GENETICS OF CAROTENOIDS IN TROPICAL-ADAPTED PROVITAMIN A MAIZE (*ZEA MAYS* L.)

BY

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GENETICS OF CAROTENOIDS IN TROPICAL-ADAPTED PROVITAMIN A MAIZE (*ZEA MAYS* L.)

BY

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DECLARATION

I declare that the work in this thesis entitled "GENETICS OF CAROTENOIDS IN TROPICAL-ADAPTED PROVITAMIN A MAIZE (*Zea mays* L.)" has been carried out by me in the Department of Plant Science. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

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Name of Student Signature Date

CERTIFICATION

This thesis entitled "GENETICS OF CAROTENOIDS IN TROPICAL-ADAPTED PROVITAMIN A MAIZE (*Zea mays* L.)" by Alhassan Dada HALILU meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my late parents: Alhaji Halilu Usman and Hajiya Simbiatu Halilu and to my late son Halilu Dada, May Allah grant them *Aljanna firdaus*. Amin.

ABSTRACT

Vitamin A is a group of fat-soluble vitamins essential for life and health. It plays vital role in vision, epithelial tissue protection, bone and brain development, and immune system functions. β-carotene (βC), β-cryptoxanthin (βCX) and α-carotene (αC) are vitamin A precursors found in plants and are referred to as provitamin A (PVA). Maize contains considerable levels of provitamin A making it an important crop to combat vitamin A deficiency through biofortification. The genetic diversity among 31 tropical-adapted maize inbred lines developed for medium to high PVA were determined using molecular markers. Genomic DNAs were extracted from the leaves and genotyped using 14 simple sequence repeat (SSR) markers. A total of 33 alleles with an average of 2.36 alleles per locus were compared among the lines. The gene diversity (D), revealed by all the SSR markers, ranged from 0.22 to 0.60 with a mean of 0.45 indicating moderate genetic diversity among the lines. Cluster and Principal Coordinate analyses based on genetic distances from the SSR data separated the lines into two main groups with 4 clusters and several subclusters consistent with their pedigrees. Nine lines were selected from the different clusters for inheritance study. The genomic DNAs of the selected lines were genotyped using five molecular markers for the two major functional genes in carotenoids biosynthesis, *lycE* and *crtRB1*. The results showed different profiles of the nine lines possessing three to five alleles of *lycE* and *crtRB1*genes that favour increased provitamin A active carotenoids (βC, βCX and PVA) in maize endosperm and indicated potential of these markers for marker assisted selection. The nine selected lines were crossed using half diallel mating design to develop 36 crosses. The crosses were evaluated in the field in two locations (Samaru and Kerawa). The first two plants in each plot were self pollinated to produce seeds for

carotenoids analysis. The crosses were assessed for variability, general combining ability (GCA), specific combining ability (SCA), heritability and correlations for agronomic traits and carotenoid contents. Analysis of variance showed that the mean squares for location were highly significant ($p<0.01$) for the agronomic traits and the carotenoids with exception of Zeaxanthin (ZEA) and βCX. The crosses showed highly significant differences for days to 50% anthesis (DA), plant height (PH), grain yield (GY), Lutein (LUT) and ZEA at Samaru and across the locations. The mean square for GCA and SCA effects were significant for DA, PH, GY, LUT and ZEA across locations, suggesting both additive and non-additive gene effects. The GCA:SCA mean squares ratios for DA, PH, LUT, ZEA and βCX were higher than unity (>1) indicating the preponderance of additive gene effect but lower than unity (<1) indicating preponderance of non-additive gene effect for GY, βC, αC and total provitamin A. The best performing crosses for GY across locations were for E26/E6 (7.33 tons/ha) and E6/E9 (7.16 tons/ha). Lines E6, E7, E8 and E9 were the common parents in the best crosses. ZEA made up 49.5% of total carotenoids across locations making it the highest carotenoids in tropical-adapted maize. Lines with high positive GCA effects for DA, PH and GY were E8, E9, E3 and E37 while lines E6, E9, E28 and E7 had high positive GCA for βC and PVA contents. Significant positive SCA effects for αC, βC and PVA across locations were observed in the crosses E3/E16 and E8/E6. Although many of the other crosses had high positive SCA effects for the PVA carotenoids, the effects were not significant. For grain yield, 53% of the crosses had positive SCA effects. Heritability (H^2) was high for grain yield ($H^2>60\%$), moderate for DA, LUT and ZEA (30-60%), and low for PH, βCX, βC, αC and PVA ($H^2<30\%$). The phenotypic correlations between GY and the carotenoids were significantly positive with βC, βCX and

αC while genotypic correlations were negative and non-significant, indicating masking of environmental effects. GY is not genotypically significantly correlated with DA, PH, αC, βC, βCX, PVA, ZEA and LUT, suggesting that GY and provitamin A carotenoids can be improved concurrently. Hybrid development and recurrent selection are, therefore, recommended for the development of maize varieties with high GY, PVA, βC and βCX for the tropical sub-saharan Africa. Direct selection for yield should also be practice while applying independent culling selection method for DA, PH, ZEA and LUT because of their negative indirect contributions on PVA.

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LIST OF ABBREVIATIONS

αC	α-Carotene
βC	β-Carotene
βCX	β-Cryptoxanthin
BHT	Butylated HydroxyToluene
CovFS	Covariance of Full Sib Relatives
CovHS	Covariance of Halfsib Relatives
CrtISO	Carotene Isomerase
CRTRB	β-Carotene Hydroxylase family
CV	Coefficient of Variation
DA	Days to 50% Anthesis
df	Degrees of freedom
DMAPP	Dimethylallyl Pyrophosphate
DXP	1-deoxy-D-xylulose 5-phosphate;
DXR	1-deoxy-d-xylulose 5-phosphate Reductoisomerase
DXS	DXP Synthase
GA3P	Glyceraldehyde 3-Phosphate;
GD	Genetic Distances
GGPP	GeranylGeranyl Pyrophosphate
GGPPS	GGPP Synthase
GY	Grain Yield
HDR	4-Hydroxy-3-Methylbut-2-Enyl Diphosphate Reductase
HPLC	High Performance Liquid Chromatography
IDP	Insertion/Deletion Polymorphisms
Indel	Insertion-Deletion Mutation
IPP	Isopentenyl Pyrophosphate
IPPI	IPP isomerase
KOH	Potassium Hydroxide
lcyB	lycopene β-cyclase
lcyE	lycopeneepsilon ε-cyclase
LUT	Lutein
M	Million

MAS	Marker Assisted Selection
MeOH	Methanol
MEP	methylerythritol 4-phosphate
MNM	Micronutrient Malnutrition
MT	Metric Tonnes
MTBE	Methyl-Tertiary-Butyl Ether
PCoA	Principal Co-ordinate Analysis
PEM	Protein Energy Malnutrition
PH	Plant Height
p*i*	Allele Frequencies of Alleles *i*
PIC	Polymorphic Information Content
p*j*	Allele Frequencies of Alleles *j*
PSY	Phytoene Synthase
PVA	Provitamin A
QTL	Quantitative Trait Locus
RAPD	Random Amplified DNA Polymorphism
RDA	Recommended Daily Allowance
RFLP	Restriction Fragment Length Polymorphisms
SCAR	Sequence Characterized Amplified Region
SCN	Standing Committee on Nutrition
SE	Standard Error
SNP	Single Nucleotide Polymorphism
SOV	Source of variation
SSA	Sub-Saharan Africa
SSR	Simple Sequence Repeats
STS	Sequence Tagged Site
TE	Transposable Element
THF	Tetrahydrofuran
UPGMA	Unweighted Pair-Group Method with Arithmetical Averages
USDA	United States Department of Agriculture and Foreign Agricultural Service
VAD	Vitamin A Deficiency
WCA	West and Central Africa

ZEA	Zeaxanthin
ZEP	Zeaxanthin Epoxidase

CHAPTER 1: INTRODUCTION

Maize (*Zea mays L.*), is a cereal crop that is cultivated all over the world. About 50 species exist and are of different colors, textures and grain shapes and sizes (Hallauer *et al.*,2010). White, yellow and red are the most common grain types. The white and yellow varieties are preferred by most people who depend on maize as staple food (Nuss and Tanumihardjo, 2010). Maize was introduced into Africa in the 1500s and has since become one of Africa's dominant food crops (Fajemisin, 2014).

In 2012, over 870 million (M) metric tons (MT) of maize grains were produced in the world, with the United States, European Union, China, Brazil, Mexico, and India being the world's leading producers (FAO, 2013). Africa contributed about 70M MT of which West Africa produced 25.7%. Nigeria produced more than half of the maize in West Africa having a production of 9.4M MT out of 18M MT produced in the region (FAO, 2013). An annual increase in maize production is more than that of any other grain in the world (FAO, 2013). Increased production of maize in West and Central Africa (WCA), Nigeria in particular has been a result of collaborative activities of scientists in the region who have been working to develop high yielding, drought tolerant, different maturity groups, as well as *Striga* resistant varieties (Ado *et al.,* 2007; Fajemisin, 2014). The land area devoted to maize production in WCA has increased significantly (an average of 2.7 percent per year for the region). In Nigeria, there has been an annual increase of 5.5 percent in the hectarage

of land allocated for maize cultivation translating to an annual increase in profit margin of 5.3 percent (Fakorede *et al.,* 2001; FAOSTAT, 2014).

The popularity of maize as a crop is largely due to the different ways that it can be consumed as food by both humans and animals. The kernels can be consumed as vegetable, boiled, fried and roasted. On the other hand maize may be dried, ground, or fermented for use in breads, porridges, gruel, cakes, and alcoholic beverages. Maize can be further processed and used as food thickeners, sweeteners, oils, and non-consumables (Nuss and Tanumihardjo, 2010). Worldwide consumption of maize is more than 116 million tons, with Africa consuming 30% of which Sub Saharan Africa (SSA) consumes 21%. Africa as a whole utilizes 95% of maize as food compared to other world regions that use most of its maize as animal feed. Thus, maize is now the most important staple food for more than 1.2 billion people in SSA and Latin America (Lutz *et al.,* 2001; USDA 2009).

The major nutritional constituents of maize kernel include starch, protein, fat and some micronutrients, which are also required for human health. Maize provides an estimated 15% of the world's protein and 20% of the world's calories (Brown *et al.,* 1988). However, excessive consumption of maize can lead to malnutrition and vitamin deficiency diseases such as night blindness and kwashiorkor, as a result of the inability of a dominant maize diet to meet up with the recommended daily allowance (RDA) of protein and essential micronutrients for the body. Micronutrient malnutrition (MNM), unlike Protein-Energy Malnutrition (PEM), often has no obvious manifestations except in extreme cases, and is for this reason often termed the "hidden hunger" (Pfeiffer and McClafferty, 2007). However, the prevalence of MNM greatly exceeds the prevalence of PEM. Compared to

the 180 million children with protein-energy malnutrition, 3.5–5 billion persons are iron-deficient, and 140–250 million persons are vitamin A-deficient (VAD) (WHO, 2001a, 2001b 2001c; SCN, 2010).

Vitamin A deficiency (VAD) disorder is a situation where tissue concentrations of vitamin A is low enough to have adverse health consequences such as xerophthalmia, anaemia, growth retardation, increased infectious morbidity and mortality, impaired iron utilization, disturbed cellular differentiation and suppressed immune response (World Bank, 2006). VAD is indicated when the liver store of retinol is below 20 μg/g (0.07 μmol/ g) (SCN, 2004). The Standing Committee on Nutrition (SCN) of the United Nations puts the number of preschool children in low-income countries that are affected by VAD at 160 million. Physical symptoms of eye problems as a result of the deficiency have a prevalence of about 1–2%. Africa accounts for 26% cases of preschool children of which 4% is in Nigeria. For the maternal night blindness cases, Africa has 4.4% cases in the world while Nigeria has 2.4% of Africa's cases (SCN, 2010).

