 196 (2881 kg ha^{-1}), TZEEIOR 218 x TZdEEI 12 (2082 kg ha^{-1}), TZEEIOR 12 x TZEEIOR 42 (1809 kg ha^{-1}), and TZEEIOR 113 x TZEEIOR 219 (1771 kg ha^{-1}). These hybrids showed, on the average, 20 to 30% dead leaf area with the anthesis-silking interval ranging from 2 to 3 days. Hybrids TZEEIOR 218 x TZdEEI 7 and TZEEIOR 76 x TZEEIOR 130 had anthesis-silking interval of zero and 1, respectively. Hybrid TZEEIOR 218 x TZdEEI 7 was identified as the worst performing hybrid based on the index. Among the five best hybrids selected under drought stress, only TZEEIOR 130 x TZEEIOR 196 and TZEEIOR 113 x TZEEIOR 219 were also selected among the 28 best performing hybrids under *Striga* infestation.

Table 5.14 Index-based performance of top 20% and 3 worst extra-early maturing single cross hybrids across drought-stress environments at Ikenne during 2016/2017 and 2017/2018 dry seasons

Hybrid	Grain Yield, Kg ha^{-1}	Ears Per plant	Anthesis Silking interval	Plant Aspect (1-9)	Ear Aspect (1-9)	Leaf death (1-9)	INDEX
TZEEIOR 100 X TZEEIOR 130	2496	1	3	5	4	4	13.73
TZEEIOR 130 X TZEEIOR 196	2881	1	3	5	5	4	11.16
TZEEIOR 218 X TZdEEI 12	2082	1	2	5	4	4	10.83
TZEEIOR 12 X TZEEIOR 42	1809	1	3	4	5	4	9.34
TZEEIOR 113 X TZEEIOR 219	1771	1	2	5	4	4	8.10
TZEEIOR 196 X TZEEIOR 222	2316	1	3	4	6	5	8.07
TZEEIOR 130 X TZEEIOR 161	1845	1	3	4	4	5	7.88
TZEEIOR 196 X TZEEIOR 218	1625	1	2	6	4	3	7.81
TZEEIOR 12 X TZEEIOR 145	1882	1	3	5	4	4	7.77
TZEEIOR 76 X TZEEIOR 113	1888	1	2	5	4	5	7.56
TZEEIOR 53 X TZdEEI 12	2225	1	5	5	4	5	7.15
TZEEIOR 130 X TZEEIOR 222	1888	1	3	4	5	4	7.15
TZEEIOR 76 X TZEEIOR 223	2196	1	3	5	4	5	7.08
TZEEIOR 161 X TZEEIOR 222	1903	1	3	5	4	5	6.87
TZEEIOR 12 X TZEEIOR 223	2234	1	5	5	5	5	6.74
TZEEIOR 53 X TZEEIOR 161	1993	1	4	6	5	4	6.35
TZEEIOR 141 X TZEEIOR 196	1832	1	4	5	4	4	6.18
TZEEIOR 76 X TZEEIOR 130	1810	1	1	5	4	5	5.90
TZEEIOR 130 X TZEEIOR 218	1547	1	3	5	5	3	5.50
TZEEIOR 12 X TZEEIOR 222	1875	1	3	5	5	4	5.46
TZEEIOR 12 X TZEEIOR 219	1805	1	3	5	4	5	5.38
TZEEIOR 130 X TZdEEI 7	1813	1	4	5	5	4	5.31
TZEEIOR 12 X TZEEIOR 161	1877	1	3	5	5	5	5.28
TZEEIOR 113 X TZEEIOR 161	1809	1	3	5	4	4	5.03
TZEEIOR 141 X TZdEEI 7	1614	1	4	5	4	5	4.99
TZEEIOR 130 X TZEEIOR 141	1758	1	4	5	5	4	4.97
TZEEIOR 141 X TZEEIOR 219	1603	1	4	6	4	5	4.78
TZEEIOR 76 X TZEEIOR 141	1767	1	4	5	4	4	4.72
TZEEIOR 100 X TZEEIOR 219	684	0	6	6	6	5	-11.46
TZEEIOR 42 X TZEEIOR 161	505	0	9	6	6	6	-14.63
TZEEIOR 218 X TZdEEI 7	516	0	0	7	8	6	-17.80
Minimum	505	0	1	4	4	3	
Maximum	2881	1	9	7	8	6	
Mean	1427	1	5	6	5	5	
S.E	257	0	1	0	0	0	

S.E = standard error.

5.3.3.3 Performance of hybrids across test environments.

Table 5.15 presents the top 20 and 5 worst hybrids based on the genetic values (GV) across test environments. Forty-eight percent (48%) of the hybrids having positive GV were selected. The top ten performing hybrids were as follow: TZEEIOR 141 x TZEERIOR 219, TZEEIOR 141 x TZEEIOR 223, TZEEIOR 145 x TZdEEI 7, TZEEIOR 12 x TZEEIOR 141, TZEEIOR 161 x TZEEIOR 219, TZEEIOR 53 x TZEEIOR 196, TZEEIOR 12 x TZEEIOR 196, TZEEIOR 53 x TZEEIOR 223, TZEEIOR 141 x TZEEIOR 218, and TZEEIOR 113 x TZEEIOR 141.

Out of the top ten performing hybrids identified based on genetic value, inbred TZEEIOR 141 was involved in six crosses. It may be recalled that TZEEIOR 141 was the inbred parent with the highest GCA effect. The best crosses with TZEEIOR 141 involved only two inbreds with positive GCA effects for grain yield, which are: TZEEIOR 12 and TZdEEI 7.

Table 5.16 presents the correlation coefficients among the indices used, SCA, and GV. Results showed that grain yield across test environments of the hybrids was significant ($p < 0.01$) and positively correlated with their specific combining ability (r = 0.857), their genetic value (r = 0.77), and also with the base index under *Striga* infestation (0.695). On the contrary, the base index under drought-induced environments was neither correlated to the grain yield of the hybrids across test environments nor any of the other parameters used for selecting the hybrids. High correlation was also observed between SCA and GV.

Table 5.15 Performance of the top 20 and 5 worst extra-early maturing single cross hybrids across test environments based on their genetic values across 11 test environments

Hybrid	Grain yield kg ha^{-1}				GCA_P1	GCA_P2	SCA	GV
	Striga	Drought	Optimal	Across				
TZEEIOR 141 X TZEEIOR 219	2944	1603	4396	3360	258.39	-17.95	383.57	624.01
TZEEIOR 141 X TZEEIOR 223	3053	1550	4435	3408	258.39	-51.82	393.37	599.94
TZEEIOR 145 X TZdEEI 7	3295	1055	5675	3970	-5.12	88.86	490.36	574.10
TZEEIOR 12 X TZEEIOR 141	2958	1519	4470	3384	90.90	258.39	206.07	555.36
TZEEIOR 161 X TZEEIOR 219	3422	1238	4628	3573	23.57	-17.95	535.01	540.63
TZEEIOR 53 X TZEEIOR 196	3110	1610	4792	3602	239.39	-56.03	285.82	469.18
TZEEIOR 12 X TZEEIOR 196	3184	1472	5411	3885	90.90	-56.03	424.31	459.18
TZEEIOR 53 X TZEEIOR 223	2698	1869	3860	3075	239.39	-51.82	261.17	448.74
TZEEIOR 141 X TZEEIOR 218	3595	798	4052	3294	258.39	-74.61	263.43	447.21
TZEEIOR 113 X TZEEIOR 141	3403	682	3682	3035	-50.28	258.39	223.11	431.22
TZEEIOR 141 X TZEEIOR 222	3207	1425	4115	3296	258.39	-151.13	323.57	430.83
TZEEIOR 53 X TZEEIOR 218	2812	2131	3507	3004	239.39	-74.61	249.09	413.87
TZEEIOR 130 X TZEEIOR 141	3674	1758	3600	3292	-73.82	258.39	195.95	380.52
TZEEIOR 53 X TZEEIOR 141	2161	1838	3332	2635	239.39	258.39	-125.59	372.19
TZEEIOR 222 X TZdEEI 7	4035	1171	4249	3611	-151.13	88.86	395.98	333.71
TZEEIOR 42 X TZEEIOR 53	2477	1534	3744	2882	19.8963	239.39	59.1312	318.42
TZEEIOR 223 X TZdEEI 7	3611	1213	4302	3489	-51.82	88.86	280.69	317.73
TZEEIOR 12 X TZdEEI 12	3108	1387	4160	3273	90.90	4.75	202.1	297.74
TZEEIOR 219 X TZdEEI 7	3198	1231	4331	3355	-17.95	88.86	220.97	291.88
TZEEIOR 141 X TZdEEI 12	3688	1437	3254	3081	258.39	4.75	9.4292	272.56
TZEEIOR 222 X TZEEIOR 223	2512	1402	1586	1889	-151.13	-51.82	-502.14	-705.09
TZEEIOR 218 X TZEEIOR 222	2280	757	1734	1755	-74.61	-151.13	-530.49	-756.23
TZEEIOR 218 X TZEEIOR 223	1856	1213	1625	1634	-74.61	-51.82	-638.99	-765.42
TZEEIOR 218 X TZEEIOR 219	1279	1017	1320	1250	-74.61	-17.95	-814.39	-906.95
TZEEIOR 219 X TZEEIOR 222	1134	954	1461	1250	-17.95	-151.13	-830.44	-999.52
S.E	458	257	496	209				

GCA_P1 and GCA_P2, general combining ability of parent 1 and parent 2, respectively; GV: Genetic value of the cross; S.E: standard error.

Table 5.16 Spearman's rho correlation coefficients among the four parameters used for selecting the F_1 hybrids and their grain yield across 11 test environments

	GY across	SCA	GV	Index *Striga*	Index Drought
GY across	1	0.857**	0.772**	0.695**	0.129
SCA	0.857**	1	0.771**	0.571**	0.147*
GV	0.772**	0.771**	1	0.482**	0.135
Index *Striga*	0.695**	0.571**	0.482**	1	0.032
Index Drought	0.129	0.147*	0.135	0.032	1

***** and ******, significance at respectively $p< 0.05$ and $p<0.01$ (one-tailed); **GV**, genetic value of hybrid F_1; **SCA**, specific combining ability; **GY across**, Grain yield of hybrids F_1 across 11 test environments.

5.3.3.4 Genetic correlation and heritability among test environments for grain yield

The genetic correlation coefficients among all test environments and their heritability for grain yield are presented in Table 5.17. Results indicated that the two drought stress environments (IK16DT, IK17DT) and *Striga*-infested environment at Abuja in 2017 (ABJ17STR) were negatively correlated with most of the rest of the environments. However, all of the environments had higher heritability than the threshold ($h \leq 0.20$) set for eliminating environment for stability analysis, in this study. Higher genetic correlations were found among optimal environments than those among *Striga* environments.

Drought stress environments and *Striga* environment at Abuja in 2017 were then removed from the stability analysis despite their relatively high heritability observed for grain yield.

Table 5.17 Genetic correlation among test environments and heritability for grain yield

	Environment	Environment										
		1	2	3	4	5	6	7	8	9	10	11
1	ABJOPT16	1										
2	ABJSTR16	0.46	1									
3	IKDT16	-0.07	-0.31	1								
4	IKOPT16	0.79	0.28	-0.13	1							
5	MKOPT16	0.48	0.26	-0.08	0.27	1						
6	MKSTR16	0.74	0.47	0.08	0.55	0.15	1					
7	ABJSTR17	-0.1	-0.17	-0.04	-0.08	-0.08	0.06	1				
8	IKDT17	-0.08	-0.06	0.42	0.16	-0.21	0.16	0.16	1			
9	IKOPT17	0.54	0.69	-0.11	0.71	0.3	0.63	-0.16	0.1	1		
10	MKOPT17	0.52	0.3	-0.07	0.47	0.21	0.38	-0.14	0	0.66	1	
11	MKSTR17	0.26	0.21	0.08	0.3	0.13	0.55	-0.2	-0.02	0.33	0.61	1
Heritability		**0.64**	**0.44**	**0.53**	**0.76**	**0.56**	**0.49**	**0.54**	**0.52**	**0.63**	**0.66**	**0.55**

ABJOPT16, ABJSTR16, ABJSTR17 = Optimal trial and *Striga*-infested trials at Abuja in 2016 and 2017; IKDT16 and IKOPT16, IKDT17 and IKOPT17 = Managed drought and Optimal trials at Ikenne in 2016 and 2017; MKOPT16 and MKSTR16, MKOPT17and MKSTR17= Optimal trials and *Striga*-infested trials at Mokwa in 2016 and 2017.