Nutritional sources of vitamin A are carotenoids. Carotenoids are a fat-soluble group of naturally occurring plant pigments. They are classified as carotenes, un-substituted hydrocarbons (e.g. β-carotene, α-carotene, and lycopene), or xanthophylls, which contain one or more oxygen functional groups (e.g. lutein, zeaxanthin, and β-cryptoxanthin). Carotenoids have a broad range of roles in relation to human health (Krinsky, 1989; Fraser and Bramley, 2004). Interest in dietary carotenoids comes from their anti-oxidant properties and the association between carotenoid deficiencies and many chronic human diseases (e.g. VAD). Some carotenoids, notably β-cryptoxanthin, α-carotene, and

β-carotene, are referred to as Provitamin A carotenoids because their cleavage by a mono-oxygenase results in one, or in the case of β-carotene, two molecules of vitamin A (Olson, 1989; Kiefer *et al.,* 2001).

1.1 Justification

Approaches to combat VAD using regular supplementation and food fortification with vitamin A in the developing world were not sustainable especially when government's attention and international funding for micronutrient issues fade (Beaton *et al.,* 1994; Mason *et al.,* 2001). Thus, a sustainable strategy to combat VAD in these regions is through biofortification which is an endogenous fortification in plant breeding of major staple crops such as maize (*Zea mays* L.). After a one-time investment in developing seeds that fortify themselves, recurrent costs are low and it is highly sustainable. Hence, to alleviate VAD, maize has been targeted for biofortification. Among the major food grains, only yellow maize contains significant levels of β-carotene, a source of vitamin A (Buckner *et al.,* 1990) and has considerable natural variability for the other carotenoids (Egesel, 1997; Wurtzel, 2004; Menkir *et al.,* 2008; Mishra and Singh, 2010). Typical yellow maize varieties have 0.5 to 1.5 μg/g PVA content (Harjes *et al.,* 2008). Current International target of combating VAD through utilizing maize, is to develop maize kernels with 15μg Provitamin A per gram dry weight (HarvestPlus, 2008).

Though considerable genetic variations in carotenoids have been observed in nature in yellow maize lines and hybrid varieties in the temperate (Weber, 1987; Wong, 1999; Egesel *et al.,* 2003b) and in the tropics (Menkir and Maziya-Dixon, 2004; Maziya-Dixon *et al.,* 2000; Menkir *et al.,* 2008), limited information is available for the range of variation of

carotenoids in tropical-adapted yellow maize lines in the sub-Saharan Africa (SSA). Information on the genetics of carotenoids in yellow maize is also limited even for temperate maize where maize carotenoids are most studied, Egesel *et al*. (2003), Burt (2010) and Suwarno *et al*. (2014) are some of the few studies on gene action and inheritance of carotenoids in temperate maize. Though some studies have been conducted for tropical-adapted yellow maize diversity (Adeyemo *et al.*, 2011) and carotenoids variability (Maxiya-Dixon and Menkir, 2004; Menkir *et al.* 2008), there is paucity of information on the genetics (e.g. gene action, combining ability, and inheritance) of carotenoids in maize of tropical SSA.

Therefore, this research was undertaken to obtain genetic information that can be used for effective pro-vitamin A maize biofortification adaptable to the tropical SSA.

1.2 Objectives

The objectives of this research were to:

1. estimate the genetic diversity among the 31 tropical-adapted provitamin A maize inbred lines using molecular markers.
2. assess the variability, heritability and genetic gain for the carotenoids contents and agronomic traits in provitamin A maize crosses.
3. assess the general and specific combining ability (GCA and SCA) for agronomic traits and carotenoids contents from crosses of selected tropical-adapted maize inbred lines.

4. assess degree of associations and cause and effect relationships among agronomic traits and carotenoids contents.

CHAPTER 2: LITERATURE REVIEW

2.1 Genetic Diversity in Maize

Maize is highly adaptable and responsive to selection pressure because of its exceptional genetic diversity. As a result, it has been cultivated in a wide bio-geographical range encompassing tremendous diversity in soil composition, climate, day length and elevation through the tropics to the temperate regions of the world (Neuffer *et al.* 1997). Numerous studies investigating genetic diversity within breeding materials for maize have been reported by Benchimol *et al.* (2000), Liu *et al.* (2003), and Chander *et al.* (2008b). Prior to crossing (hybridization) and line development, cultivar identity, assessment of genetic diversity and parent selection, and confirmation of hybrids have been done based on visual selection and analysis of data based on quantitative and morphological characteristics. Typically breeding programmes usually grow hundreds or even thousands of populations, and many thousands or millions of individual plants (Ribaut and Hoisington, 1998). Given the extent and complexity of selection required in breeding programmes and the number and size of populations, the development of new tools to ease the process of plant selection by breeders, is an appreciated development.

2.1.1 Genotyping tools for genetic diversity

The development of Molecular DNA markers has provided a less cumbersome platform for assessing genetic diversity among maize inbred lines and help in assigning them to different heterotic groups (Livini *et al.*, 1992; Menkir *et al.*, 2004, 2005; Senior *et al.*, 1998). Many studies have reported estimates of correlations between marker-based genetic

distances (GD) and hybrid performance in maize (Boppenmaier *et al.,* 1993; Lanza *et al.,* 1997; Ajmone-Marsan *et al.,* 1998).

Several DNA markers have been used to determine maize genetic diversity. These include restriction fragment length polymorphisms (RFLPs) (Benchimol *et al.,* 2000), and the PCR-based techniques such as simple sequence repeats (SSR) (Taramino and Tingey, 1996), insertion/deletion polymorphisms (IDP) (Harjes *et al.,* 2008). Others are Sequence tagged site (STS), sequence characterized amplified region (SCAR) or single nucleotide polymorphism (SNP) (Yan *et al.,* 2010) markers that are derived from specific DNA sequences of markers (e.g. RFLPs) that are linked to a gene or quantitative trait locus (QTL).

Simple Sequence Repeat (SSR) markers

Among the PCR-based markers, SSR has been used extensively for genetic diversity assessment in maize (Senior and Heun, 1993; Chin *et al.,* 1996; Taramino and Tingey, 1996; Matsuoka *et al.,* 2002a, 2002b; Liu *et al.,* 2003). Previous studies have shown that maize contains abundant simple sequence repeats and that these SSRs are highly polymorphic even among small samples of maize inbreds. They are highly reliable (i.e. reproducible), co-dominant in inheritance, relatively simple and cheap to use.

Analysis to determine the genetic structure and diversity among 260 maize inbred lines using SSR markers was reported by Liu *et al.* (2003). Ninety four SSRs were used to partition the lines into five groups and to define the relationship between pedigree and genetic distance. The findings were based on calculated observed

heterozygosity, gene diversity (or expected heterozygosity), number of private alleles, number of group-specific alleles, pairwise F-statistics, and stepwise mutation model index. The mean gene diversity of all SSRs reported by Liu *et al.* (2003) was 0.82 and allelic variation was 21.7 alleles per locus over the 94 loci. Other findings based on different sets of maize germplasm and number of SSRs reported values of allelic variation of 5.21 (Senior *et al.,* 1998), 6.6 (Taramino and Tingey, 1996), 4.9 (Lu and Bernardo, 2001), and 6.9 (Matsuoka *et al.,* 2002a) alleles per locus. While results of gene diversity reported were 0.59 (Senior *et al.,* 1998), 0.76 (Taramino and Tingey, 1996), 0.59 (Smith *et al.,* 1997) and 0.62 (Matsuoka *et al.,* 2002a). Adeyemo *et al.* (2011) also reported genetic diversity in 38 tropical adapted maize inbred lines of high Provitamin A activity using 75 SSR markers. The polymorphic information content (PIC) values they obtained for the polymorphic SSR markers varied from 0.17 to 0.84 with an average of 0.56.

Allele specific or functional markers (lycε and crtRB1)

In maize, many genes have been noted to influence the production of carotenoids in the kernel and leaf (reviewed by Xu *et al.,* 2009). In most cases when a plant is homozygous recessive for alleles of these genes the kernel will accumulate carotenoid precursors (Figure 1) (Parry and Horgan, 1992). Over the past decade, most of the genes encoding carotenoid biosynthetic enzymes in plants have been cloned and characterized (Cunningham *et al.,* 2007; Fraser and Bramley, 2004). Phytoene synthase (PSY) has been shown to be rate-limiting for carotenoid content in several plant tissues, including maize and rice endosperm (Gallagher *et al.,* 2004; Paine *et al.,* 2005; Bai *et al.,* 2009). *Psy1* locus

has been a target of selection resulting in the accumulation of seed carotenoids in yellow maize varieties (Palaisa *et al.,* 2003). The first branch point in maize carotenoid biosynthesis occurs during cyclization of lycopene (Figure 1), and directs substrate towards the production of β,ε-carotenoids (e.g. α-carotene) or β,β-carotenoids (e.g. β-carotene).

The chemical analysis of carotenoids in maize is the major hurdle limiting progress in Provitamin A improvement programs. This is because the accepted method of analysis is the high performance liquid chromatography (HPLC) which is very expensive, time-consuming and has low throughput (Pfeiffer and McClafferty, 2007). Visual selection based on kernel color is also not reliable due to poor correlation and low coefficient of determination (R^2) between endosperm colour and Provitamin A carotenoid content (Mishra and Singh, 2010). Marker assisted selection (Moose and Mumm, 2008) using allele specific markers, thus, can help to overcome these challenges by offering a strategy to quickly and accurately predict phenotypes (Anderson and Lübberstedt, 2003). These markers are based on polymorphic sequences situated inside the gene of interest and bearing causal effects on the variation of the phenotype controlled by the gene (Gupta and Rustig 2004; Varshney *et al.,* 2007). Unlike other classes of DNA based markers like random markers (e.g. RAPD), functional markers are derived from the conserved portion of the genome (Varshney *et al.*, 2007).

At least two important allele mining works have been reported for two maize carotenoid biosynthesis genes; the lycopene epsilon cyclase gene, *lcyE* (Harjes *et al.*, 2008), and a carotenoid hydroxylase gene, *crtRB1 or HYD3* (Vallabhaneni *et al.*, 2009; Yan *et al.*, 2010). These genes have been demonstrated to play major roles in dictating variations in

maize endosperm Provitamin A contents. The specific sequence polymorphisms associated to the variations have also been clearly described.

Harjes *et al.* (2008) found the gene encoding lycopene epsilon cyclase (*lcyE*) to significantly affect the level of Provitamin A in maize endosperm through control of the bifurcation of the pathway into β and α branches (Figure 1). Four polymorphisms in *lcyE* were found to explain significant proportion of the variation between the β and α branches and a threefold difference in Provitamin A compounds (Table 1). Yan *et al*. (2010) reported another critical gene (*crtRB1*) encoding the enzyme β-carotene hydroxylase which catalyzes oxidization of β-carotene to carotenoids having half and none Provitamin A activities, β-cryptoxanthine and zeaxanthine, respectively. Three of the polymorphic sites in *crtRB1* were shown to significantly regulate the concentration and conversion of β-carotene in maize endosperm. Validation of the effects of the molecular marker polymorphisms in *lcyE* and *crtRB1* on Provitamin A concentrations had been reported in some maize inbreds (Safawo *et al.,* 2010). The unfavorable allele for both *lcyE* and *crtRB1* is named 'allele 2' and it is always an insertion, while all other alleles are considered favorable for their respective genes. The others are therefore called 'allele 1'.

Table 1. Functional polymorphism classification for *lcyE* and *crtRB1*.and their effects on carotenoids contents in maize

Gene	Allele	Nomenclature	Description
lcyE	*lcyE*-5'TE	2 or I	Unfavorable
		1 & 4	Favorable
	lcyE-3'Indel	2 or I	Unfavorable
		1 or D	Favorable
crtrB1	*crtRB1*-3'TE	2 or I	Unfavorable
		1 or D	Favorable
	crtRB1-5'	1 or I	Unfavourable
		2&3	Favorable

The nomenclature for different functional polymorphism sets were according to Harjes *et al.* (2008) for *lcyE* and Yan *et al.* (2010) for *crtRB1*.
(Source: Babu *et al.*, 2013).

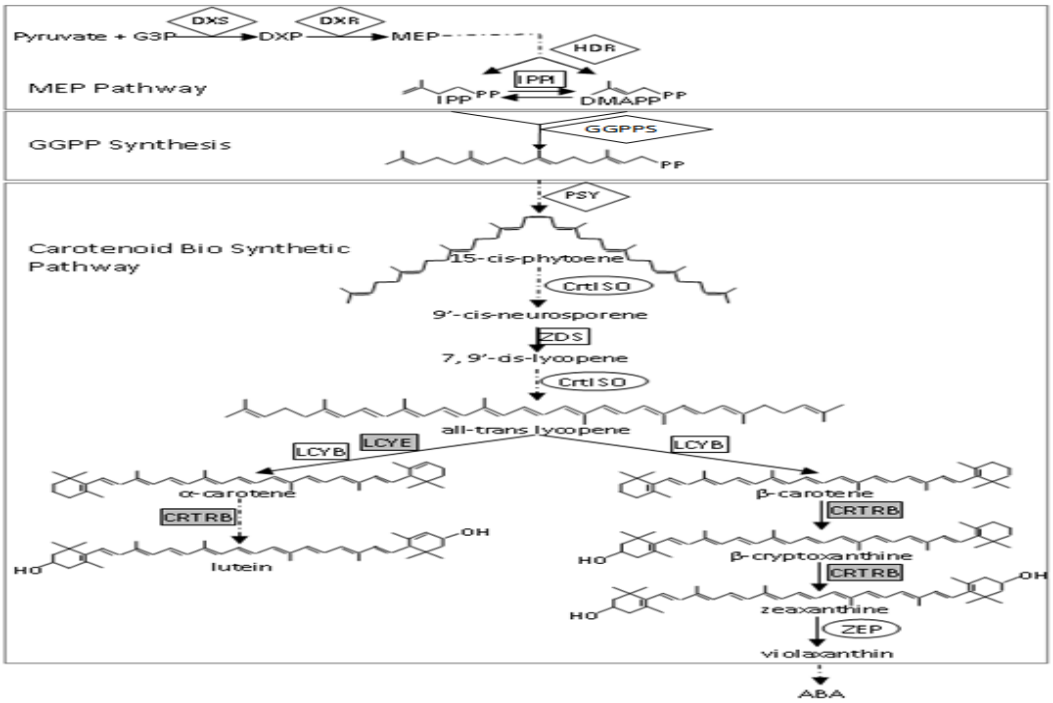


Figure 1. Schematic diagram of the carotenoid biosynthesis pathway. Arrows and boxes represent steps of enzymatic reactions. Enzymes indicated in shaded rectangular boxes are shown to have significant influence on β-carotene content in maize kernel by Harjes *et al.* (2008) and Yan *et al.* (2010).

Source (Vallabhaneni *et al.*, 2009 and Rodriguez-Amaya, 2010)

2.2 Genetic Variability for Provitamin A Carotenoids in Maize

Yellow maize is the only grain crop that naturally accumulates a significant amount of carotenoids in its seed (Buckner *et al.*, 1990). Significant genetic variations for carotenoids content have been reported mostly for temperate maize (Forgey, 1974; Egesel *et al.*, 2003b; Mishra and Singh, 2010). These studies have shown significant differences among maize inbred lines for the different carotenoids levels. Blessin *et al.* (1963) reported ranges of 0.9 to 4.1 µg/g for carotenes and 18.6 to 48.0 µg/g Xanthophylls for 39 maize inbred lines. Quackenbush *et al.* (1963) also reported provitamin A contents ranging from a trace to 7.3 µg/g, lutein from 2 to 33 µg/g for 125 inbred lines. In another study, among the diverse panel of inbred lines analysed, β-carotene level was found to be up to 13.6 µg/g whereas most of the yellow maize grown and consumed throughout the world have only 0.5 to 1.5 µg/g β-carotene (Harjes *et al.*, 2008). Mishra and Singh (2010) also reported total carotenoids content to vary from minimum of 0.027 µg/g to maximum of 25.75 µg/g dry weight with overall mean of 18.11 µg/g dry weight in a diverse panel of maize inbred lines. Based on these findings the dominant carotenoids in maize kernels, in decreasing order of concentration, are: lutein, zeaxanthin, β-carotene, β-cryptoxanthin and α-carotene. In a similar study of tropical adapted maize inbred lines developed in the pre-breeding activities of IITA in her effort to biofortify maize, the yellow-endosperm maize genotypes exhibited significant differences for all the traits measured. Seventy percent of the total variation observed was due to β-carotene (Menkir and Maziya-Dixon, 2004). The mean β-carotene content averaged over six environments varied from 0.45 µg/g to 2.18 µg/g. Further study of many yellow maize inbred lines adapted to the tropics (Menkir *et al.*, 2008) revealed that carotenoid concentrations were not strongly affected by the

differences in replications or locations or genotype by environment interaction, and there were large differences among the tropical-adapted yellow maize inbred lines in lutein, zeaxanthin, β-carotene, β-cryptoxanthin, α-carotene and total provitamin A contents. Lutein content was found to be 0.1-19 µg/g, Zeaxanthin 0.3-24.5 µg/g, β-cryptoxanthin 0.3-5.5 µg/g, α-carotene 0-1.9 µg/g, β-carotene 0.3-4.7 µg/g, and provitamin A was 0.4-19 µg/g (Menkir *et al.,* 2008). These levels were similar to findings of Chander *et al.* (2008b) for maize inbred lines in China, where β-carotene, β-cryptoxanthin, α-carotene, lutein, zeaxanthin, and total carotenoids ranged between 0.016-1.726 µg/g, 0.012-3.666 µg/g, 0.004-0.852 µg/g, 0.040-17.504 µg/g, 0.022-6.728 µg/g, and 0.096-22.495 µg/g, respectively. Though limited studies are available for tropical adapted maize lines and hybrids, these findings demonstrate potentials for improving the provitamin A contents of the tropical-adapted inbred lines.

2.3 Combining Ability for Carotenoids in Maize

Combining ability of a genotype is an important criterion in developing improved hybrids. General and specific combining ability (GCA and SCA) are useful in testing procedures where the objective is to compare hybrid performance of crosses and lines (Griffing, 1956). The concepts of GCA and SCA were introduced by Sprague and Tatum (1942), who partitioned the genetic variability among crosses into effects due primarily to either additive (GCA) or non-additive (SCA) effects. The relative importance of GCA and SCA depend on the extent of previous testing of the parents included in the crosses (Hallauer *et al.,* 2010). Jenkins (1940) reported that additive genetic effects were of greater importance and that selection should emphasize GCA. Hull (1945), however, had

contrary opinion and suggested that selection should emphasize for non-additive effects or SCA. Comstock *et al.* (1949), however, suggested a selection method that was equally effective for both GCA and SCA. This information can be useful for the selection of parents that have good general combining ability in a series of crosses and good specific combining ability for specific pairs of parents. This type of information is quite useful to maize breeders, particularly if the selected set of parents represents an elite group of inbred lines that are possible candidates as parent seed stock for the production of single-cross hybrids.

Several combining ability studies have been conducted in maize for yield, prolificacy (Mhike *et al.,* 2010), flowering time and agronomic characters (Dickert and Tracy, 2002; Halilu *et al.,* 2012), growth characters (Rana and Kapoor 2003; Subramanian and Subbaraman, 2006), protein and tryptophan contents (Machida *et al.,* 2010), drought tolerance (Durães *et al.,* 2002), cold tolerance (Rodríguez *et al.,* 2007), downy mildew resistance (Kim *et al.,* 2003) and corn earworm resistance (Fajemisin *et al.,* 1985). However, limited report is available on combining ability in maize carotenoids contents. A pioneering work on hybrid carotene performance in maize was by Aurand *et al.* (1947). They found that 10 yellow dent inbred lines resulted in hybrids with high carotene levels. The maize lines varied widely in carotene content. Combining ability for carotenoids and tocopherol was reported by Egesel *et al.* (2003a). Ten parents and 45 hybrids of temperate maize were evaluated and it was found that variation for all the carotenoids was more attributable to GCA than to SCA effects, indicating a major role for additive gene action (Egesel *et al.,* 2003a). No such

information on gene action for tropical maize hybrids carotenoids was obtained.

2.4 Heritability and Genetic Gain for Carotenoid and Agronomic Traits in Maize

Heritability estimates are useful in predicting gain from selection and comparing the gain from selection under different experimental designs for optimal breeding strategies (Hallauer and Carena, 2009). Heritability can also be used, based on different family structures derived from the same base population, to determine the best family structure for maximizing genetic gain over unit of time, thus, heritability may vary among populations. Its estimates from different populations can be useful for choosing appropriate base populations in which selection will be most effective (Hallauer *et al.*, 2010). Efficiency of selection depends on heritability of the trait under consideration, which can be estimated from evaluation trials based on progeny mean or by imposing mating designs on the population(s) of interest to calculate the genetic components (Hallauer, 2007; Hallauer and Carena, 2009).

Inheritance of carotenoid content has been a topical issue in various crops like maize (Hauge and Frost, 1928; Ford, 2000), tomatoes (Stommel and Haynes, 1994), wheat (Santra *et al.,* 2005), and cassava (Akinwale *et al.,* 2010). In general, carotenoid content is heritable and thus can further be improved through plant breeding. The effect of maternal contribution to maize endosperm carotenoid was reported by Egesel *et al.* (2003b) to be supposedly due to the diploid contribution of the mother plant to the triploid nature of the endosperm. The effect of environment on carotenoids was also found to be little (Egesel *et al.,* 2003a; Menkir and Maziya-Dixon, 2004; Menkir *et al.,* 2008), indicating possibility of

developing maize hybrids with consistently higher levels of Provitamin A. Chander *et al.* (2008b) reported heritability estimates observed for nutritional traits in maize to be medium (65.6% for protein) to high (92.5% for R and γ tocopherol ratio). Similar results reported earlier were within the range (Galliher *et al.,* 1985; Wong *et al.,* 2004; Clark *et al.,* 2006).

Chander *et al.,* (2008b) obtained for high heritability estimates for carotenoid (84-96%) whereas Wong *et al.* (2004) reported medium to high levels of heritability for fractions of carotenoid (48-87%). The genetic advance as percent of mean (GAM) for nutritional traits of maize kernels ranged from 6.1% for starch to 222.5% for β-carotene (Chander *et al.,* 2008b). The higher amount of GA as percent of mean for carotenoids and tocopherols suggested possibility of maximum scope of improvements for these nutritionally important compounds.

Since heritability estimates must refer to a defined population of genotypes and specified population of environments (Nyquist, 1991), there will be need to study heritability of the different carotenoids of the maize breeding populations developed by IITA for PVA biofortification activities. This is expected to provide guide for effective selection and populations development.

2.5 Correlations Among the Different Carotenoids Contents in Maize

Concurrent improvement of the various carotenoids and agronomic traits is important to maximize health benefits derived from yellow maize without compromising grain yield. Genetic correlations are useful if indirect selection gives greater response to selection for a trait than direct selection for the same trait. This usually happens when the target/primary

traits are less heritable (Diallo *et al.,* 1989; Hallauer, 2007). Several studies have been reported on correlations among various traits in maize (Bernardo, 1996; Carena and Cross, 2003). In tropical-adapted maize inbred lines, lutein was found to be significantly correlated with α-carotene while correlations among α-carotene with the β, β-ring provitamin A carotenoids, β-cryptoxanthin and β-carotene, were weak but significant (Menkir *et al.,* 2008). Zeaxanthin, α,β, ring carotenoid, was reported to be significantly correlated with β-cryptoxanthin and β-carotene ($r = 0.43$ to $r = 0.70$, $p < 0.0001$) (Menkir *et al.,*2008*).* Lower level of positive association was found between non-Provitamin A carotenoids (lutein and zeaxanthin). The correlation between β-cryptoxanthin and β-carotene was also significant and positive. Brunson and Quackenbush (1962), Egesel *et al.* (2003a) and Chander *et al.* (2008b) reported similar findings in different temperate maize inbred lines.