5.3.3.5 Stability of the performance of hybrids across test environments

Figure 5.7 presents the "which won where" genotype main effect plus genotype x environment biplot of 26 top performing hybrids plus the 4 experimental hybrid checks. The genotype main effects and the genotype x environment interaction (GGE) biplot explained 77.3% of the total variation in grain yield across eight environments with the two axes (PC1 and PC2) constituting the biplot explaining 66.4 and 10.9%, respectively, of the variation captured. The perpendicular lines to the polygon sides divide the biplot into sectors, which are mega-environments suggesting that different genotypes won in different sectors and thus genotype x environment interaction or crossover patterns existed in the data. Environments E8, E10, E1 (Optimal environments) plus E11 (*Striga*-infested) were grouped as one mega-environment, that is these environments consistently shared the best set of genotypes. Similarly, E7 and E5 (optimal) plus E9 (*Striga*-infested) shared the same set of best genotypes. However, the *Striga*-infested environment at Abuja in 2016 (E2) did not share the best genotypes with any of the two mega-environments identified.

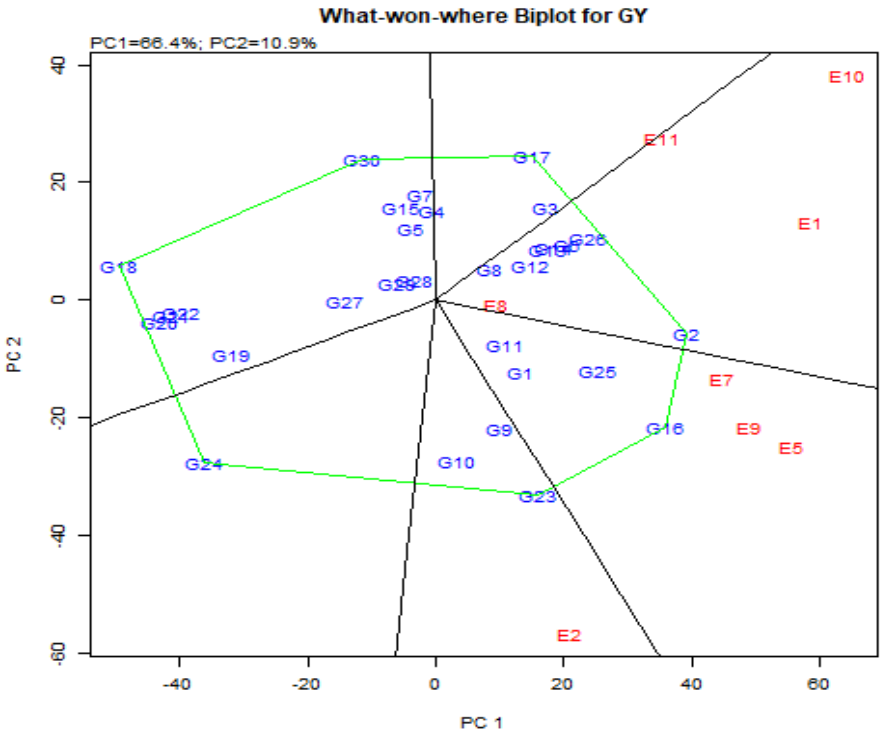


Figure 5. 7 A 'which won where' genotype main effect plus genotype x environment biplot of 30 extra-early maturing maize hybrids evaluated for grain yield across *Striga* infested and optimal environments in 2016 and 2017

LEGEND

Code	Hybrid	Code	Hybrid	Code	Environment
G1	TZEEIOR 12 X TZEEIOR 141	G16	TZEEIOR 145 X TZdEEI 7	E1	Abuja optimal 2016
G2	TZEEIOR 12 X TZEEIOR 196	G17	TZEEIOR 161 X TZEEIOR 219	E2	Abuja *Striga*-infested 2016
G3	TZEEIOR 12 X TZdEEI 12	G18	TZEEIOR 218 X TZEEIOR 219	E5	Ikenne optimal 2016
G4	TZEEIOR 42 X TZEEIOR 53	G19	TZEEIOR 218 X TZEEIOR 222	E7	Ikenne optimal 2017
G5	TZEEIOR 53 X TZEEIOR 141	G20	TZEEIOR 218 X TZEEIOR 223	E8	Mokwa optimal 2016
G6	TZEEIOR 53 X TZEEIOR 196	G21	TZEEIOR 219 X TZEEIOR 222	E9	Mokwa *Striga*-infested 2016
G7	TZEEIOR 53 X TZEEIOR 218	G22	TZEEIOR 219 X TZEEIOR 223	E10	Mokwa optimal 2017
G8	TZEEIOR 53 X TZEEIOR 223	G23	TZEEIOR 219 X TZdEEI 7	E11	Mokwa *Striga*-infested 2017
G9	TZEEIOR 113 X TZEEIOR 141	G24	TZEEIOR 222 X TZEEIOR 223		
G10	TZEEIOR 130 X TZEEIOR 141	G25	TZEEIOR 222 X TZdEEI 7		
G11	TZEEIOR 141 X TZEEIOR 218	G26	TZEEIOR 223 X TZdEEI 7		
G12	TZEEIOR 141 X TZEEIOR 219	G27	TZdEEI 1 x TZdEEI 12		
G13	TZEEIOR 141 X TZEEIOR 222	G28	TZEEI 79 x TZEEI 82		
G14	TZEEIOR 141 X TZEEIOR 223	G29	(TZEEI 79 x TZEEI82) x TZEEI 95		
G15	TZEEIOR 141 X TZdEEI 12	G30	TZEE-Y Pop STR C5 x TZEEI 58		

The vertex genotype, that is the winning genotype, for E2 was hybrid TZEEIOR 219 x TZdEEI 7. In the mega-environment constituted of E7, E9, and E5, hybrid TZEEIOR 145 x TZdEEI 7 was the winner while TZEEIOR 12 x TZEEIOR 196 was the winner in the mega-environment composed of E1, E8, E10, and E11.

Figure 5.8 presents the mean grain yield versus stability of genotype main effect plus genotype x environment biplot of 26 top performing hybrids plus the 4 experimental hybrid checks. The ideal genotype is represented by the head of the arrow on the average environment coordinate (AEC) abscissa (horizontal axis). The arrow shown on the AEC abscissa points in the direction of higher mean performance of genotypes. Results showed that hybrids TZEEIOR 12 x TZEEIOR 196, TZEEIOR 145 x TZdEEI 7, TZEEIOR 222 x TZdEEI 7, TZEEIOR 223 x TZdEEI 7, and TZEEIOR 219 x TZdEEI 7 were the top 5 highest yielding among the hybrids selected for stability analysis. In addition, hybrids TZEEIOR 12 x TZEEIOR 196 and TZEEIOR 222 x TZdEEI 7 had a near-zero projection onto the AEC ordinate (vertical axis), meaning that the rank of these two genotypes was highly consistent across environments. Because these hybrids combined both high grain yield and stability, they were identified as the most stable hybrids.

Figure 5.9 presents the discriminating power against the representativeness of the environment in identifying the superior hybrids in mega-environments. Results revealed the optimal environment at Mokwa in 2016 (E8) as the least discriminating of the hybrids with the shortest vector from the biplot origin. All the test environments had similar discriminating power, with the most discriminating environment as the optimal environment at Mokwa in 2017. Moreover, E7 had the smallest angle with the AEC abscissa in the mega-environments composed of E7, E9, and E5. The environment E1 had the smallest angle with the AEC abscissa in the mega-environments composed of E8, E10, E11 and E1. The environments E7 and E1 were, therefore, the most representative of their respective mega-environments.

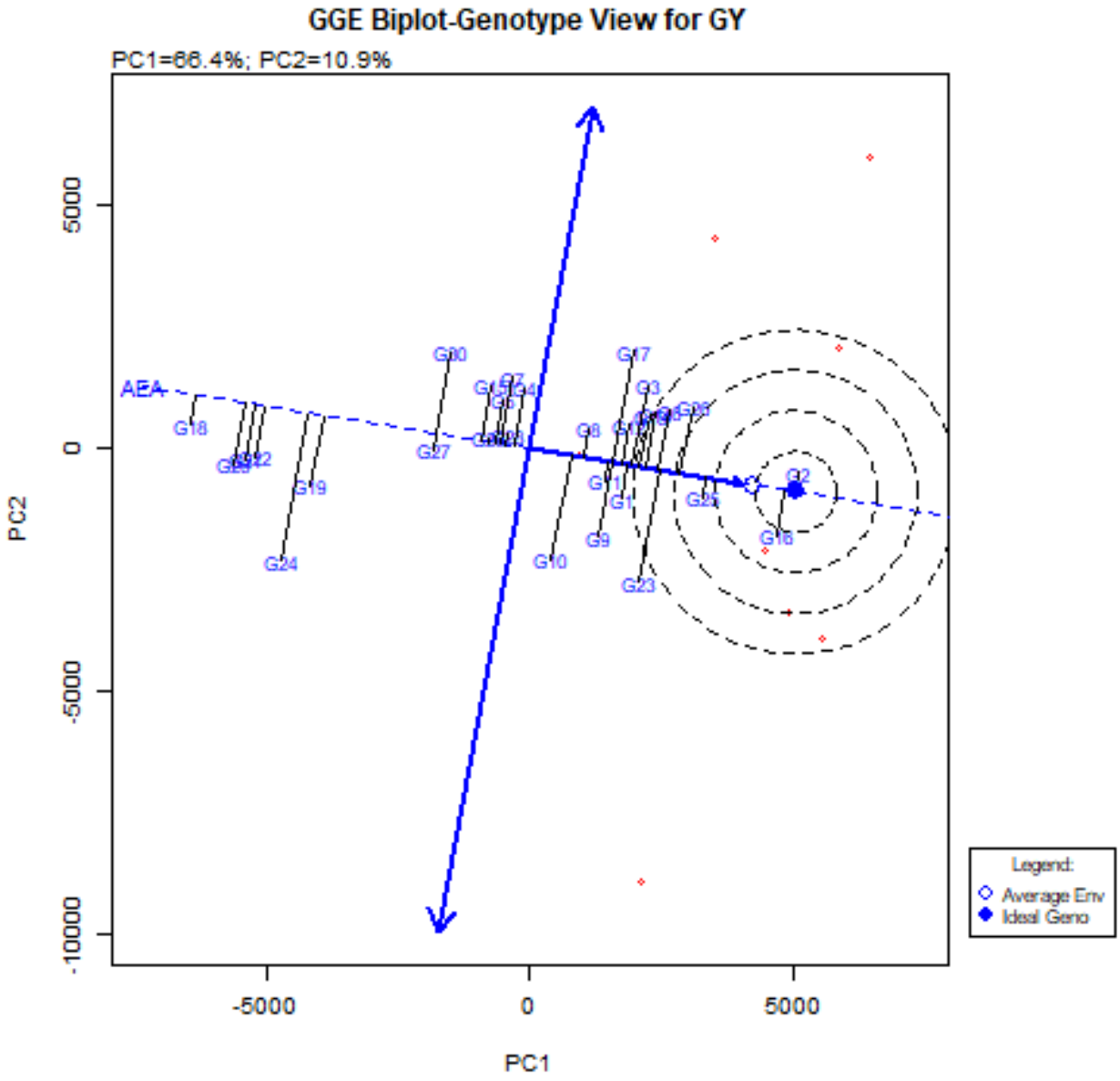


Figure 5. 8 Mean vs. stability view of genotype main effect plus genotype x environment biplot of grain yield of 30 selected extra-early maturing maize hybrids evaluated across *Striga*-infested and optimal environments in 2016 and 2017