2.6 Methods of Genetic Studies in Crops

2.6.1 Mating designs

The estimation of variance components in genetically broad-based populations provides the breeders information on genetic structure. As a consequence, breeding methodologies that are adequate to each population based on the genetic information are estimated. From the analysis of variance of experimental designs, expectations are expressed in terms of the appropriate components of variance; from the components of variance, translations are made to the appropriate relationships (covariances) of relatives based on the mating design used (Searle, 1971; Hallauer *et al.,* 2010).

Some mating designs are used more extensively than others, but each has its own advantages and disadvantages depending on the reference population under consideration and the information desired. Common mating designs include North Carolina design I, II and III introduced by Comstock and Robinson (1948). Others include diallel mating designs introduced by Sprague and Tatum (1947) which is the most commonly used mating designs in maize.

2.6.2 Diallel

Diallel mating designs permit estimation of the magnitude of additive and non-additive components of heritable variance (Griffing, 1956; Mather and Jinks, 1971). Diallel mating involves making crosses in pairs for n number of parents in a similar fashion to NC design II. However, the main difference with design II is that the same individuals are used as parents for the diallel mating design (individuals are used both as male and female). There are four methods of producing progenies in diallel design as demonstrated by Griffing (1956)

a) Method I = n^2. It includes all possible crosses and parents.

b) Method II = n(n+1)/2. This method is the most widely used and it includes one set of crosses and the parents (no reciprocals).

c) Method III = n(n−1). It includes two sets of crosses without parents.

d) Method IV = n(n−1)/2. It only includes one set of crosses with neither reciprocals nor parents.

The appropriateness of the various kinds of diallel crossing methods depends on the experimental material and the objectives of the experiment (Griffing, 1956). According to Griffing (1956) "When information on general and specific combining ability for a specific set of lines is desired in connection with a plant or animal breeding problem, experimental methods 3 or 4 are most applicable. In plant material, if it can be assumed that there will be no genotypic reciprocal effects (this is usually the case) method 4 is most suitable. If there is any doubt about whether or not reciprocal genotypic effects will occur, then both sets of F_1's should be included and diallel crossing method 3 used. Data obtained from diallel crosses are analysed based on the procedure proposed by Hayman (1954) and Griffing (1956). If 10 or less number of parents are included in the diallel crosses, a randomized complete block design should be satisfactory in most instances where uniform land is present (Griffing, 1956). Incomplete block designs should be considered if the number of crosses and the environmental variability among experimental units are large.

2.6.3 Model I vs Model II

There are four sets of assumptions which can be considered with regard to the variety (genotypic) and block effects (Griffing, 1956). These are (1) the variety and block effects are constants, (2) the variety effects are random variables and the block effects are constants, (3) the variety effects are constants, and the block effects are random variables, and (4) the variety and block effects are both random variables. The first and last two classes of models have been designated as models I and II respectively by Eisenhart (1947). The second and third sets of assumptions lead to mixed models which we designate as mixed A and mixed B respectively.

In model I the experimental material is to be regarded as the population about which inferences are to be made. The objectives are to compare combining abilities of the parents when the parents themselves are used as testers, and to identify the higher yielding combinations. Thus the interest is in estimating combining ability effects and computing appropriate standard errors for differences between effects.

In model II the assumption is that we are dealing with random samples from some parent population, and the inferences are not to be made about the individual lines in the sample but about the parameters in the parent population. The interest is in estimating the genetic and environmental components of the complex population variances (Griffing, 1956).

CHAPTER 3: MATERIALS AND METHODS

3.1 Genetic Materials and their Pedigrees

Yellow maize inbred lines developed at IITA, Ibadan were used for this research. The inbred lines were derived from tropical populations and back crosses with temperate germplasm. The materials had been tested across the several ecologies in Nigeria and selected for medium to high Provitamin A contents and adaptability to the tropics (Menkir *et al.*, 2008). Table 2 shows the 31 inbred lines used for this study, their descriptions and pedigrees. The materials were increased through selfing and sib-mating by hand pollination in the 2010 and 2011 wet seasons at Institute for Agricultural Research (IAR) Farm, Samaru (Appendices 1 and 2). The inbred lines with sufficient seeds from the 2010 seed increase were evaluated for agronomic performance in the 2011 wet season in a randomised complete block design with two replications using one row plot of 5.0m long and 0.75m inter-row spacing. The result is presented in Appendix 3. In September to December 2011 the lines were analysed for genetic diversity using SSR markers and functional markers of *lcyE* (Harjes *et al.*, 2008) and *crtRB1* (Yan *et al.*, 2010). The functional markers were used to genotype nine inbred lines selected from the 4 clusters derived from the SSR based genotyping of the 31 maize inbred lines (Figure 2). This was done by randomly selecting two inbred lines from each of clusters 1, 2, and 3 and 3 lines from cluster 4. The nine selected lines were used for population development for inheritance studies. Details of the procedures are described sections 3.2 – 3.6.

Table 2. Seed description and pedigree of 31 maize inbred lines with medium to high Provitamin A (PVA) contents.

S/No	Code Name	Pedigree	Seed type	Seed color	β-carotene (µg/g)	*Provitamin A (µg/g)
1	E1	(9450xCM 116x9450)-3-3-1-2-1-B-B-B-B-B-B-B	dent	orange	4.749	7.752
2	E2	9450xKI 21-1-4-1-2-1-B-B-B-B-2-B-B-B	flint	orange	2.501	4.506
3	E3	9450xKI 21-1-4-1-2-1-B-B-B-B-1-B-B-B	dent	orange	2.501	4.506
4	E5	TZMI214 x A619LPA x TZMI214-10-3-B-B-B-B-B-B	flint	orange	3.613	4.961
5	E6	SYN-Y-STR-34-1-1-1-1-2-1-B-B-B-B-B-B-B-B-B-B	flint	orange	8.277	9.792
6	E7	9450xKI 21-7-3-1-2-5-B-B-B-B-B-B	flint	orange	3.071	5.085
7	E8	9450xKI 21-7-2-2-1-1-B-B-B-B-B-B	flint	orange	3.838	5.877
8	E9	(9071 x 4058)-8-2-1-1-B-B-B-B-B-B	dent	orange	4.579	7.872
9	E10	KU1409/KU1414-SR/CI187-B-B-B-B-B-B	flint	orange	2.535	4.229
10	E11	KU1414-SR/KVI43-4-1-B-B-B-B-B-B	flint	orange	2.799	4.573
11	E12	KU1414-SR/KVI43-6-1-B-B-B-B-B-B	flint	orange	2.854	5.051
12	E14	(9450 x KI 28)-5-1-1-1-B-B-B-B-B-B-B	flint	orange	2.521	4.688
13	E16	KU1414-SR/KVI43-6-4-B-B-B-B-B-B	flint	orange	3.019	5.287
14	E18	KU1409/KU1414-SR/SC55-B-B-B-B-B-B	flint	orange	2.27	4.307
15	E19	ACR97TZL-CCOMP1-Y-S3-33-5-B-B-B-B-B-B-B-B	flint	orange	2.275	4.052
16	E20	ACR97TZL-CCOMP1-Y-S3-13-1-B-B-B-B-B-B-B-B	flint	orange	2.586	4.511
17	E21	9450xKI 21-1-4-1-1-2-B-B-B-B-B-B-B	flint	orange	2.385	5.317
18	E22	POP 61-SR - 11-2-3-3-1-B-B-B-B-B-B-B-B	flint	orange	3.027	4.703
19	E23	(MP420 x 4001 x MP420)-3-1-3-1-B-B-B-B-B-B-B-B	flint	deep orange	2.818	4.309
20	E24	9450xKI21-1-5-3-2-1-B-B-B-B-B-B-B	flint	orange	3.006	5.484
21	E25	9450xKI21-1-5-3-2-2-B-B-B-B-B-B-B	flint	orange	3.101	5.951

Table 2. Continued

S/No	Code Name	Pedigrees	Seed type	Seed color	β-carotene (µg/g)	*Provitamin A (µg/g)
22	E26	KU1409 x MO17LPA x KU1409-11-4-1-B-B-B-B-B-B	flint	orange	2.058	4.178
23	E27	9450xKI 21-3-1-1-2-1-B-B-B-B-B-B-B	flint	orange	1.867	4.157
24	E28	KU1414-SR/KVI11-7-1-B-B-B-B-B-B	flint	orange	3.243	5.736
25	E30	ACR97SYN-Y-S1-27-B-B-B-B-B-B-B-B	flint	orange	2.386	4.271
26	E31	ACR97SYN-Y-S1-38-B-B-B-B-B-B-B-B	flint	deep orange	2.567	4.044
27	E32	KU1409 x MO17LPA x KU1409-27-3-1-1-B-B-B-B-B-B-B-B	flint	orange	2.815	4.881
28	E33	KU1409 x MO17LPA x KU1409-27-3-1-1-B-B-B-B-B-B-B	flint	orange	2.815	4.881
29	E35	(GT-MAS:Gk x BABANGOYO x GT-MAS:Gk)-1-1-1-3-B-B-B-B-B-B-B-B-B	flint	orange	2.062	3.167
30	E36	(MP420 x 4001 x MP420)-3-1-3-1-B-B-B-B-B-B-B-B-B	flint	orange	2.148	3.52
31	E37	KU1409 x MO17LPA x KU1409-27-3-4-1-B-B-B-B-B-B-B	dent	orange	2.264	3.426

*Typical yellow maize varieties have 0.5 to 1.5 µg/g PVA content (Harjes *et al.*, 2008); Medium PVA ≥ 3.0 ≤ 5.5 µg/g, High PVA >5.5 µg/g.

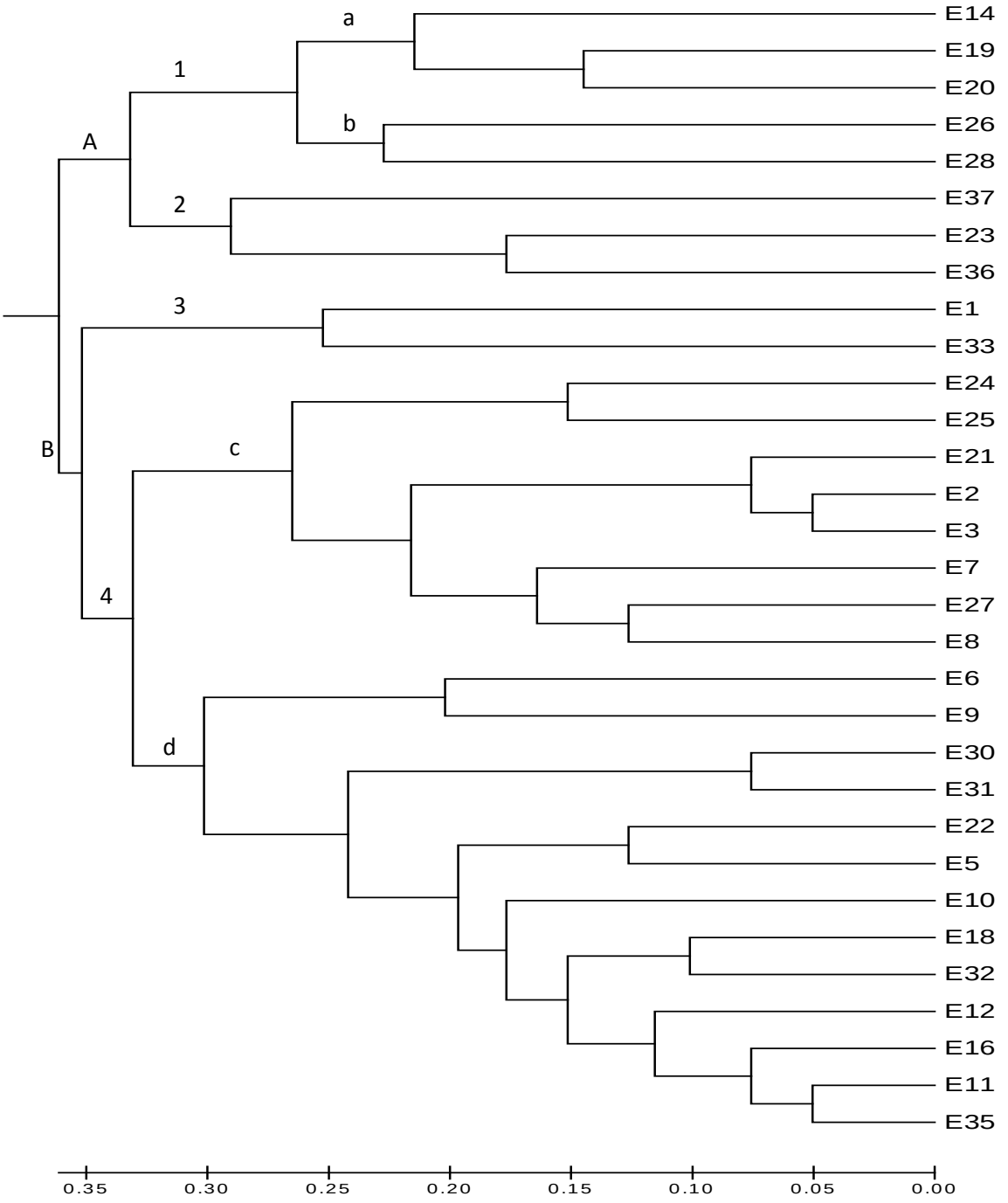


Figure 2: Dendrogram of 31 tropical-adapted maize inbred lines containing medium to high Provitamin A content. Relationships were based on the Euclidean genetic distances calculated using 14 polymorphic simple sequence repeat (SSR) markers

3.2 Simple Sequence Repeat –Polymerase Chain Reaction Analysis

Seeds of the 31 maize inbred lines were germinated in a screen house at room temperature using normal garden soil (comprising of topsoil, sand and organic matter in ratio 3:2:1). Approximately 5 cm fresh leaf tissue of two weeks old individual seedlings were excised. Sampled leaves were freeze-dried in eppendorf tubes at -80°C for total genomic DNA extraction. DNA was isolated from 5 individual seedlings per sample using cTAB method (Dellaporta *et al.,* 1983) as modified by George and Regalado (2004). The quality and quantity of DNA was assessed using NanoDrop spectrophotometer (ND-1000® Technologies, Wilmington, Delaware, USA). Extracted DNA concentrations were normalised to 25ng/µl.

3.2.1 SSR-PCR amplification and electrophoresis

A total of 14 SSR maize primers were used for the PCR amplification of the extracted genomic DNA. The primers were selected from the 52 core SSR primers of maize based on their Bin numbers to cover the 10 chromosomes in maize after Warburton *et al.* (2002) and Adeyemo *et al.* (2011). Names and chromosome loci (Bin number) of the SSR primers used are presented in the results section (Chapter 4). The primers were diluted to a working concentration of 5 µM with sterile water and stored at -20°C. PCR amplification was carried out in a 25 µl reaction mixture containing 1 µl template DNA, 13.2 µl H_2O, 5.6 µl dNTP (1.25mM), 2.6 µl Taq buffer (10X), 2.0 µl $MgCl_2$ (2mM), 0.4 µl primer (5 µM) and 0.2 µl Taq polymerase (5u/ µl). The PCR program was 4 minutes preheating and initial denaturation at 94^{o}C; then nine cycles of annealing at the higher annealing temperature decreased by -1 ^{o}C per cycle followed by 26 cycles of annealing at constant lower

temperature of the "Touch Down" annealing temperature range (65-55°C). The extension was run at 72°C for 1.30 minutes at each cycle and a final extension of seven minutes at 72°C (Appendix 5). The PCR reactions products were stored at 4°C until electrophoresis.

The amplified DNA fragments were separated on 2% (w/v) superfine agarose gels (Amresco) ran at 100 V for 2 hours. The gel was stained by soaking in 10 mg/ml ethidium bromide solution for one minute. The stained gels were photographed under UV transilluminator by a gel documentation system (Bio-Rad, Hercules, CA). Clear polymorphic SSR fragments (bands) were scored in a binary form of 1 or 0 for presence or absence of a band, respectively.

PowerMarker software (Liu and Muse, 2005) was used to calculate standard diversity statistics of observed heterozygosity, gene diversity (D or expected heterozygosity), mean number of alleles per locus (A) and polymorphic information content (PIC). Data used were from 14 SSR primers found to be polymorphic (which are reported to be adequate to uniquely fingerprint and distinguish inbred lines (Liu *et al.*, 2003)).

Gene diversity at a given locus was calculated as:

$$D = (1-\sum p^2_i)/ (1-(1-F_{IS}/n))$$

Where p_i is the frequency of the allele i, F_{IS} is the population inbreeding coefficient estimated from genotype frequencies and n is the sample size (Weir, 1996).

The PIC of a given primer, which is closely related to *D*, provides an estimate of the discriminatory power of a primer and was calculated according to Bolstein *et al.* (1980) as:

$$PIC = 1-\sum p^2_i -\sum \sum 2p^2_i p^2_j$$

Where p_i and p_j are the allele frequencies of alleles *i* and *j*, respectively.

Pattern of the genetic diversity was assessed using cluster analysis and Principal Co-ordinate Analysis (PCoA). The cluster analysis is implemented in the PowerMarker V3.25 software which estimated Euclidean genetic distance (Appendix 4) from allele frequencies (Kaufman and Rousseeuw, 1990). The genetic matrices generated by the Euclidean distance were used to reveal the patterns of genetic relationships among the lines. Dendrograms were constructed based on unweighted pair group method with arithmetic averages (UPGMA) using the software MEGA version 5 (Tamura *et al.,* 2011).

3.3 *lycE* and *crtRB1* PCR Analysis

Extracted DNAs of the selected nine maize inbred lines were amplified using functional markers. The primer combinations for *lcyE* and *crtRB1* (Table 3) were used for genotyping the nine selected lines. The PCR and gel-electrophoresis conditions were conducted according to Harjes *et al*. (2008) and Yan *et al*. (2010). Detailed PCR conditions for the different markers are presented in Appendix 5. Generally, the PCR program was four minutes preheating and initial denaturation at 94^{o}C; then nine cycles of annealing at the higher annealing temperature decreased by -1 ^{o}C per cycle followed by 26 cycles of annealing at constant lower temperature of the "Touch Down" annealing temperature ranges. Variable "touch down" annealing temperatures (65-55°C, 70-63°C and 60-50°C) were used for the different functional markers. The extension was run at 72°C for 1.30 minutes at each cycle and a final extension of seven minutes at 72°C. The PCR reactions products were stored at 4°C until electrophoresis. The amplified DNA fragments were separated on 2% (w/v) superfine agarose gels (Amresco) ran at 100 V for

two hours. The gel was stained by soaking in 10mg/ml ethidium bromide solution for one minute. The stained gels were photographed under UV transilluminator by a gel documentation system (Bio-Rad, Hercules, CA). The resultant amplicons (bands) were scored according to Harjes *et al.* (2008) and Yan *et al.* (2010).

3.4 Population Development

The nine selected inbred lines from a population of 31 medium to high PVA maize lines were used as parents in population development using half diallel mating design method 4 in which only crosses were considered. The lines which were used as both male and female parents were planted in pairs of male and female as designed using maize fieldbook v8.5.1 software (www.cimmyt.cgiar.org /assessed 2011). They were planted at IAR Samaru on 13 and 20 December, 2011 with one week interval to allow synchrony of anthesis and silking (flowering) among the plants under irrigation. Thirty six crosses were made (no selfs and no reciprocals), using half diallel $n(n-1)/2$, where n is the number of parents involved (Griffing, 1956; Hallauer *et al.,* 2010), by repeating each parent for each combination of crosses and making paired-row crosses by hand pollination. The seeds produced on each parent was bulked for each cross-combination. The crosses were harvested on 17 May, 2012. The harvested progenies (F_1) were used for field evaluation in 2013 rainy season.

Table 3. The functional markers of *lcyE* (Harjes *et al.*, 2008) and *crtRB1* (Yan *et al*., 2010) used for genotyping nine selected Provitamin A maize inbred lines in 2011

Gene	Polymorphic site/Markers	Primer names	Sequences
lcyE (Harjes *et al.*, 2008)	*lcyE* 5'TE	TE103PF F-1	CGCTAGCAAGCCCATTATTTTTA
		TE103PR R-1	CGGTATGGTTTTTGGTATACGG
		TE105PR R1	GAGAGGGAGACGACGAGACAC
		ZGt111204-976R(F1)	AAGCATCCGACCAAAATAACAG
	lcyE SNP (216)	S216-L1	GCGGCAGTGGGCGTGGAT
		S216-R1	TGAAGTACGGCTGCAGGACAACG
	lcyE 3'indel	3pINDL-L1 LEFT	GTACGTCGTTCATCTCCCGTACCC
		3pINDL-R1	CTTGGTGAACGCATTTCTGTTGG
		3pINDL-L2	GGACCGGAACAGCCAACTG
		3pINDL-R2	GGCGAAATGGGTACGGCC
crtRB1 (Yan *et al.*, 2010)	*crtRB1* 5'TE	crtRB1 HF	TTAGAGCCTCGACCCTCTGTG
		crtRB1 HR	AATCCCTTTCCATGTTACGC
	crtRB1 InDel4	crtRB1 DF	ACCGTCACGTGCTTCGTGCC
		crtRB1 D4R	CTTCCGCGCCTCCTTCTC
	crtRB1 3'TE	crtRB1 65F	ACACCACATGGACAAGTTCG
		crtRB1 62R	ACACTCTGGCCCATGAACAC
		crtRB1 66R	ACAGCAATACAGGGGACCAG

3.5 Field Evaluation

The 36 crosses developed and two commercial yellow hybrid maize designated as checks 1 and 2 (Obasuper2 and Obasuper4, respectively) were evaluated in 2013 wet season for agronomic traits under field conditions at Samaru (N11°11.297', E07°37.078', 673m) and Kerawa (N10°59.145', E07°25.081', 605m). Each experiment was conducted using randomized complete block design with two replications. Entries were planted in one row plots (5.00 x 0.75 m). Fertilizers (120kg N, 60kg P_2O_5 and 60kg K_2O per hectare) were applied using NPK 15:15:15 and urea. P_2O_5 and K_2O were applied at planting while N was applied in two split doses at planting and at five weeks after planting. The experiments were kept weed-free by application of 5.0 litres per hectare each of a mixture of gramaxone, a contact herbicide and atrazine, a pre-emergence herbicide. Subsequently, manual weeding was performed four weeks after sowing. The first 2-3 plants per plot were self-pollinated by hand and their seeds harvested separately for carotenoids analyses. Field data on agronomic traits were taken on plot basis as described below.

1. Days to Anthesis (DA): The number of days from planting to the day when 50% of the plants have their tassels emerged.
2. Days to Silk (DS): The number of days from planting to the day when 50% of plants have their silks emerged.
3. Plant Height (PH): Average height of 5 plants in centimeters (cm) from the base of the plant to where tassel branching begins.
4. Ear Height (EH): Average height of 5 plants in cm from the base of the plant to

the node bearing the upper ear.