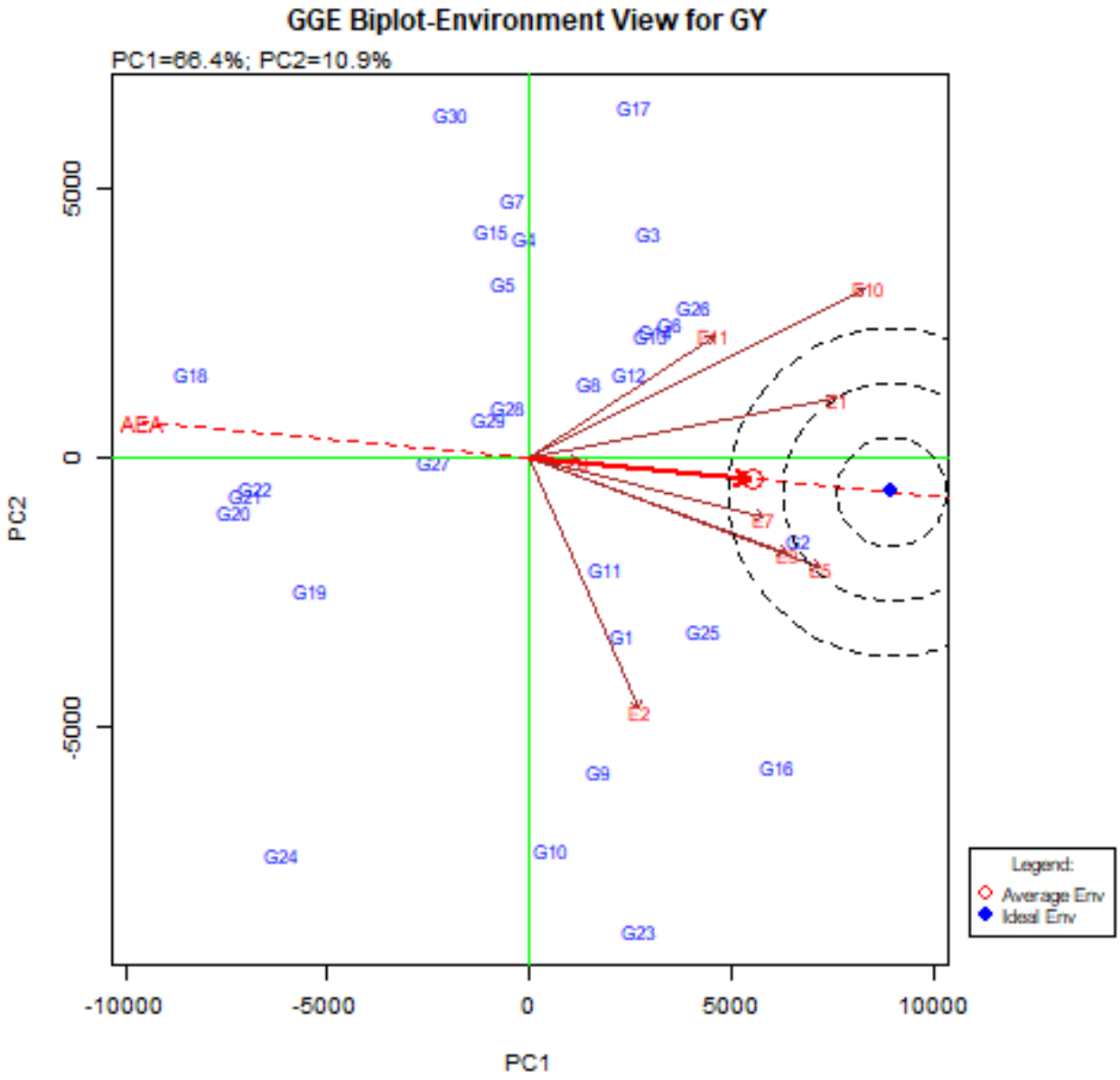


Figure 5. 9 Discriminating vs. representativeness view of GGE biplot of 30 selected extra-early maturing hybrid maize for grain yield across *Striga*-infested and optimum environments in 2016 and 2017

5.4 Discussion and conclusion

The observed significant variances for both GCA and SCA interactions with environments, for all the measured traits including grain yield under each trial management type and across test environments, indicated a change in ranking among genotypes in each environment and across test environments. The results also suggest that the environments were discriminating enough to uncover the genetic variability among the hybrids, which is the basis of selection for improvements in the measured traits. Previous studies (Badu-Apraku *et al.,* 2015; Annor and Badu-Apraku, 2016; Talabi *et al.,* 2017) reported genetic variability among hybrids developed in IITA needed for selection of high performing genotypes. However, there were both genetic and environmental variations implying that selection of superior genotypes may be difficult for few evaluations. This result suggested that extensive evaluations may be needed to be able to identify stable genotypes.

As indicated by the significant GCA and SCA variances and GCA/SCA ratio, both additive and non-additive gene actions were important in this set of orange maize inbreds for grain yield and other agronomic traits under each of the three groups of environments regarding the management and under test environments. Under *Striga* infestations, *Striga* damage at 8 WAP and number of emerged *Striga* plants at 8 and 10 WAP had GCA/SCA ratio greater than 2 with Baker ratio for each close to unity. This indicated a predominance of GCA over SCA for these traits and implied that additive genetic effects were the primary type of the gene action for these traits, hence improvement for *Striga* resistance indicator traits in maize could be successfully achieved based on the predictive values of GCA alone. Under drought stress, the results showed that only staygreen characteristic can be improved based solely on GCA predictions of parents. Grain yield under each trial management type and across test environments had a GCA/SCA ratio of less than 0.5, indicating the predominance of non-additive genetic effects in its inheritance. These results disagree with those of Makumbi *et al.* (2011) and Badu-Apraku and Oyekunle (2012) who reported

additive gene action to be predominant in the inheritance of grain yield in early-maturing maize inbred lines. However, it supports the findings of Betran *et al.* (2003) and Meseka *et al.* (2006). The difference in gene action for grain yield reported compared to other findings may be explained by the difference in the genetic material used.

The important effects of SCA revealed in this study suggests that one should consider not only GCA but also the deviations from predicted values of GCAs in selecting hybrids parents. GCA effects of inbred lines for a given trait are the part of the genetic effect that can be transmitted to progeny. This is particularly important in population development such as synthetics. Results showed that only TZdEEI 7 consistently combined positive GCA effects for grain yield under test environments and negative GCA effects for ASI and STGC, and *Striga* related traits under drought stress and *Striga* infestation, respectively. The inbred lines with favourable GCA effects for traits under a specific group of environments could be used as parents to improve such traits in population development for the specific environment (Makumbi *et al.*, 2011, Badu-Apraku *et al.*, 2012b; 2015). For instance, TZEEIOR 42 combined positive GCA effects for grain yield across test environments and negative GCA effects for ASI, and LD while TZEEIOR 141 and TZEEIOR 53 combined positive GCA effects for grain yield across test environments and negative GCA effects for *Striga* damage rating at 8 and 10 WAP.

Narrow sense heritability for a trait is an estimator of the expected genetic variance of a parent in its progeny. It allows measurement of how effective a trait can be inherited. Results of this study showed low to moderate narrow sense heritability for all the measured traits under *Striga* infestation and across test environments. Under drought stress, low heritability was, on the contrary, observed for all traits except for stay green characteristic with values lower than those reported by Mhike *et al.* (2011) and Oyekunle and Badu-Apraku (2014). However, the heritability values obtained were relatively higher under optimal conditions. These results highlighted the

relative importance of non-additive genetic effects over additives genetic effects discovered for most of the traits, in this set of inbred parents and also the high heterogeneity among error variances.

In order to maximise their potential usefulness for the development of productive synthetic varieties and hybrids, the newly developed extra-early orange inbreds needed to be classified into appropriate heterotic groups. This has, also, the advantage of helping to identify the potential extra-early orange testers which are completely lacking in SSA. The inbreds classified into the same heterotic group in the present study may be recombined to form heterotic populations that could be improved through recurrent selection while superior hybrids could be obtained in crosses involving inbreds from different heterotic groups. In this study, TZEEIOR 53 and TZEEIOR 141 had the highest positive and significant GCA effects and appertained to the same heterotic group. Inbred TZEEIOR 141 was identified as inbred tester and, therefore, could be used for grouping new inbreds into different heterotic groups in a line by tester mating scheme. In addition, the cross between TZEEIOR 53 and TZEEIOR 141 was ranked fourteenth among the top fifteen performing hybrids. Therefore, hybrid TZEEIOR 53 x TZEEIOR 141 could be used as a single-cross tester for the development of three-way and double cross hybrids.

Maize has been successfully introduced to savannas through extra-early and early varieties. However, currently, only few commercial extra-early maturing orange maize hybrids with significant levels of resistance/tolerance to *Striga* and drought tolerance are available. Results of the present study indicated that hybrids TZEEIOR 12 x TZEEIOR 96, TZEEIOR 145 x TZdEEI 7, TZEEIOR 222 x TZdEEI 7, TZEEIOR 223 x TZdEEI 7, and TZEEIOR 219 x TZdEEI 7 out-yielded the best check (TZEEI 79 x TZEEI 82) by 35-60% across environments. These outstanding hybrids are also among the top performing hybrids under *Striga* infestation and could, therefore,

be used in these areas to increase the production, incomes, and nutrition of less privileged maize farmers in SSA.

Different superior hybrids were identified under different stresses. There is, hence, the need for introgression of new sources of genes responsible for resistance to *Striga* and tolerance to drought in this set of inbred parents.

The high correlation between hybrid performance and the respective SCA and GV is an indication that SCA among parental lines and also GCA and SCA combined can predict hybrid performance, as well as the base index does.

In conclusion, only TZEEIOR 53 and TZEEIOR 141 had positive and significant general combining ability effects for grain yield across test environments. Non-additive genetic effects were the primary type of gene action for grain yield and most of the other agronomic traits in all environments except for staygreen characteristic under drought stress and *Striga* resistance indicator traits, which could be improved based solely on GCA predictions of parental lines.

The inbred line TZEEIOR 141 was identified as inbred tester while the cross TZEEIOR 53 x TZEEIOR 141 was identified as hybrid tester.

Superior hybrids identified in this study including TZEEIOR 12 x TZEEIOR 196 which was identified as the most stable hybrid could successfully be used to improve maize productivity, production, and well-being of less privileged farmers in sub-Saharan Africa, especially in *Striga* endemic and drought prone environments.

CHAPTER SIX

6.0 Estimate of combining abilities and heterosis for carotenoids in maize kernels of extra-early orange inbreds

6.1. Introduction

Breeding for maize cultivars with elevated provitamin A carotenoids is a sustainable and effective way to alleviate provitamin A deficiency in sub-Saharan Africa (SSA). The use of markers for favourable alleles at both *lycE* and *crtRB1* loci, in MAS, allowed increases exceeding the breeding target of 15 µg g^{-1} such as up to 17.25 µg g^{-1} (Azmach *et al.*, 2013), from 15 to 20 µg g^{-1} (Babu *et al.*, 2013) and more recently, up to 22.6 µg g^{-1} (Menkir *et al.*, 2017) mostly in maize inbred lines. Despite the excellent progress in breeding for higher levels of provitamin A, the current released cultivars contain an average of 6-8 µg g^{-1} of provitamin A (HarvestPlus, 2017; Menkir *et al.*, 2017). The existence of up to 58 genes involved in the biosynthesis of carotenoids in maize (Owens *et al.*, 2014) makes the trait a complex quantitative trait whose inheritance need to be elucidated. The predominance of additive genetic effects and high heritability were reported (Egesel *et al.*, 2003b) and yet, OPVs with high levels of provitamin A are lacking. On the contrary, Halilu *et al.* (2016) found non-additive genetic effects to be predominant for all carotenoids and this finding supports that of Burt *et al.* (2011) who reported that heterosis in carotenoids exist even though rare. This could justify the difficulties in developing hybrids with high levels of provitamin A. The present study aims at adding knowledge to the problem and was designed to:

i. determine the combining abilities of selected orange inbreds and the gene action of accumulation of carotenoids in maize kernels; and
ii. determine the heterosis and heterotic patterns of carotenoids in single crosses.

6.2. Materials and Methods

6.2.1 Plant material

One hundred and thirty-six (136) hybrids from a diallel mating design together with their seventeen parental lines plus three inbred checks and four experimental hybrid checks were planted under optimal growing conditions to produce fresh kernels. Each entry was planted in a single row of one meter (1 m) length with a spacing of 0.2 m between hills, giving a total of five plants per inbred line or per hybrid. The spacing between rows was 0.75 m. All the five plants of each inbred or hybrid in the row were self-pollinated. Each cob was harvested with the husks and kept in the pollination bags to avoid contact with sunlight. The harvested cobs were dried in a glass house for five days. Representative cobs of each genotype were selected, shelled and a sample of hundred kernels was randomly drawn from the bulk to constitute the material for carotenoids analysis. Each sample was packed in a white paper envelop and dispatched for carotenoid analysis at the Institute of Biological Chemistry and Nutritional Science at the University of Hohenheim, Stuttgart, Germany.