5. Grain Yield (GY): In Kg/plot converted to tons per hectare at 15 % moisture.
6. Anthesis-Silking Interval (ASI): the difference between DA and DS.
7. Ear to Plant height ratio (EPH): the ratio of EH to PH.
8. Number of Ears per plant (EPP): the ratio of number of ears to number of plants harvested.

Mean performance of each entry was calculated from the replicated plot means for the traits.

3.6 Carotenoid Analysis Using HPLC

Ten grams of random samples of 20 to 30 seeds of each of the self-pollinated ears were freeze-dried at -80 ^{0}C, ground to a fine powder (0.5µm) and used for carotenoid analysis using high performance liquid chromatography (HPLC). The extraction protocol used was the method of Granado *et al.* (2001), as modified by Menkir *et al.* (2008) for analysis of dried maize kernels.

3.6.1 Carotenoids extraction

Fine powder of 1.0 g of the ground selfed seeds was transferred to a 40 ml screw-capped test tube. Then 6.0 ml of methanol (MeOH) (0.01% BHT) and a magnetic stirrer were added, and the tube was loosely capped. Multiple test tubes were placed in a beaker of water and incubated with stirring on a magnetic hotplate for 15 minutes at 50 ^{0}C. After cooling to room temperature, 6.0 ml of tetrahydrofuran (THF) were added to each test tube, and the tube was vortexed for 90 seconds. The test tubes were then allowed to stand for five

minutes to allow the fine particles to settle. Aliquot of 0.5 ml of the MeOH/THF extract was transferred to a 15 ml screw capped test tube. Then 1.0 ml of 40% KOH in MeOH, containing 0.1 M pyrogallol, was added. The tube was flushed with argon, tightly capped, and vortexed for three minutes. To each tube, 2.0 ml of HPLC-grade water were added and the tube was vortexed for 30 seconds. An internal standard, bapo-80-carotenal, in MeOH was added. The carotenoids were partitioned into 4.0 ml of hexane/methylene chloride (5:1 by volume containing 0.01% BHT) by vortexing for 60 seconds. The sample was centrifuged for five minutes at 700xg to obtain visible phase separation, and the upper phase was dried under vacuum.

3.6.2 HPLC analysis

The dried extract containing total carotenoids was re-dissolved in 100 µl of methyl-tert-butyl ether (MTBE), followed by 300 µl of MeOH. A 100 µl aliquot was injected into the HPLC system for analysis of α-carotene (αC), β-carotene (βC) (cis and trans isomers), β-cryptoxanthin (βCX), lutein (LUT), and zeaxanthin (ZEA) contents. The HPLC components included a 717 Plus autosampler with temperature control set at 5 ^{o}C, two 515 solvent-delivery systems, and a 2996 photodiode array detector (Waters Corporation). The system was operated with Empower 1 Software (Waters Corporation). Carotenoids were separated on a 5.0 µm C30 Carotenoid Column (4.6 x 250 mm; Waters Corporation) eluted by a mobile phase gradient from 100% methanol (containing 1.0 g ammonium acetate/l) to 100% MTBE over 60 minutes. The flow rate was 1.0 ml/minute. Solvents were HPLC grade. To maximize detection of carotenoids, absorbance was measured at 453 nm. Lutein, ZEA, βCX and βC were identified through their characteristic spectra and comparison of

their retention times with known standard solutions. Total Provitamin A content (PVA) was calculated for each sample as the sum of βC plus one-half of βCX and one-half of αC. This was on the basis of molecular structures; αC and βCX are considered to have 50% of the Provitamin A activity of βC (U.S. Institute of Medicine, 2001).

3.7 Data Analysis

3.7.1 Analysis of variance, GCA and SCA

Analyses of variance (ANOVA) for the crosses (with and without check) for each location and for combined locations were performed on plot means for all measured agronomic traits and the carotenoids (αC, βC, βCX, LUT and ZEA and PVA). Fisher's LSD was used for the mean separation. Diallel analysis of variance and estimation of the general combining ability (GCA) of parents and specific combining ability (SCA) of crosses for three agronomic traits (DA, PH and GY) and for all the measured carotenoids were performed using Griffing Method 4 Model I (Griffing, 1956). The forms of ANOVA and expected mean squares for each location and for the combined locations are, respectively, presented in Tables 4and 5 for diallel Method 4 Model I. The analyses were performed for each trait using the linear model:

$X_{ijk} = \mu + r_k + g_i + g_j + s_{ij} + e_{ijk}$ for single location.

For multi-location diallel analysis the model is:

$X_{ijk} = \mu + r(l) + l + g_i + g_j + s_{ij} + l*g_i + l*g_j + l*s_{ij} + e_{ijk}$

$g_i = \frac{1}{p(p-2)}(pX_{i.} - 2X..)$

$$s_{ij} = x_{ij} - \frac{1}{p-2}\left(X_{i.} + X_{j.}\right) + \frac{2}{(p-1)(p-2)} \mathrm{X}_{..}$$

where X_{ijk} is the observation for the cross between parents i and j in replication k (i.e. replicate 1 or 2), μ is the grand mean, r_k is the replication effect, g_i and g_j are the GCA effects, s_{ij} is the SCA effect, $l*g_i$ and $l*g_j$ are location by GCA effects interactions, $l*s_{ij}$ is location by SCA effect interaction, *r(l)* is replication nested within location effect, l is the location effect and e_{ijk} is the experimental error for the X_{ijk} observation (k = 1, 2;

$i = j$ = 1, 2,..,9), p is number of parents.

The ratio of GCA:SCA mean squares indicating whether a character is controlled by additive or non additive gene was calculated for the lines.

The GCA effect variance associated with the ith line was estimated as follows:

$$\sigma^2{}_{gi} = g_i^2 - \frac{p-1}{p(p-1)}\sigma^2$$

Table 4. Form of Analysis of Variance (ANOVA) of half diallel set of crosses among n parents evaluated in one location based on Griffing Method IV, Model I

Source of Variation	dfa	MS	EMS
Replications	$r-1$		
Crosses	$[n(n-1)/2]-1$	$M2$	$\sigma^2 + rK^2_c$
GCA	$n-1$	$M21$	$\sigma^2 + [r(n-2)/(n-1)]K^2_{gi}$
SCA	$n(n-3)/2$	$M22$	$\sigma^2 + \{2r/[n(n-3)]\} K^2_{sij}$
Error	$(r-1)\{[n(n-1)/2]-1\}$	$M1$	σ^2
Total	$r[n(n-1)/2]-1$		

r = number of replications, n=number of parents, K_c=variance for crosses effects, σ^2=error variance, K_{GCA}= GCA effects variance, K^2_{SCA}=SCA effects variance, (Source: Hallauer *et al*., 2010).

Table 5. Form of Analysis of variance (ANOVA) of half diallel set of crosses among n parents evaluated in multiple locations based on Griffing Method IV, Model I

Source of Variation	Df	MS	Model I (EMS)
Environments (L)	l−1		
Replications/L	l(r−1)		
Crosses (C)	[n(n-1)/2]−1	M3	σ^2 + $r\sigma^2$lc+ lrK^2c
GCA	n−1		
SCA	n(n−3)/2		
C × L	(l−1){[n(n-1)/2]−1}	M2	σ^2 + $r\sigma^2$lc
GCA × L	(l−1)(n−1)		
SCA × L	(−1)[n(n−3)/2]		
Pooled error	l(r−1){[n(n-1)/2]−1}	M1	σ^2
Total	[lrn(n-1)/2]−1		

r = number of replications, n=number of parents, K_c=variance for crosses effects, σ^2=error variance, K_{GCA}= GCA effects variance,K^2_{SCA}=SCA effects variance, σ^2lc= crosses by location interaction variance.

(Source: Hallauer *et al*., 2010).

Heritability estimates were obtained as follows:

For single location:

$$H^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma^2}$$

For combined location:

$$H^2 = \frac{\sigma_c^2}{\frac{\sigma_c^2}{rl} + \frac{\sigma_{lc}^2}{r} + \sigma^2}$$

Correlations

Correlation coefficients were estimated to determine degree of association between traits and how they may enhance selection. The estimates were determined according to Singh and Chaudhary (1985) and Evans *et al.* (2002).

$$\text{Genotypic correlation } (r_{gxixj}) = \frac{\sigma_g^2 xi.xj}{\sqrt{(\sigma_g^2 xi)(\sigma_g^2 xj)}}$$

Where $\sigma_g^2 xi.xj$ is the genotypic covariance between two traits i and j, $\sigma_g^2 xi$ is the genetic variance of trait i and $\sigma_g^2 xj$ is the genetic variance of trait j.

The phenotypic correlation coefficients are also calculated as:

$$\text{phenotypic correlation } (r_{pxixj}) = \frac{\sigma_p^2 xi.xj}{\sqrt{(\sigma_p^2 xi)(\sigma_p^2 xj)}}$$

Where $\sigma_p^2 xi.xj$ is the phenotypic covariance between two traits i and j, $\sigma_p^2 xi$ is the phenotypic variance of trait i and $\sigma_p^2 xj$ is the phenotypic variance of trait j.

Phenotypic and genotypic correlations among the traits were estimated using program based on covariances (Holland *et al.*, 2003) in SAS software version 9.2 (SAS Institute, 2001).

Path analysis

The cause and effect relationships between PVA and other carotenoids were estimated and decomposed into direct and indirect effects using Path analysis according to Wright (1934). Path coefficients were calculated from the following simultaneous equations using inversion of genotypic correlations matrix in Microsoft Excel.

$r(x_1,Y)=a+ r(x_1 x_2)b+ r(x_1 x_3)c+ r(x_1 x_4)d+ r(x_1 x_5)e$

$r(x_2,Y)=a(x_2x_1)+ b+ r(x_1 x_3)c+ r(x_1 x_4)d+ r(x_1 x_5)e$

.

CHAPTER 4: RESULTS

4.1 Genetic Diversity

4.1.1 SSR markers

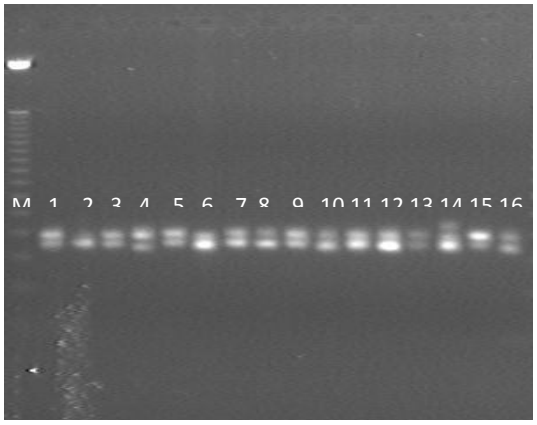
The results of analysis of the 31 yellow maize inbred lines for genetic diversity using 14 SSRs markers is presented in Table 6. Electrophoregram of six of the 14 markers (phi034, phi053, phi059, phi064, nc133 and Umc1136) are shown in Plate 1. A total of 33 alleles or average of 2.36 alleles per locus were compared among the lines. The gene diversity (D) of all the SSRs ranged from 0.22 to 0.60 with a mean of 0.45 indicating moderate genetic diversity among the lines. The polymorphic information content (PIC) ranged from 0.20 to 0.53 with a mean of 0.37 showing the extent of discrimination achievable using these SSR markers.

Dendrogram of the 31 tropical-adapted maize inbred lines is shown in Figure 2. The dendrogram consisted of two main groups, *A* and *B.* Each of the main groups was divided into two clusters. Main group *A* consisted of clusters *1* and *2*, group *B* consisted of clusters *3* and *4.* Cluster *1* is divided into subclusters *a* and *b.* Subcluster *a* contained line E14 *(9450* x *KI28)* developed from bi-parental cross containing the temperate line *9450.* Subcluster *a* also contained another subcluster containing lines E19 and E20 both derived from ACR97TZL-COMP1-Y population. Subcluster *b* contained E26 and E28. Cluster *2* contained E37, E23 and E36. All the lines in main group *A* contained the temperate lines KU1409 and MO17LPA in their pedigree except the lines in subcluster *a*. Main group *B* cluster *3* is

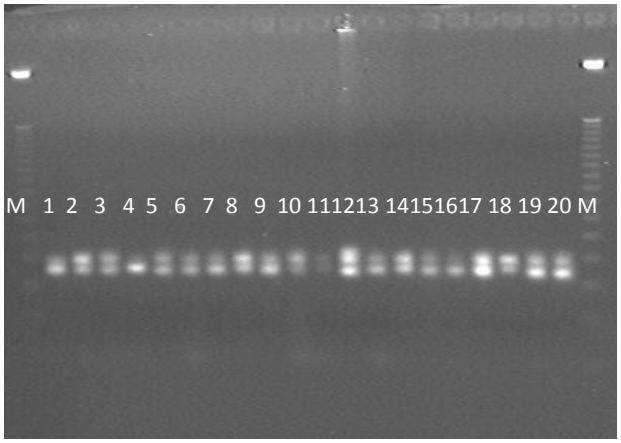
made up of lines E1 and E33. Cluster 4 contained subclusters *c* and *d.* Subcluster *c* had 3 subclusters containing lines E24 and E25 in one, lines E21, E2 and E3 in another and lines E7, E27 and E8 in the other. All the lines in subcluster *c* were developed from a population of bi-parental cross involving *9450*x*KI21*. Subcluster *d* of cluster 4 was also divided into 2 more subclusters, one contained lines E6 and E9 while the other contained lines E30 and E31 in one subcluster, lines E22 and E5 in another, and lines E18 and E32; E12, E16, E11 and E35 in the other subclusters. These were developed from diverse source germplasm of IITA and backcrosses involving temperate lines *KU1424, GT-MAS:Gk, 9071* and *4058*. The groupings, though related, showed considerable levels of genetic diversity.

Table 6. Diversity statistics for the SSR loci used to genotype 31 tropical-adapted Provitamin A maize inbred lines

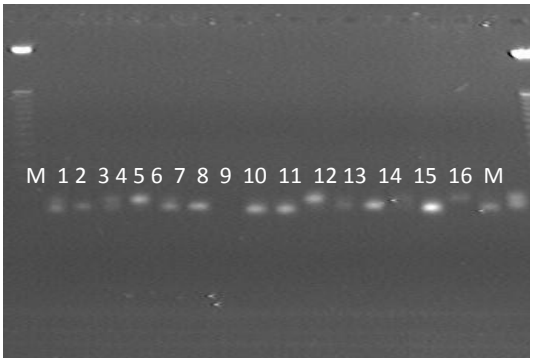
SSR locus	Bin no.	Major Allele Frequency	Number of Alleles (A)	Gene Diversity (D)	Heterozygosity	Polymorphic Information Content (PIC)
phi034	7.02	0.4677	3	0.5583	0.3548	0.4589
phi053	3.05	0.5161	3	0.6035	0.4516	0.5288
phi056	1.01	0.4839	3	0.5578	0.0645	0.4586
phi059	10.02	0.5161	2	0.4995	0.0000	0.3747
phi011	1.09	0.8710	2	0.2248	0.0000	0.1995
phi032	9.04	0.5323	2	0.4979	0.0323	0.3740
phi072	4.01	0.5323	3	0.5499	0.0323	0.4541
phi087	5.06	0.7258	2	0.3980	0.0968	0.3188
phi328175	7.04	0.7258	2	0.3980	0.1613	0.3188
phi374118	3.02	0.6452	2	0.4579	0.0645	0.3530
umc1136	3.09	0.6774	3	0.4558	0.3226	0.3773
phi064	1.11	0.6935	2	0.4251	0.1613	0.3347
nc133	2.05	0.7903	2	0.3314	0.0968	0.2765
phi029	3.04	0.7419	2	0.3829	0.0000	0.3096
Mean		0.6371	2.3571	0.4529	0.1313	0.3670



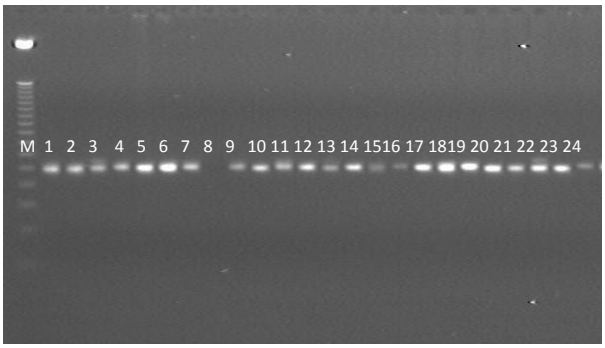
Phi034



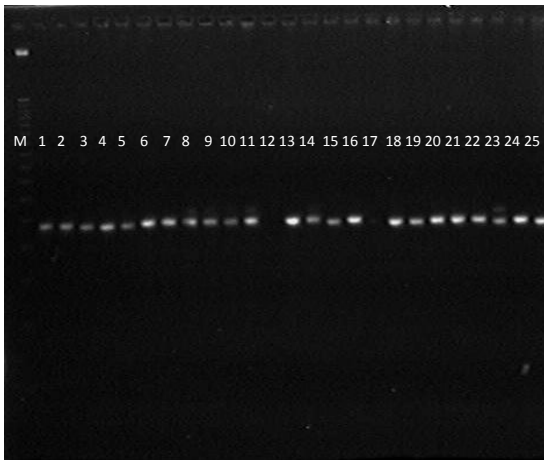
phi053



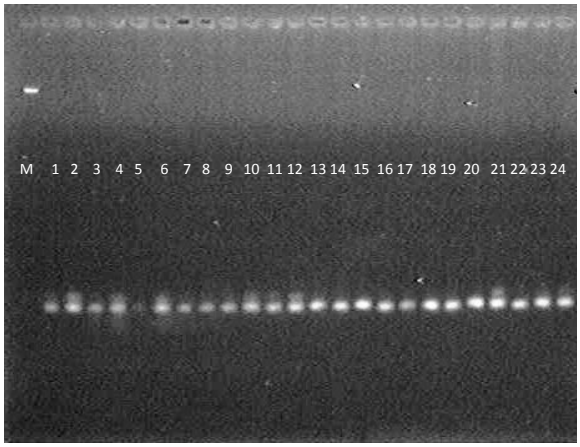
Umc1136



phi059



nc133



phi064

Plate 1. Electrophoregram of DNA fragments of six SSR primers amplification of the tropical-adapted PVA maize inbred lines. M is DNA ladder, numbers represent maize lines.

4.1.2 *lycE* and *crtRB1* markers

The polymorphisms in *lycE* and *crtRB1* marker alleles were used to obtain the genetic fingerprints and to determine presence of alleles favouring PVA build up in maize endosperm of nine selected lines. The inbred lines expressed different polymorphisms and profiles for the different alleles' markers which are read and interpreted according to Harjes *et al.* (2008) and Yan *et al.* (2010) (Table 7).

Three different amplicons (amplified DNA fragments) designated alleles *1*, *2* and *4* polymorphisms (variants) of *lcyE*-5'TE marker were observed in the 9 inbred lines screened. All the lines contained the favourable alleles for PVA (alleles *1* and *4*) with exception of line E6 (SYN-Y-STR-34) that contained the unfavorable allele *2*. The two polymorphisms of *lycE*-3'TE indel, allele *2* or Insertion (*I*) which is unfavourable and allele *2* or deletion (*D*) which is favourable, were observed. Four lines E8, E26, E7 and E16 contained the favourable *D* allele while the remaining 5 lines have the insertion type, I allele. The third *lycE* marker, *lycE*-SNP216, showed single nucleotide polymorphisms (SNPs) at two nucleotide positions: thymine (T) (favourable) and Guanine (G) nucleotide (unfavourable) in the lines. All the lines showed the favourable *T* substitution except E37 and E7. The *crtRB1* gene markers, on the other hand, expressed allele 1 (favourable) for βC accumulation in all the lines for the *crtRB1*-5'TE polymorphisms. The *crtRB1*-3'TE showed two polymorphisms as allele *1* and allele *2*. Only line E3 contained the allele *2*, all the other lines contained allele *1* favourable for βC and consequently PVA concentration. As in Harjes *et al. (*2008*),* the *crtRB1* nomenclature designated allele *2* as the unfavourable allele while the other alleles *1, 3* and *4* are considered favourable (Yan *et al.*, 2010). Electrophoregrams of the tested markers are shown in Plates 2 to 4.

Generally, all the tested lines expressed at least 3-4 favourable alleles for increasing PVA activity with different profiles. This also indicate variability in these lines with respect to functional markers in carotenoids anabolism.

Table 7. Observed polymorphisms for the functional markers in carotenoids biosynthesis (lycE and crtRB1) for 9 selected tropical-adapted PVA maize inbred lines

Line	*lycE*			*crtRB1*	
	lycE-5'TE	*lycE*-3'TE indel	*lycE*-SNP216	*crtRB1*-5'TE	*crtRB1*-3'TE
E8	4	D	T	1	1
E26	1	D	T	1	1
E6	1	I	T	1	1
E9	2	I	T	1	1
E3	1	I	T	1	2
E28	1	I	T	1	1
E37	1	I	G	1	1
E7	4	D	G	1	1
E16	1	D	T	1	1

D=deletion, I=insertion, T=thymine, G=guanine, 1, 2, 4, D and T are favorable for Provitamin A (PVA) expression in maize, 2, I and G are unfavorable for PVA.

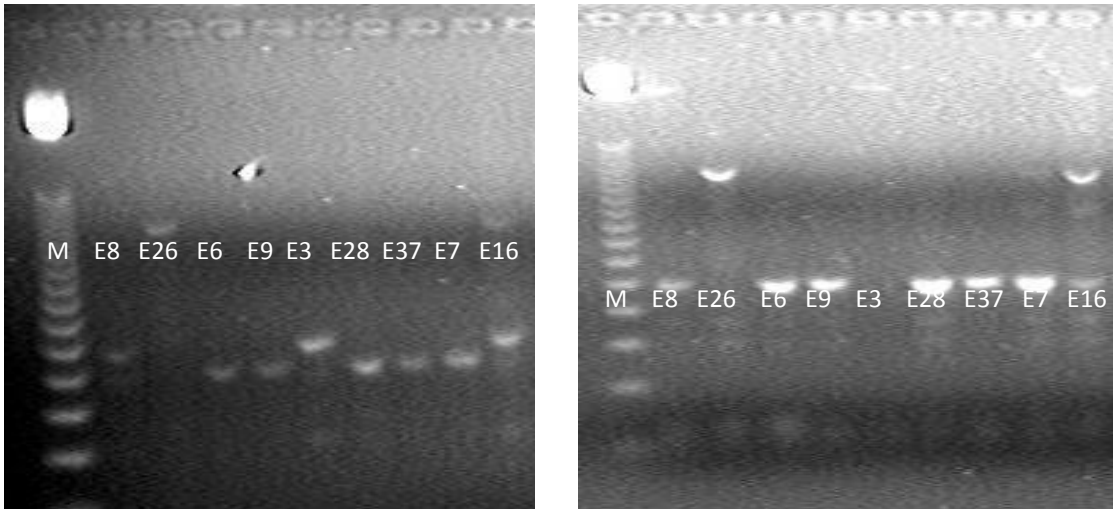


Plate 2. Electrophoregram of DNA polymorphisms in 9 tropical-adapted PVA maize inbred lines for *lycE* 5'TE using 4 primers (left) and 2 primers (right); (M=DNA ladder).