6.2.2 Sample preparation

Liquid nitrogen was added to the samples, then ground to a fine powder (≈ 0.5 mm) in a Foss CT 1093 Cyclotec sample mill. A drying test was performed to determine the necessary time for the process. In this regard, eight samples were weighed before and after freeze-drying for 8 h, 12 h, and 24 h to generate a curve and determine the appropriate duration of the drying process. The results indicated 12 h to be the appropriate duration. After drying, the samples were transferred into white plastic bags, sealed, and kept at -80 °C from where they were progressively drawn for carotenoid extraction.

6.2.3. Carotenoid extraction and quantification

The total carotenoids were extracted from each sample following the Reverse-Phase-HPLC method developed by Wald *et al.* (2018). The sample size was modified to 200–210 mg per each repetition. Three repetitions were used for each genotype. The HPLC machine described by the same authors with the same setting were used for the chromatographic separation and detection of different compounds of carotenoids at room temperature.

The injection volumes were also modified to 10 µL for the standards and 30 µL for the samples to enable clear detection of the cis-isomers of β-carotene.

6.2.4 Parameters measured and data analysis process

Quantitative evaluation of standards and samples was performed via peak area using the chromatography software LabSolutions (version 5.71 SP1; Shimadzu Corporation, Kyoto, Japan). A regression line obtained from the eight different concentrations of each carotenoid compound in the standard mixture was used to calculate the carotenoid content in the samples. The concentrations obtained were then transferred to an Excel sheet where the formula below (Equation 6.1) was used to convert the concentrations read by the software to actual concentrations in the samples.

Equation 6.1 $$: CC = \frac{Con.LAB \times V_d \times 100000}{SW} \times \frac{I_{std}}{I_{sample}}$$

Where CC is carotenoid compound concentration (µg per 100g), $Con._{LAB}$ is concentration of carotenoid compound read by the software (µg per L), V_d is the final dilution volume of the extract, SW is dried sample weight (mg), I_{std} is injection volume of the standard mixture, I_{sample} is injection volume of the extract.

The contents of the carotenoid compounds were used to calculate the total carotenoid content (TC, as the sum of the seven compounds) and the provitamin A content (PVA), as given by the formula below (Equation 6.2).

Equation 6.2 $$pVA = \beta C + \frac{1}{2}(\beta CX + \alpha C + 13cis\beta C + 9cis\beta C)$$

Where pVA is provitamin A carotenoids content, βC is beta carotene, βCX is beta cryptoxanthin, αC is alpha carotene, $13cis\beta C$ and $9cis\beta C$ are, the *cis* isomers of beta carotene.

Eight ratios (Venado *et al.*, 2017) described in Table 6.1 were calculated from the compounds and used together for further analysis.

Table 6.1 Derived ratios used to assess inbreds and hybrids in 2018 and their significance

Ratio code	Ratio formula	Significance
R1	β-branch/α-branch	Flux between the carotenoids in the β-branch versus the α-branch
R2	βC/βCX	Conversion of βC into the next product βCX
R3	βC/ (βCX +ZEA)	Conversion of βC into the next two products βCX and ZEA
R4	βCX/ZEA	Conversion of βCX into the next product ZEA
R5	(βC+βCX)/ZEA	Conversion of βC and βCX into ZEA
R6	βC/PVA	Contribution of βC to the concentration of PVA
R7	βCX/PVA	Contribution of βCX to the concentration of PVA
R8	βCX/TC	Contribution of βCX to the total concentration of carotenoids

LUT. = Lutein, ZEA. = Zeaxanthin, βCX = beta cryptoxanthin, 13cis-βC = 13-cis-beta carotene, βC = beta carotene, 9cis-βC = 9-cis-beta carotene.

The R software was used to perform a principal component analysis of the raw data of inbreds to determine their carotenoids profiles. On the other hand, the GENES (Genes, 2016) and the SASHAYDIALL macro (Makumbi *et al.*, 2018) were used to perform Gardner and Eberhart (1966) analysis II and Hayman diallel analysis, respectively. Gardner and Eberhart (1966) analysis II provided information on additive and nonadditive genetic effects and heterosis while Hayman

analysis decomposed total variance into additive and dominance effects of genes, average degree of dominance, proportion of dominance, direction of dominance, distribution of genes, number of groups of genes that control a trait and exhibit dominance, ratio of dominant to recessive alleles in all the parents, and broad-sense (H^2) and narrow-sense (h^2) heritability estimates (Hayman 1954).

6.3 Results

6.3.1 Carotenoids profile of the selected parental lines

Table 6.2 presents the results of the analysis of variance of carotenoid compounds for the inbred lines. There were highly significant differences $p(\alpha < 0.001)$ in all the measured parameters among the inbreds.

Table 6.3 presents the content of carotenoid compounds and different ratio values in the inbreds. The total carotenoid content ranged from 11.19 µg g^{-1} (TZdEEI 7) to 42.12 µg g^{-1} (TZEEIOR 42) with an average of 26.77 µg g^{-1}. On the other hand, the total provitamin A carotenoids content ranged from 0.97 µg g^{-1} (TZEEI 79, yellow inbred check), to 10.53 µg g^{-1} (TZEEIOR 100) with an average of 5.54 µg g^{-1}. The inbred lines in this set showed different levels of various compounds within each of the two branches. However, α-carotene was not found in almost all the 18 inbreds with an average of 0.05 µg g^{-1}. Zeaxanthin was the most abundant carotenoid compound and represented, on average, more than twice the content of lutein (5.94 vs 13.08 µg g^{-1}). Levels of β-cryptoxanthin ranged from 0.27 µg g^{-1} (TZdEEI 7) to 6.97 µg g^{-1} (TZEEIOR 141) whereas those of β-carotene varied from 0.55 µg g^{-1} (TZEEI 79) to 6.93 µg g^{-1} (TZEEIOR 100).

Table 6.2 Mean squares of different compounds of carotenoids in 18 tropical inbreds lines

Source of variation	DF	LUT.	ZEA.	βCX.	13βC	αC.	βC.	αC.	PVA	TC
Rep	2	0.86^{ns}	4.66^{ns}	0.33^{ns}	0.02^{ns}	0.00^{ns}	0.12^{ns}	0.01^{ns}	0.56*	16.90*
Entry	19	20.00***	41.50***	10.88***	0.12***	0.05***	7.78***	0.34***	17.93***	144.15***
Error	38	0.37	1.67	0.12	0.01	0.00	0.04	0.00	0.17	5.10
R-Square		0.96	0.93	0.98	0.92	0.96	0.99	0.98	0.98	0.93
CV, %		10.51	9.97	9.36	14.67	48.54	6.65	7.41	7.42	8.44

'ns' non-significance; "*", '***' significance at $p(\alpha$ <0.05) and $p(\alpha$ <0.001); DF, degree of freedom; LUT, lutein; ZEA, zeaxanthin; βCX., β-cryptoxanthin; 13βC, 13-cis-β-Carotene; α-Carotene; β-Carotene; PVA, pro vitamin A carotenoids; TC; total carotenoids.

Table 6.3 Profile of the 17 parental inbred lines and one checks in carotenoid compounds and derived parameters and ratios

Entry	LUT	ZEA	β-CX	13βC	αC	βC	9βC	PVA	TC	R1	R2	R3	R4	R5	R6	R7	R8
TZEEIOR 12	3.27	11.17	2.21	0.33	0.01	1.98	0.25	3.37	19.21	0.20	0.90	0.15	0.20	0.38	0.59	0.66	0.12
TZEEIOR 42	13.83	18.62	3.87	0.67	0.40	4.07	0.67	6.87	42.12	0.51	1.05	0.18	0.21	0.43	0.59	0.56	0.09
TZEEIOR 53	5.00	12.08	2.54	0.47	0.00	2.44	0.68	4.28	23.21	0.28	0.96	0.17	0.21	0.41	0.57	0.59	0.11
TZEEIOR 76	4.54	13.66	2.29	0.58	0.00	2.58	1.03	4.53	24.68	0.23	1.13	0.16	0.17	0.36	0.57	0.51	0.09
TZEEIOR 100	2.86	13.40	5.30	0.76	0.00	6.93	1.14	10.53	30.38	0.10	1.31	0.37	0.40	0.91	0.66	0.50	0.17
TZEEIOR 113	4.47	13.68	2.69	0.37	0.00	1.99	0.61	3.83	23.82	0.23	0.74	0.12	0.20	0.34	0.52	0.70	0.11
TZEEIOR 130	6.40	10.51	1.66	0.38	0.00	2.15	0.59	3.47	21.69	0.42	1.30	0.18	0.16	0.36	0.62	0.48	0.08
TZEEIOR 141	6.18	16.52	6.97	0.78	0.04	3.43	1.19	7.92	35.10	0.22	0.49	0.15	0.42	0.63	0.43	0.88	0.20
TZEEIOR 145	8.28	19.02	4.43	0.40	0.13	2.19	0.66	5.00	35.10	0.32	0.49	0.09	0.24	0.35	0.44	0.89	0.13
TZEEIOR 161	7.82	14.05	2.17	0.28	0.00	1.89	1.02	3.63	27.23	0.40	0.87	0.12	0.16	0.29	0.52	0.60	0.08
TZEEIOR 196	7.13	16.69	3.03	0.41	0.00	3.00	0.81	5.12	31.06	0.30	0.99	0.15	0.18	0.36	0.59	0.59	0.10
TZEEIOR 218	5.40	12.59	5.18	0.49	0.21	2.55	0.49	5.74	26.91	0.26	0.49	0.14	0.41	0.61	0.45	0.90	0.19
TZEEIOR 219	4.92	13.98	4.97	0.51	0.08	2.73	0.49	5.75	27.67	0.22	0.55	0.14	0.36	0.55	0.47	0.86	0.18
TZEEIOR 222	4.41	13.45	6.03	0.57	0.00	3.43	0.79	7.13	28.69	0.18	0.57	0.18	0.45	0.71	0.48	0.85	0.21
TZEEIOR 223	4.51	14.77	6.63	0.67	0.00	3.50	0.82	7.56	30.89	0.17	0.53	0.16	0.45	0.69	0.46	0.88	0.22
TZdEEI 7	4.08	4.13	0.27	0.48	0.00	1.62	0.61	2.30	11.19	0.57	6.02	0.37	0.07	0.46	0.70	0.12	0.02
TZdEEI 12	4.70	11.42	2.98	0.57	0.00	2.63	0.89	4.84	23.18	0.25	0.88	0.18	0.26	0.49	0.54	0.62	0.13
TZEEI 79	9.08	5.69	0.65	0.07	0.00	0.55	0.14	0.97	16.17	1.28	0.84	0.09	0.11	0.21	0.56	0.66	0.04
Mean	5.94	13.08	3.55	0.49	0.05	2.76	0.72	5.16	26.57								
Minimum	2.86	4.13	0.27	0.07	0.00	0.55	0.14	0.97	11.19								
Maximum	13.83	19.02	6.97	0.78	0.39	6.93	1.19	10.53	42.12								
S.E.M	0.33	0.49	0.24	0.03	0.01	0.21	0.05	0.32	0.91								

"*", "**", "***", = significance at p(α <0.05), p(α <0.01), and p(α <0.001) respectively; "ns" = non-significant. LUT. = Lutein, ZEA. = Zeaxanthin, βCX = beta cryptoxanthin, 13cis-βC = 13-cis-beta carotene, βC = beta carotene, 9cis-βC = 9-cis-beta carotene; S.E.M, standard error of mean. R1 = Flux between the carotenoids in the β-branch versus the α-branch, R2 = Conversion of βC into the next product βCX, R3 = Conversion of βC into the next two products βCX and ZEA, R4 = Conversion of βCX into the next product ZEA, R5 = Conversion of βC and βCX into ZEA, R6 = Contribution of βC to the concentration of PVA, R7 = Contribution of βCX to the concentration of PVA, R8 = Contribution of βCX to the total concentration of carotenoids.

Figure 6.1 describes the inbred lines regarding the content of carotenoid compounds and the importance of different ratios in determining the variation observed among the lines. The principal component (PCA) analysis biplot indicated that lutein and α-carotene contributed less to the 72.1% variation explained by the principal components 1 and 2, from the total variation among the inbreds. β-cryptoxanthin and total provitamin A carotenoids contributed most to the variation explained by PCA1 while lutein content and the conversion of β-carotene to β-cryptoxanthin and zeaxanthin were most determinant of the PCA2. Inbreds TZEEIOR 141, TZEEIOR 222, and TZEEIOR 223 were characterized by high levels of β-cryptoxanthin, high contribution of β-cryptoxanthin to total provitamin carotenoids, and low conversion of β-cryptoxanthin to zeaxanthin. On the other hand, inbreds TZEEIOR 42, TZEEIOR 145, TZEEIOR 218, and TZEEIOR 219 were characterized by high levels of total carotenoids and zeaxanthin. TZEEIOR 42 had, in addition, the highest level of lutein with 13.83 μg g^{-1}.

Figure 6.2 presents the inbred lines in the different groups based on their profiles. Three different groups were suggested by Elbow method. Inbred TZEEI 79 and TZdEEI 7, characterised by low levels in all carotenoid compounds in the β-branch of the carotenoid biosynthetic pathway, were grouped together in Group I. The inbreds TZEEIOR 42, TZEEIOR 100, TZEEIOR 141, TZEEIOR 145, TZEEIOR 218, TZEEIOR 219, TZEEIOR 222, and TZEEIOR 223 constituted the Group II. This group was composed of the inbreds with high total carotenoids with moderate to high levels of carotenoids in the β-branch. This group was also characterised by equal contribution of β-cryptoxanthin and β-carotene to the total provitamin A carotenoids level. The remaining inbreds were clustered together in Group III and were characterized by low to moderate levels of total carotenoids and variable content of carotenoids in the β-branch.

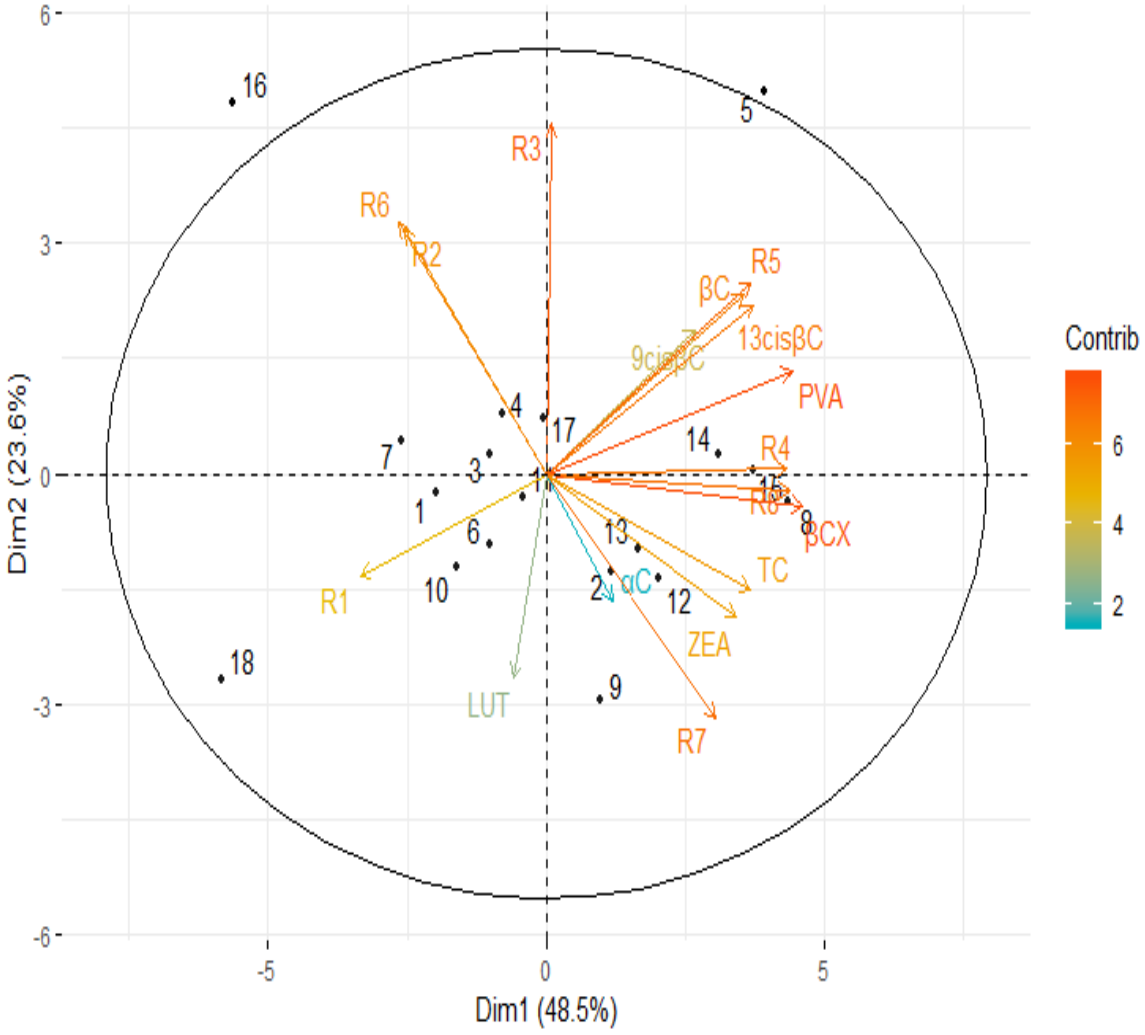


Figure 6.1 Carotenoids profile of 18 maize inbred lines using Principal Components Analysis (PCA) biplot

Legend

Code	Name	Code	Name
1	TZEEIOR 12	10	TZEEIOR 161
2	TZEEIOR 42	11	TZEEIOR 196
3	TZEEIOR 53	12	TZEEIOR 218
4	TZEEIOR 76	13	TZEEIOR 219
5	TZEEIOR 100	14	TZEEIOR 222
6	TZEEIOR 113	15	TZEEIOR 223
7	TZEEIOR 130	16	TZdEEI 7
8	TZEEIOR 141	17	TZdEEI 12
9	TZEEIOR 145	18	TZEEI 79

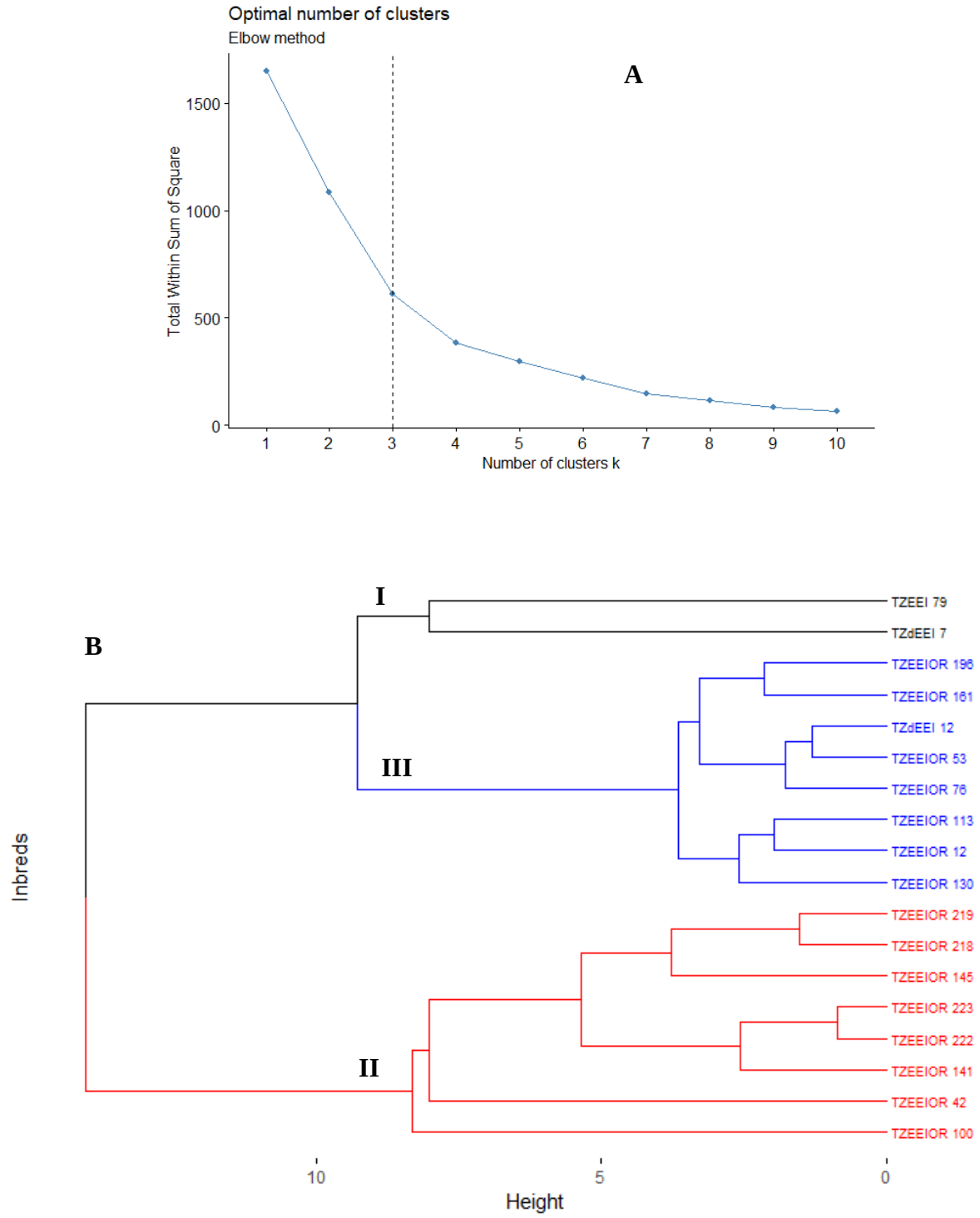


Figure 6.2 Optimal number of clusters suggested by Elbow method (A) and carotenoids profile-based grouping of 18 maize inbred lines (B)

6.3.2 Heterosis for carotenoid accumulation in maize kernels

Table 6.4 presents the results of Gardner & Eberhart analysis II and Hayman analysis of diallel of carotenoid traits among extra-early maize inbreds. There were highly significant ($p < 0.001$) differences among inbreds effects and effects of their derived crosses for all the carotenoid compounds, total provitamin A, and total carotenoids content. Average heterosis was significant ($p < 0.001$) for lutein and zeaxanthin, on one hand, and highly significant ($p < 0.001$) for beta-carotene and its cis isomers, on the other hand. The contribution of heterosis by each inbred to its crosses was also highly significant for all the measured parameters except for lutein while the specific heterotic effect in each cross was highly significant for all parameters.

Table 6.5 presents the summary statistics for the mid-parent heterosis, the better parent heterosis, and the worst parent heterosis. There were small and positive heterotic effects for all the traits for heterosis and heterobeltiosis. The average heterotic effects were 35.98%, 5.68%, 2.78%, 1.3%, 0.86%, and 0.09% for 9-cis-β-carotene, 13-cis-β-carotene, β-cryptoxanthin, PVA, zeaxanthin, and total carotenoids, respectively.

Table 6.6 presents the heterosis and the heterobeltiosis of β-cryptoxanthin for the 136 single crosses. The highest heterosis effect for β-cryptoxanthin was obtained for TZEEIOR 113 x TZEEIOR 130 with value of 67.4%. The highest heterosis obtained among high-level parents for β-cryptoxanthin was 20.37% between TZEEIOR 218 and TZEEIOR 219. The Inbred TZEEIOR 113 showed a consistent positive and significant heterosis effect in all the crosses except its crosses with TZEEIOR 141 and TZEEIOR 223. A similar trend was observed for the better parent heterosis with the highest being 49.69% between TZEEIOR 53 and TZEEIOR 113. The cross TZEEIOR 100 x TZEEIOR 218 exhibited 24.75% heterobeltiosis, the highest among high-level parents for β-cryptoxanthin.

Table 6.4 Analysis of variance for carotenoid compounds, provitamin A (PVA), and total carotenoids (TC) of selected extra-early maturing orange maize

Source of variation	DF	Gardner & Eberhart (1966) mean squares of Carotenoids compounds and derived parameters							
		LUT.	ZEA.	βCX	13βC	βC	9βC	PVA	TC
Replication	2	0.02^{ns}	2.28^{*}	0.15^{ns}	0.00^{ns}	0.09^{ns}	0.01^{ns}	0.29^{ns}	5.06^{ns}
Genotype	152	11.4^{***}	22.25^{***}	6.24^{***}	0.06^{***}	1.82^{***}	0.16^{***}	6.31^{***}	95.53^{***}
Inbreds	16	72.2^{***}	166.82^{***}	50.67^{***}	0.34^{***}	13.44^{***}	0.68^{***}	49.45^{***}	722.43^{***}
Hybrids	136	4.2^{***}	5.24^{***}	1.01^{***}	0.03^{***}	0.46^{***}	0.10^{***}	1.24^{***}	21.77^{***}
H.Mean	1	3.8^{**}	0.29^{ns}	0.15^{ns}	0.02^{**}	1.12^{***}	2.49^{***}	0.001^{ns}	0.16^{ns}
H.Inbreds	16	4.8	9.41^{***}	1.98^{***}	0.05^{***}	0.87^{***}	0.16^{***}	2.67^{***}	39.26^{***}
H.Specific	119	4.1^{***}	4.72^{***}	0.88^{***}	0.03^{***}	0.40^{***}	0.07^{***}	1.06^{***}	19.60^{***}
Error	304	0.4	0.66	0.1	0.002	0.04	0.01	0.14	2.79
		Hayman (1954) mean squares of Carotenoids compounds and derived parameters							
Replication	2	0.02^{ns}	2.28^{*}	0.15^{ns}	0.00^{ns}	0.09^{ns}	0.01^{ns}	0.29^{ns}	5.06^{ns}
Genotype	152	11.36^{***}	22.25^{***}	6.24^{***}	0.06^{***}	1.82^{***}	0.16^{***}	6.31^{***}	95.53^{***}
a	16	30.51^{***}	32.17^{***}	8.61^{***}	0.14^{***}	4.05^{***}	0.36^{***}	11.76^{***}	179.36^{***}
b	136	9.10^{***}	21.08^{***}	5.96^{***}	0.05^{***}	1.56^{***}	0.14^{***}	5.67^{***}	85.66^{***}
b1	1	1.14^{ns}	2.03^{*}	8.08^{**}	0.01^{ns}	0.24^{*}	0.02^{ns}	4.42^{*}	16.62^{*}
b2	16	3.75^{***}	22.66^{***}	7.34^{***}	0.09^{***}	2.51^{***}	0.22^{***}	9.41^{***}	104.28^{***}
b3	119	9.89^{***}	21.03^{***}	5.75^{***}	0.05^{***}	1.45^{***}	0.13^{***}	5.18^{***}	83.74^{***}
Residual	304	0.36	0.66	0.1	0.0	0.04	0.01	0.14	2.79
a x Rep	32	0.38	0.8	0.13	0	0.06	0.01	0.21	3.63
b1 x Rep	2	0.17	0.03	0.01	0	0.01	0	0.03	0.13
b2 x Rep	32	0.39	0.43	0.05	0	0.03	0.01	0.11	2.29
b3 x Rep	238	0.35	0.68	0.1	0	0.04	0.01	0.14	2.77
b x Rep	272	0.35	0.64	0.09	0	0.04	0.01	0.14	2.69
Total x Rep	304	0.36	0.66	0.1	0	0.04	0.01	0.14	2.79
Mean		5.5	13.6	3.8	0.53	2.7	1	5.4	27.1
CV %		10.9	6	8.3	8.6	7.6	10.4	7	6.2

"**", "***", = significance at p(α =0.01), and p(α =0.001) respectively; "ns" = non-significant. LUT. = Lutein, ZEA. = Zeaxanthin, βCX = beta cryptoxanthin, 13cis-βC = 13-cis-beta carotene, βC = beta carotene, 9cis-βC = 9-cis-beta carotene. a, additive effect; b, dominance effect; b_1, measure of directional dominance; b_2, measure of ambi-directional dominance; b_3, residual dominance.

Table 6.5 Minimum, maximum, and average heterosis (H) and heterobeltiosis (Hb) for carotenoids compounds, provitamin A (PVA), and total carotenoids (TC) of selected extra-early maturing orange maize

Trait	H%			Hb(>P) %			Hb(<P) %		
	Min.	Max.	Avr.	Min.	Max.	Avr.	Min.	Max.	Avr.
Lutein	-66.16	52.05	-3.02	-70.36	39.40	-17.49	-64.35	116.57	24.71
Zeaxanthin	-36.17	27.80	0.86	-53.05	24.71	-10.67	-24.87	168.42	24.44
β-Cryptoxanthin	-93.10	67.40	2.78	-95.46	49.69	-18.77	-85.67	978.63	105.81
13cis-β-Carotene	-68.72	84.19	5.68	-71.72	71.54	-8.69	-65.05	178.57	29.31
β-Carotene	-45.48	84.04	-3.64	-63.17	67.40	-17.26	-25.78	121.08	21.53
9cic-β-Carotene	-53.50	175.08	35.98	-71.91	106.80	15.00	-13.93	312.12	78.17
PVA	-46.01	74.83	1.30	-61.50	65.70	-14.85	-19.81	142.67	31.95
TC	-35.53	35.02	0.09	-66.16	52.05	-3.02	-26.21	132.33	20.78

Hb(>P) = heterobeltiosis higher parent; Hb(<P) = heterobeltiosis lower parent; Min. = minimum; Max. = maximum; Avr. = average.

Table 6.7 presents the heterosis and the heterobeltiosis of β-carotene for the 136 single crosses. The highest heterobeltiosis, 67.4%, was observed in the cross involving TZEEIOR 53 and TZEEIOR 113. The same parents showed the highest heterosis in their cross with the effect estimated at 84.04%.

Table 6.6 Estimates of heterobeltiosis (upper triangle) and heterosis (lower triangle) effects (%) for β-cryptoxanthin of 136 F_1's derived from half diallel crosses among 17 extra-early maturing orange maize inbreds

	Inbred	Heterotic effect expressed in percentage (%)																
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
1	TZEEIOR 12		-2.93	-2.49	22.85*	-28.46**	24.10*	-56.11**	-95.46**	-38.71**	-20.36	-20.71*	-22.26**	-6.04	-35.86**	-36.37**	-47.66**	9.63
2	TZEEIOR 42	23.58**		-9.04	-6.55	4.79	9.30	-14.39*	-25.78**	-13.63*	-2.67	-1.55	31.72**	3.29	-1.66	-12.43**	-50.04**	18.00**
3	TZEEIOR 53	4.35	9.77		13.90	-24.37**	49.69**	4.98	-39.65**	-23.87**	17.69	12.22	-20.53**	-31.81**	-36.19**	-39.03**	-42.20**	-15.01
4	TZEEIOR 76	25.04**	17.43*	19.86*		-17.95**	33.37**	7.13	-31.71**	-22.89**	15.14	-25.99**	-33.14**	-24.16**	-35.64**	-41.85**	-39.88**	-5.49
5	TZEEIOR 100	0.93	21.06**	2.17	14.55*		-0.76	-28.40**	-0.43	6.23	-22.23**	-26.26**	24.75**	10.83*	-11.71**	-8.05	-59.76**	-24.24**
6	TZEEIOR 113	36.42**	28.83**	54.07**	44.25**	31.50**		35.23**	-30.56**	-6.10	12.24	29.40**	9.52	-6.98	-9.56*	-28.22**	-32.26**	34.71**
7	TZEEIOR 130	-49.87**	19.83*	27.05**	24.22*	9.01	67.40**		-66.86**	-59.34**	3.68	-15.86	-33.46**	-41.68**	-41.11**	-48.59**	-51.81**	-20.49*
8	TZEEIOR 141	-93.10**	-4.55	-11.56*	2.81	13.18**	0.14	-46.47		-31.80**	-36.92**	-37.97**	-18.99**	-26.45**	-20.37**	-24.10**	-58.73**	-35.29**
9	TZEEIOR 145	-18.23**	-7.83	-3.30	1.63	15.71**	16.71**	-40.86	-16.58**		-43.37**	-19.13**	-12.10*	-16.58**	-29.67**	-33.75**	-60.92**	-14.68*
10	TZEEIOR 161	-19.70**	24.66**	26.93**	18.15	10.27	24.30**	17.57	-3.83	-24.04**		-17.84*	-15.31**	-34.36**	-43.54**	-43.66**	-41.26**	-12.65
11	TZEEIOR 196	-8.34	10.49	21.96**	-15.74	-6.17	36.87**	8.68	-13.51**	-3.94	-4.36		-13.06**	-19.93**	-35.03**	-36.97**	-56.28**	17.40*
12	TZEEIOR 218	8.98	50.79**	6.60	-7.27	26.10**	44.05**	0.78	-7.05	-5.21	19.31**	9.75		17.89**	6.46	0.20	-48.52**	-7.92
13	TZEEIOR 219	30.05**	16.11**	-9.81	3.81	14.36**	20.57**	-12.57	-14.10**	-11.78*	-8.68	-0.50	20.37**		1.55	-6.99	-57.92**	-1.28
14	TZEEIOR 222	-6.10	19.83**	-10.22*	-6.69	-5.94	25.01**	-7.62	-14.64**	-18.87**	-16.98**	-13.47**	14.56**	11.39**		-6.39	-68.67**	-29.61**
15	TZEEIOR 223	-4.57	10.57*	-11.89*	-13.57**	2.24	2.04	-17.78**	-22.19**	-20.57**	-15.15**	-13.47**	12.48**	6.32	-2.00		-66.90**	-35.06**
16	TZdEEI 7	-6.60	-6.53	4.63	7.69	-23.38**	23.28**	-16.96	-20.50**	-26.28**	4.65	-19.64	-2.08	-20.13*	-40.00**	-36.37**		-42.78**
17	TZdEEI 12	25.83**	33.40**	-8.33	6.83	-3.02	41.36**	2.08	-9.32*	2.03	0.97	18.38*	16.96**	23.46**	-5.73	-10.38*	5.03	

"*", "**" = significance at p(α <0.05) and p(α <0.01), respectively.

Table 6.7 Estimates of heterobeltiosis (upper triangle) and heterosis (lower triangle) effects for β-carotene of 136 F_1's derived from half diallel crosses among 17 extra-early maturing orange maize inbreds

Inbred		Heterotic effect expressed in percentage (%)																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	TZEEIOR 12		-24.80**	-5.48	-9.55	-49.23**	26.26**	42.64**	-26.75**	2.74	-26.10**	-34.19**	1.70	6.23	-24.66**	-14.38**	-15.82	-5.08
2	TZEEIOR 42	1.21		-29.87**	-24.39**	-20.77**	-21.36**	-6.46	-27.41**	-29.46**	-14.73**	9.49	-4.42	-24.39**	-9.66*	-10.72*	-13.34*	-16.04**
3	TZEEIOR 53	4.23	-12.19**		13.94*	-63.17**	67.40**	25.34**	-18.68**	-6.85	-4.93	2.79	-4.96	-9.90	-18.64**	-17.91**	-31.09**	-18.04**
4	TZEEIOR 76	2.41	-7.46	17.34**		-44.90**	2.19	5.29	-22.28**	-27.35**	-8.90	-17.59*	-1.94	-17.60**	-20.87**	-8.00	-13.29*	-12.32
5	TZEEIOR 100	-21.02**	-0.18	-45.48**	-19.72**		-36.44**	-46.88**	-45.62**	-43.61**	-46.92**	-36.01**	-37.31**	-39.95**	-51.92**	-39.09**	-54.61**	-46.25**
6	TZEEIOR 113	26.68**	5.60	84.04**	15.37*	-1.27		-1.24	-28.41**	-11.26	7.02	9.02	3.13	-11.74	-17.57**	-22.29**	6.52	5.97
7	TZEEIOR 130	48.51**	22.44**	33.09**	14.93*	-18.90**	2.50		-39.88**	-7.31	-0.62	-5.46	-6.27	-17.12**	-20.48**	-21.43**	-20.78**	-9.27
8	TZEEIOR 141	-7.15	-21.15**	-4.89	-11.37*	-27.22**	-9.47	-26.12**		-29.77**	-23.25**	-33.56**	-40.37**	-24.81**	-25.92**	-23.14**	-38.81**	-39.10**
9	TZEEIOR 145	7.91	-8.25	-1.95	-21.36**	-14.29**	-7.09	-6.45	-14.30**		-25.11**	-22.49**	-9.01	-20.42**	-40.68**	-37.05**	-28.46**	-29.99**
10	TZEEIOR 161	-24.38**	16.49**	7.02	5.21	-16.58**	9.87	5.78	-1.06	-19.61**		-37.86**	-7.57	-23.35**	-44.17	-33.81**	-8.47	-25.16**
11	TZEEIOR 196	-20.78**	26.23**	13.39*	-11.53*	-10.61**	30.88**	10.05	-29.07**	-10.48	-23.82**		-2.45	-17.48**	-20.48**	-14.09**	-41.31**	-3.01
12	TZEEIOR 218	14.56*	17.51**	-2.67	-1.36	-8.36	15.84*	1.77	-31.66**	-2.04	6.23	5.29		11.86	1.26	3.05	-11.62	7.62
13	TZEEIOR 219	23.09**	-9.41*	-4.78	-15.38**	-13.80**	1.98	-7.31	-16.25**	-11.73*	-9.46	-13.64**	15.53**		-14.08**	-11.71*	-33.62**	8.44
14	TZEEIOR 222	-4.43	-1.95	-4.77	-9.69	-35.69**	4.30	-2.21	-25.85**	-27.56**	-27.99**	-15.04**	16.15**	-4.22		-10.57*	-32.91**	-20.39**
15	TZEEIOR 223	9.37	-3.96	-3.15	5.86	-19.04**	-0.97	-2.65	-22.33**	-22.56**	-14.04*	-7.39	19.16**	-0.75	-9.71*		-32.95**	-19.14**
16	TZdEEI 7	-7.41	24.01**	-17.27*	6.59	-26.42**	17.52*	-9.64	-16.91**	-17.76*	-1.42	-23.84**	8.15	-16.72*	-8.84	-8.33		-15.25*
17	TZdEEI 12	8.19	2.14	-14.96*	-11.65*	-22.01**	20.43**	-0.28	-31.02**	-23.68**	-13.00*	3.38	9.08	10.53	-9.74*	-7.57	4.79	

"*", "**" = significance at p(α <0.05) and p(α <0.01), respectively.

6.3.3 Gene action and inheritance of carotenoids in 17 orange maize inbred lines

Table 6.8 presents the genetic parameter estimates from the Hayman diallel analysis for carotenoid traits. The ratio of the total number of dominant to recessive genes, estimated by $\widehat{K_D}/\widehat{K_R}$ indicated that there were more dominant than recessive genes for all the traits in all the parents. For β-carotene this ratio was 2.36. On the other hand, the component of variation due to dominance effects of genes was found to be greater than the dominance component indicating asymmetry of positive and negative effects of genes for all the traits ($\widehat{H}1 > \widehat{H}2$). Also, there was not equal distribution of alleles with positive and negative effects in the parents ($\widehat{H}2/4\widehat{H}1 < 0.5$) at loci exhibiting dominance. The proportion of genes with positive and negative effects was less than 0.2 for all the traits in this set of inbreds.

The results showed that two genes or block of genes which exhibited total dominance for 9-cis-β-carotene were present in the parents ($\hat{h}2/\widehat{H}2 = 1.88$). For the rest of the measured traits, there were no genes or blocks of genes with complete dominance, at the loci exhibiting dominance.

Table 6.9 presents the general combining ability estimates of inbred parents for carotenoid compounds. Expected small GCA effects were observed for all measured traits but differences among inbreds were highly significant. Only the inbred TZEEIOR 42 expressed positive and highly significant effect for all the traits. The inbred TZEEIOR 100 showed a positive and significant effect for carotenoids in the β-branch. The highest additive effect for β-cryptoxanthin was observed for TZEEIOR 218, TZEEIOR 223, and TZEEIOR 100 with 1.18, 1.09, and 1.06 $\mu g\, g^{-1}$, respectively. For β-carotene, the highest effect was found in TZEEIOR 100 with 1.40 $\mu g\, g^{-1}$, followed by TZEEIOR 42 with a far lower effect, 0.8 $\mu g\, g^{-1}$.

Table 6.8 Genetic parameter estimates for carotenoid compounds, provitamin A carotenoids (PVA), and total carotenoids (TC) of 17 selected extra-early maturing orange maize inbreds and their 136 hybrids

Parameter	PVA	TC	Carotenoid compounds					
			LUT	ZEA.	βCX	13βC	βC	9βC
$\hat{D}$	4.22 ± 0.16	48.38 ± 2.59	6.42 ± 0.57	11.50 ± 0.73	3.55 ± 0.26	0.02 ± 0.0	1.52 ± 0.09	0.06 ± 0.01
$\hat{H}_1$	2.21 ± 0.28	36.52 ± 4.61	6.48 ± 1.01	8.78 ± 1.3	1.77 ± 0.46	0.05 ± 0.01	0.79 ± 0.16	0.15 ± 0.02
$\hat{H}_2$	1.37 ± 0.22	24.44 ± 3.66	5.02 ± 0.8	5.89 ± 1.03	1.14 ± 0.36	0.03 ± 0.01	0.52 ± 0.12	0.10 ± 0.01
$\hat{h}_2$	0.00 ± 0.15	0.00 ± 2.44	0.27 ± 0.54	0.00 ± 0.69	0.00 ± 0.24	0.00 ± 0.0	0.08 ± 0.08	0.19 ± 0.01
$\hat{E}$	0.05 ± 0.04	0.94 ± 0.61	0.12 ± 0.13	0.22 ± 0.17	0.03 ± 0.06	0.00 ± 0.0	0.01 ± 0.02	0.00 ± 0.0
$\hat{F}$	1.59 ± 0.34	8.35 ± 5.69	2.85 ± 1.25	2.40 ± 1.61	0.56 ± 0.56	0.01 ± 0.01	0.89 ± 0.19	0.06 ± 0.02
$\sqrt{\hat{H}1/\hat{D}}$	0.72	0.87	1	0.87	0.7	1.51	0.72	1.61
$\hat{H}2/4\hat{H}1$	0.16	0.17	0.19	0.17	0.16	0.17	0.16	0.17
$\widehat{K_D}/\widehat{K_R}$	1.71	1.22	1.57	1.27	1.25	1.37	2.36	1.84
$\hat{h}2/\hat{H}2$	0	0	0.05	0	0	0.05	0.16	1.88
r	0.69	056	0.86	0.35	0.94	-0.24	0.65	-0.15
H_n^2	0.82	0.79	0.65	0.78	0.85	0.58	0.76	0.47
H_b^2	0.98	0.97	0.97	0.97	0.98	0.97	0.98	0.94

$\hat{D}$, component of variation due to additive effect of genes; $\hat{H}_1$, component of variation due to dominance effects of genes; $\hat{H}_2$, dominance component indicating asymmetry of positive and negative effects of genes; $\hat{h}_2$, overall mean dominance effect of heterozygous loci; $\hat{F}$, relative frequency of dominant and recessive alleles in the parents; $\hat{E}$, replicate variation. $\sqrt{\hat{H}1/\hat{D}}$ = Mean Degree of Dominance, $\hat{H}2/4\hat{H}1$ = Proportion of genes with positive and negative effects in the parents, $\widehat{K_D}/\widehat{K_R}$ = Proportion of dominant & recessive genes in parents, $\hat{h}2/\hat{H}2$ = Number of groups of genes which control the character and exhibit dominance, H_n^2 = Narrow-sense Heritability, H_b^2 = Broad-sense Heritability. LUT. = Lutein, ZEA. = Zeaxanthin, βCX = beta cryptoxanthin, 13cis-βC = 13-cis-beta carotene, βC = beta carotene, 9cis-βC = 9-cis-beta carotene.

Table 6.9 General combining ability (GCA) estimates of selected extra-early maturing orange maize for different carotenoid compounds and derived traits

Inbred	GCA effect								
	LUT	ZEA	βCX	13βC	αC	βC	9βC	PVA	TC
TZEEIOR 12	-0.84***	-1.42***	-0.94***	-0.13***	-0.03***	-0.28***	-0.23***	-0.94***	-3.87***
TZEEIOR 42	3.40***	3.18***	0.56***	0.18***	0.11***	0.80***	0.16***	1.30***	8.38***
TZEEIOR 53	0.40***	-0.19^{ns}	-0.52***	0.07***	-0.02***	-0.11**	0.03*	-0.32***	-0.32^{ns}
TZEEIOR 76	-1.40***	-0.40*	-0.60***	-0.03***	-0.04***	-0.11***	0.06***	-0.42***	-2.52***
TZEEIOR 100	-1.10***	1.53***	1.06***	0.12***	-0.03***	1.40***	0.24***	2.10***	3.23***
TZEEIOR 113	-0.24*	0.53***	0.21***	0.01*	0.00*	-0.06*	0.00^{ns}	0.05^{ns}	0.45^{ns}
TZEEIOR 130	0.70***	-0.76***	-1.17***	-0.03**	-0.02***	-0.18***	0.04**	-0.77***	-1.42***
TZEEIOR 141	-0.32**	0.83***	0.90***	0.03***	-0.03***	-0.11**	-0.04*	0.32***	1.26***
TZEEIOR 145	0.15^{ns}	0.76***	-0.02^{ns}	-0.06***	0.00*	-0.50***	-0.08***	-0.58***	0.26^{ns}
TZEEIOR 161	0.49***	-0.05^{ns}	-0.78***	-0.09***	0.02***	-0.51***	0.05***	-0.91***	-0.87**
TZEEIOR 196	1.19***	0.86***	-0.41***	-0.05***	-0.03***	0.07*	0.02^{ns}	-0.17**	1.65***
TZEEIOR 218	0.48***	-0.13^{ns}	1.18***	0.01^{ns}	0.10***	0.07*	-0.06**	0.68***	1.65***
TZEEIOR 219	-0.15^{ns}	0.65***	0.75***	-0.02*	0.02***	-0.07*	-0.10***	0.26***	1.09***
TZEEIOR 222	-0.32***	0.07^{ns}	0.95***	0.01^{ns}	-0.02***	0.10**	-0.01^{ns}	0.56***	0.78*
TZEEIOR 223	-0.69***	0.71***	1.09***	0.05***	-0.03***	0.27***	0.01^{ns}	0.83***	1.41***
TZdEEI 7	-0.95***	-5.25***	-2.05***	-0.06***	0.00*	-0.64***	-0.15***	-1.77***	-9.09***
TZdEEI 12	-0.79***	-0.92***	-0.21***	-0.01^{ns}	-0.03***	-0.14***	0.03*	-0.25***	-2.06***
Baker ratio	0.66	0.79	0.85	0.57	0.54	0.77	0.44	0.83	0.8

"*", "**", "***", = significance at p(α <0.05), p(α <0.01), and p(α <0.001) respectively; "ns" = non-significant. LUT. = Lutein, ZEA. = Zeaxanthin, βCX = beta cryptoxanthin, 13cis-βC = 13-cis-beta carotene, βC = beta carotene, 9cis-βC = 9-cis-beta carotene.

6.4 Discussion and conclusion

The observed range of levels for all the carotenoid compounds among the orange maize inbred lines in this study are within the ranges reported by previous studies involving yellow or orange endosperm colour maize (Egesel *et al.*, 2003b; Menkir *et al.*, 2008). These inbreds are characterised by significantly improved levels of all the β-branch carotenoids with zeaxanthin and β-cryptoxanthin being the most abundant. This is in line with the method used for their development. Indeed, these inbreds were developed from a converted yellow population to orange using only visual scores of the grain colour for carotenoids improvement. Egesel *et al.* (2003b) found zeaxanthin to be a major pigment in endosperm colour. Thus, the results of this study support the idea that grain colour can be used as a secondary trait for indirect selection for zeaxanthin and β-cryptoxanthin (Chandler *et al.*, 2013). The moderate levels of β-carotene in this set of inbreds suggest that there was not much improvement of the trait during the selection process. Moreover, the orange colour source, STR-34-1-1-1-1-2-1-B*5/NC354/SYN-Y-STR-34-1-1-1-1-2-1-B*5 (OR1), used to convert 2004 TZEE-Y STR C4 to an orange population from which the inbreds were derived was, later on, discovered to carry none of the functional alleles of the *crtRB1* gene (Azmach *et al.*, 2013). In a different study that involved the inbred parents in this research, it was observed that only TZEEIOR 196 and TZdEEI 7 contained the favourable allele at the 3'TE of *lycE* loci while all of the other inbreds contained the unfavourable allele at 5'TE of *crtRB1* loci. It is clear that the 3'TE functional allele found in TZEEIOR 196 and TZdEEI 7 is not coming from the donor (OR1). The presence of this functional allele in these genotypes is a validation of the finding of Yan *et al.* (2010), who reported 3'TE favourable allele to be present in tropical germplasm at 4.6%. Even though TZEEIOR 196 possesses the 3'TE functional allele, which has proved to be correlated with increased levels of β-carotene, its level in β-carotene is three times

lower than that of TZEEIOR 100 and two times lower than that of TZEEIOR 42. Discovering genotypes with no known functional allele of the most used major genes in marker-assisted breeding (PSY1, *lcyE*, and *crtRB1*) having good levels of carotenoids is not a surprising event. Indeed, Azmach (2013) identified a line (entry 50) having unfavourable alleles at *lcyE SNP, crtRB1* 5'TE, *crtRB1* InDel4, and *crtRB*1 3'TE (T, 1, 0, 3), which exceeded by 23.48 $\mu g\, g^{-1}$ that of the average total carotenoid of those genotypes carrying the favourable alleles. The existence of up to 58 genes involved in the carotenoids biosynthesis (Owens *et al.*, 2014b) may explain the performance of some genotypes whose genome has been explored for the well-known candidate genes used up to date. Beside, Yan *et al.* (2010) found the most pronounced effect leading to higher βC concentrations could be attributed to the 206-bp insertion allele of 5'TE, a rare allele detected only in temperate germplasm at a frequency of 2.9% , although additive genetic effects were also reported for combinations of favourable alleles at 5'TE, InDel4, and 3'TE and for combinations of favourable alleles of *crtRB1* and *lcyE* genes. Thus, the presence of only 3'TE favourable alleles in TZEEIOR 196 and TZdEEI 7 was not enough to result in higher increase of β-carotene.

The variability discovered, even in this small set of inbred lines, indicates the potential to select parental lines for genetic improvement of the compounds for which they perform.

Principal component biplot on the carotenoid composition of the inbred lines revealed that some inbred lines had higher concentrations of provitamin A carotenoids, as well as the xanthophylls (lutein, and zeaxanthin). The inbred TZEEIOR 42 can be used as a parent for improvement of both lutein and β-branch provitamin A carotenoids while all the inbreds in group II can be used purposely for all β-branch carotenoids. But, the fact that most of the inbreds in this study did not possess favourable alleles of the known candidate genes despite their performance makes them the best candidates for gene introgression.

Both additive and non-additive genetic variances were observed to be significantly different from zero revealing the complexity in the inheritance of the studied traits. The values of Baker's ratio (>0.5) indicated the predominance of additive genetic variance for all the traits except for 9-cis-β-carotene (0.44). However, the predictability of selecting superior progeny based on estimates of GCA alone can only be possible for β-cryptoxanthin for which Baker's ratio was close to unity. As the levels of PVA in this set of inbreds were mainly determined by their levels in β-cryptoxanthin, selecting superior progeny for PVA is also found to be predictable based on estimates of GCA alone. High narrow sense heritabilities were also observed for the majority of the traits. This report on the predominance of additive genetic effects and high heritability, as also reported in earlier studies (Egesel *et al.*, 2003a; Halilu *et al.*, 2016; Menkir *et al.*, 2014), suggests that progress in breeding maize for increased carotenoid content especially, provitamin A carotenoids to be achievable. But, from the discovery of favourable alleles of *lcyE* (Harjes *et al.*, 2008) and that of *crtRB1* (Yan *et al.*, 2010) to their use in marker-assisted selection for elevated levels of provitamin A carotenoids (Babu *et al.*, 2013; Menkir *et al.*, 2017), high levels of PVA exceeding the actual breeding target (15 $\mu g\, g^{-1}$) were only reported in inbreds. Released PVA hybrids contained a range of 6 to 8 $\mu g\, g^{-1}$ of PVA. Given the availability of inbreds with much higher levels suggest some constraints to a realized heterosis in hybrid development. This may possibly be due to the presence of significant non-additive genetic effects as found in this study and also reported by Halilu *et al.* (2016).

Dominance genetic effects were found to significantly contribute to the inheritance of most carotenoid traits in the present study. There were more dominant genes than recessive genes in the parents with the ratio of the dominant to recessive being greater than 2 (2.36) for β-carotene. Hayman diallel analysis revealed that at loci exhibiting dominance, recessive alleles were mostly

positive for β-cryptoxanthin, lutein, and to some extent for β-carotene while dominant genes carried both effects for the rest of the traits. This supports the importance of the ambi-directional dominance detected. The presence of more dominant alleles with negative effects for β-cryptoxanthin and β-carotene could explain the negative to low heterotic effects observed for these traits in this set of inbreds. The negative effects of dominant genes might not have only nullified the effect of the recessive homozygotes in the parents but also could have significantly reduced the effects of additive gene effects over loci. This finding, consequently, suggests that effort in hybrid development for elevated levels of provitamin A can be hampered by the predominant negative genetic effects of dominant genes and could explain the difficulties in developing such hybrids needed to compensate for post-harvest degradation of carotenoids, degradation during processing, and storage.

Using molecular markers, Menkir *et al.* (2017) developed inbred lines with provitamin A levels ranging from 0.2 to 22.6 $\mu g\, g^{-1}$, from 2013 to 2016. At the same time, best hybrids developed from these lines have provitamin A levels ranging from 10.1 to 11.1 $\mu g\, g^{-1}$. This depicts the unavoidable need of the usage of markers to support selection based on colour rating, as recommended by Chander *et al.* (2008).

In conclusion, this study showed a similar trend in carotenoid levels in both parental lines and crosses derived from them. The genetic analysis revealed presence of negative dominance effects across loci which reduces the effects of the predominantly additive effects discovered for all carotenoid traits. Consequently, heterosis and heterobeltiosis were found to be mostly negative with the highest values obtained from crosses involving parents with low-level of the considered trait.

Finally, genotypic selection based on gain colour rating alone can be effective for increased levels of β-cryptoxanthin and zeaxanthin in maize kernels. However, effective breeding for much higher carotenoids content may require the use of molecular markers to support and guide selection based on endosperm colour. Validation of introgressed favourable alleles of the major genes could also be considered in hybrids before their evaluation to determine the overall genetic effect of favourable alleles over other genes with possible epistatic effects.

Development of hybrids with increased levels of carotenoids should consider the finding of this study by opting for a progressive gene pyramiding or simultaneous targeting of major genes and other genes with epistatic effects in a genomic selection programme. The latter option could be achieved only if maximum genes or QTLs directly or indirectly influencing the accumulation of different carotenoids are discovered and well known. In this regard, QTL analysis of the genetic pool from which the inbreds involved in this study are derived will bring clarification on the genomic regions that are linked to their performance for β-cryptoxanthin.

CHAPTER SEVEN

7.0 CONCLUSIONS AND RECOMMENDATIONS

Conclusion

The main goal of this research was to develop extra-early maturing maize hybrids that combine high yield, resistance to *Striga*, tolerance to drought and elevated levels of carotenoids, especially provitamin A carotenoids for *Striga* endemic and drought-prone areas of the savanna agro-ecologies of SSA in order to alleviate food shortage in the sub-region. Towards this end, four experiments were conducted.

Adequate genetic variability among the selected 152 orange inbreds with respect to each trial management (*Striga* infestation, drought stress and optimal) was observed. The residual variability measured was due to different levels of resistance to each of the stresses. The predicted breeding values (Pedigree-based BLUPs) under *Striga* infestation were almost thrice greater than that under drought stress for all the inbreds, indicating that there was a higher level of resistance/tolerance to *Striga* than the level of tolerance to drought. However, the higher additive genetic effects' contribution to the total genetic variability observed confirmed the genetic basis of the resistance levels to each of the stresses and the fact that the traits are highly heritable. Across all test environments, the breeding values of the inbreds were similar to those under *Striga* infestation indicating that their performance across test environments may be driven by the performance under *Striga*. Predictions over pedigree allowed identification of several inbreds not evaluated with breeding values that fell among the top best selected and even greater than those of some previously selected inbreds.

Molecular characterisation of the 152 orange maize inbred lines using 4620 SNPs revealed high homogeneity in 92% of the inbreds. However, TZEEIOR 75, TZEEIOR 76, TZEEIOR 161 and

TZEEIOR 38 were highly heterogeneous with heterozygosity level greater than 12.5%. Also, the study revealed that the inbreds were closely related regarding the genetic distance and the kinship coefficient with the latter confirming their origin from a shared pedigree. The unknown relationship between the markers used in this study and the traits for which the inbreds were selected could explain the results obtained. This suggests that specific markers may be needed to explore the true genetic relationship among these inbreds. However, model-based population structure and neighbour-joining clustering concorded in distinguishing four groups in 71% of the inbreds while 39% shared properties of different groups, probably due to the low genetic distances observed among the inbreds. Distant inbreds could be used in planned crosses to maximise the performance in their progenies.

In the evaluation of the seventeen selected inbreds for their performance in crosses, there were significant variations among environments, components of genetic variance (GCA and SCA), and environments by variance components interactions among inbred parents for grain yield and most of the traits. This indicated the presence of adequate variability among inbred parents and also among hybrids for breeding purposes and also the need for testing in several environments for selecting performing inbred parents and hybrids. Additive and non-additive genetic effects were both important in controlling grain yield and most of the measured traits in each trial and across environments. However, for leaf senescence and *Striga* related traits, additive genetic effects were the primary type of gene action suggesting that selection for these traits could easily be done based on predictions of GCA alone. Again, GCA values under *Striga* infestation were greater than those under drought stress indicating that inbred parents contributed more to the hybrid performance under *Striga* than under drought. The performance of the hybrids across test environments was highly correlated to the SCA and to their genetic values. This highlighted not only the importance

of SCA in predicting hybrid performance but also the contribution of GCA to the performance. That is, a hybrid from two parents with high GCA and SCA will yield higher than a hybrid from two parents with low GCA effects compared to the first parents but the same SCA value as the first hybrid. Among the selected outstanding hybrids across test environments, TZEEIOR 12 x TZEEIOR 196 and TZEEIOR 222 x TZdEEI 7 were the most stable and yielded respectively, 3885 kg ha^{-1} and 3611 kg ha^{-1} across environments and 5411 kg ha^{-1} and 4249 kg ha^{-1} under optimal conditions. They could thus be proposed for on-farm evaluations or included in mother-baby trials to test their adaptability to farmers conditions and acceptance.

Analysis of inbred parents and their hybrids for carotenoid levels revealed three different profiles in the inbreds: inbreds with high levels of total carotenoids, inbreds with high levels of beta-branch carotenoids, and inbreds with almost equal levels of β-carotene and β-cryptoxanthin. Inbred TZEEIOR 42 had both high levels of β-cryptoxanthin and xanthophylls. The levels of provitamin A in this set of inbreds was mainly due to the equal contribution of β-carotene and β-cryptoxanthin. Dominance genetic effects were found to significantly contribute to the inheritance of most carotenoid traits. Also, there were more dominant genes than recessive genes in the parents with the ratio of dominant to recessive being greater than 2 (2.36) for β-carotene. Dominant alleles were found to have negative effects on most of the traits, especially for β-cryptoxanthin and β-carotene. That explains the negative to low heterotic effects observed for these traits in this set of inbreds. The negative effects of dominant genes in the parents might have reduced the effects of additive gene effects over loci, thus yielding low levels in most of the hybrids. Thus, breeding for elevated levels of carotenoids in maize using colour rating improves significantly the level of zeaxanthin and β-cryptoxanthin but not in β-carotene. Success in breeding heterosis for provitamin A might need to consider the genetic background of inbred parents with one important step being the

reduction of dominant genes with negative effects by increasing the number of favourable alleles through introgression.

Recommendations to:

- **IITA-MIP**

1. The best inbreds not evaluated but selected by pedigree-based BLUPs should be evaluated to confirm their performance;
2. The level of tolerance to drought of inbreds should be increased through introgression of novel resistance alleles from different sources;
3. Specific markers such as validated drought tolerance and resistance to *Striga* should be used to further characterize the inbreds;
4. Request for the readily available sources of 5'TE favourable alleles from CIMMYT to be introgressed into the inbreds which have acceptable levels of β-branch carotenoids;

- **IITA-MIP and other research institutes**

5. Adopt a more progressive programme for gene pyramiding or simultaneous selection of major genes in a genomic selection programme for elevated levels of carotenoids in maize.

- **IITA collaborators**

6. Hybrids TZEEIOR 12 x TZEEIOR 196, TZEEIOR 145 x TZdEEI 7, and TZEEIOR 222 x TZdEEI 7 should be tested in regional trials and if suitable, released for adoption and commercialization in the sub-region;

- **Further researche**

7. Study the interaction between genes for tolerance to drought or resistance to *Striga* and genes for increased levels of β-branch carotenoids.

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