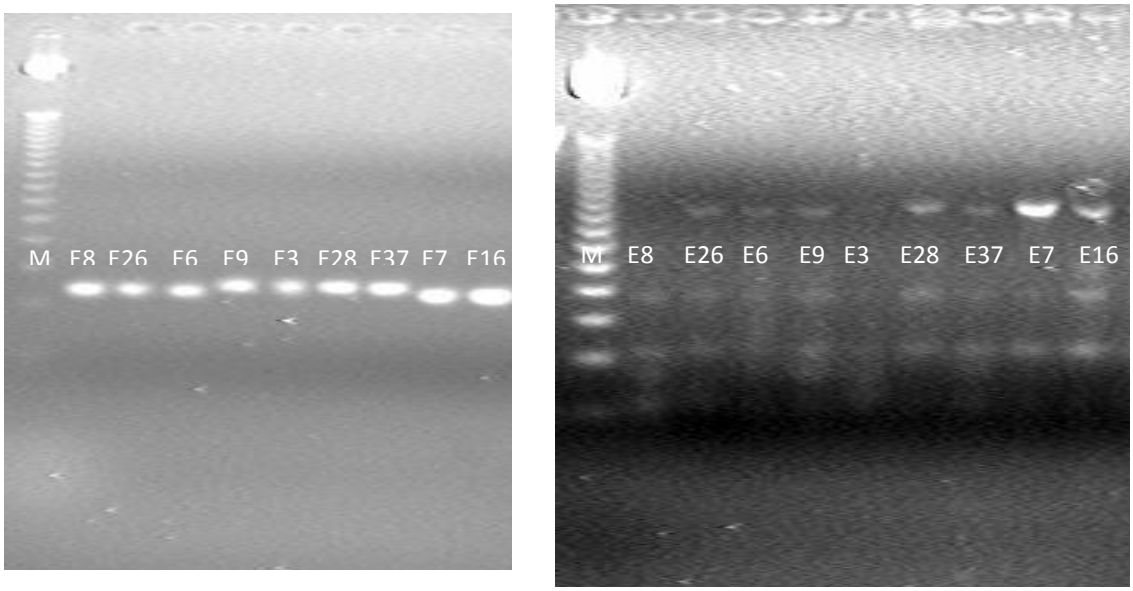


Plate 3. Electrophoregram of DNA polymorphisms in 9 tropical-adapted PVA maize inbred lines for *lycE* 3'TE (left) and *lycE* SNP216 (right); (M=DNA ladder).

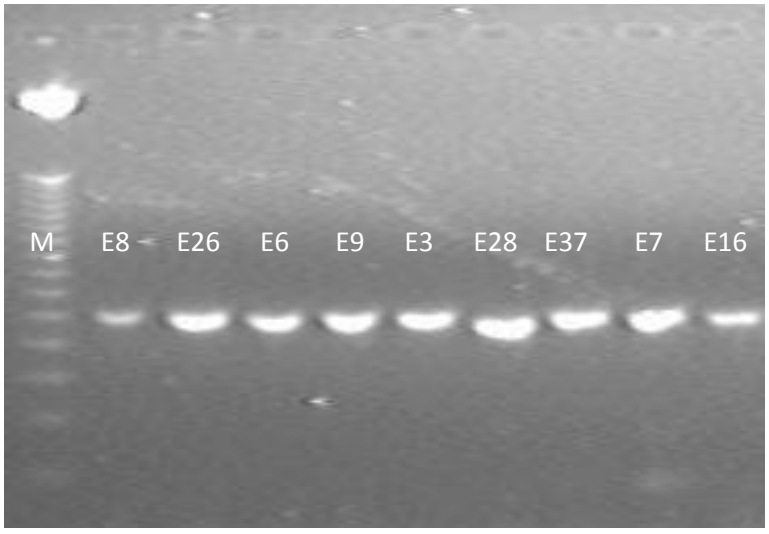


Plate 4. Electrophoregram of DNA polymorphisms in 9 tropical-adapted PVA maize inbred lines for *crtRB1* 3'TE; (M=DNA ladder).

4.2 Variability and Mean Performance

4.2.1 Analysis of variance (ANOVA) and mean performance for agronomic traits of the 36 crosses and the two commercial checks

The mean squares (MS) and mean performance for grain yield and other agronomic traits of the 36 crosses and two commercial checks (Obasuper2 and Obasuper4) evaluated in Samaru and Kerawa are presented in Tables 8-11. Analysis of variance for the entries indicated significant differences for EH, PH, EPH, EPP and GY at Samaru and DA, DS and GY at Kerawa (Table 8). The mean squares for entry were significantly different for DA, DS, PH and GY in the combined locations (Table 9).

The mean performance for days to anthesis (DA) at Samaru ranged from 55 days for E8/E7 to 69 days for E6/E28 with a mean of 61.3 days; DS from 57 days for E8/E7 to 71.5 days for E6/E28 with a mean of 63.8 days; Anthesis Silking Interval (ASI) from 1.5 days for E8/E9 and E26/E6 to 3.5 days for E26/E9 and E3/E7 with a mean of 2.5 days (Table 10). Ear height (EH) ranged from 51.5cm for E26/E37 to 111.0 cm for E8/E26 with an average of 82.5 cm; Plant height (PH) from 142.5 cm for E26/E3 to 228.5 cm for the tallest cross E8/E3 with an average of 186.8 cm while the ear position measured as ear to plant height ratio (EPH) ranged from 0.33 for E26/E37 to 0.54 for E8/E26 with a mean of 0.44. Number of ears per plant (EPP) ranged from 1 to 1.35 with a mean of 1.16. The GY for the two checks, Obasuper2 (check1) and Obasuper4 (check2), were 5.06 tons/ha and 6.77 tons/ha, respectively, in Samaru (Table 10). Thus, check 2 was designated the better check. The grain yields (GY) for all the entries at Samaru ranged from 2.74 tons/ha for E26/E37 to 8.14 tons/ha for E37/E7 with an average of 4.85 tons/ha

Table 8. Mean Squares for Grain yield (GY) and other Agronomic Traits for 36 provitamin A maize crosses and two commercial hybrid maize checks evaluated at Samaru and Kerawa in 2013

					SAMARU			
Source of Variation	Df	DA	DS	ASI	EH	PH	EPH	EPP	GY
Rep	1	82.01*	96.43**	0.58	3.44	0.01	0.00043	0.0058	0.36
Entry	37	18.86	20.39	0.45	386.57**	642.37*	0.0043**	0.037**	4.34**
Error	37	12.96	13.11	0.49	188.71	367.6	0.0021	0.013	0.43
					KERAWA			
Source of Variation	Df	DA	DS	ASI	EH	PH	EPH	EPP	GY
Rep	1	20.01**	33.44**	1.71	657.44	3132.96**	0.00034	0.0019	2.68
Entry	37	30.55**	33.69**	0.81	328.04	333.32	0.0053	0.032	3.17**
Error	37	2.4	3.15	0.62	300.05	280.89	0.0053	0.042	1.1

Rep=replication; df= degrees of freedom; DA= Days to 50% anthesis; DS=Days to 50% silking; ASI= Anthesis Silking Interval; PH= Plant height; EH= Ear height; EPH= Ear to Plant height ratio; EPP= Ears per plant; GY= Grain yield; *=significant at 0.05; **=significant at 0.01.

Table 9. Mean Squares for Grain yield (GY) and other Agronomic Traits for 36 provitamin A maize crosses and two commercial hybrid maize checks evaluated across locations (Samaru and Kerawa) in 2013

Source of Variation	Df	DA	DS	ASI	EH	PH	EPH	EPP	GY
Loc	1	126.88**	45.05**	20.72**	4588.60**	192.86	0.17	0.043	12.21**
Rep(Loc)	2	51.01**	64.93**	1.15	330.44	1566.49**	0.00039	0.0039	1.519**
Entry	37	30.66**	33.02**	0.45	354.05	536.45*	0.0047	0.033	5.57**
Entry*Loc	37	18.75**	21.07**	0.81	360.56	439.24	0.0049	0.036	1.945**
Error	74	7.68	8.13	2.86	244.38	324.24	0.0037	0.027	0.76

Loc=Location; Rep=replication; df= degrees of freedom; DA= Days to 50% anthesis; DS=Days to 50% silking; ASI= Anthesis Silking Interval; PH= Plant height; EH= Ear height; EPH= Ear to Plant height ratio; EPP= Ears per plant; GY= Grain yield; *=significant at 0.05; **=significant at 0.01.

.

Table 10. Mean performance of 36 crosses and two commercial checks for Agronomic traits at Samaru and Kerawa in 2013 wet season

	Samaru									Kerawa								
Crosses	DA (day)	DS (day)	ASI	EH (cm)	PH (cm)	EPH	EPP	GY (t/ ha)	GY* (%)	DA (day)	DS (day)	ASI	EH (cm)	PH (cm)	EPH	EPP	GY (t/ ha)	GY* (%)
E8 /E26	61.00	63.00	2.00	111.00	205.00	0.54	1.35	5.06	-25	56.00	59.50	3.50	97.50	192.50	0.50	1.35	6.12	3
E8 /E6	57.00	59.50	2.50	95.00	195.50	0.49	1.35	4.72	-30	60.50	63.50	3.00	77.50	152.50	0.51	1.05	4.45	-25
E8 /E9	59.00	60.50	1.50	90.50	197.50	0.46	1.25	5.57	-18	61.50	64.50	3.00	72.50	175.00	0.42	1.25	6.96	17
E8 /E3	59.50	62.00	2.50	108.50	228.50	0.48	1.00	3.94	-42	55.50	57.50	2.00	105.00	195.00	0.54	1.15	4.45	-25
E8 /E28	59.50	61.50	2.00	98.00	222.50	0.44	1.10	6.25	-8	58.50	62.00	3.50	82.50	167.50	0.49	1.20	5.85	-2
E8 /E37	62.00	65.00	3.00	67.50	170.00	0.39	1.20	5.31	-22	60.50	63.50	3.00	95.50	187.50	0.51	0.95	5.20	-12
E8 /E7	55.00	57.00	2.00	69.00	177.50	0.39	1.05	5.14	-24	58.00	62.00	4.00	85.00	175.00	0.49	1.10	6.49	9
E8 /E16	60.50	63.00	2.50	97.50	205.00	0.48	1.15	5.92	-13	53.00	56.00	3.00	120.00	205.00	0.59	1.15	5.56	-6
E26 /E6	57.50	59.00	1.50	85.00	201.00	0.42	1.05	5.31	-22	60.00	63.50	3.50	80.00	190.00	0.41	1.00	5.56	-6
E26 /E9	61.50	65.00	3.50	82.50	170.00	0.49	1.15	6.77	0	58.00	60.50	2.50	65.00	177.50	0.37	1.35	7.88	33
E26 /E3	60.00	62.00	2.00	56.50	142.50	0.40	1.10	3.34	-51	57.00	60.00	3.00	112.50	187.50	0.60	1.00	3.99	-33
E26 /E28	59.50	62.00	2.50	84.00	170.00	0.49	1.05	4.20	-38	55.00	58.00	3.00	102.50	187.50	0.55	1.05	4.18	-30
E26 /E37	63.50	66.00	2.50	51.50	155.00	0.33	0.95	2.74	-60	55.00	59.00	4.00	90.00	177.50	0.51	1.15	4.27	-28
E26 /E7	61.00	64.00	3.00	67.50	167.50	0.41	1.30	3.00	-56	59.00	62.50	3.50	90.00	185.00	0.49	1.00	5.10	-14
E26 /E16	64.50	67.00	2.50	85.00	200.00	0.43	1.05	3.00	-56	56.50	59.00	2.50	67.50	150.00	0.44	1.10	2.97	-50
E6 /E9	58.50	61.00	2.50	74.00	166.50	0.45	1.05	3.68	-46	57.50	60.00	2.50	92.50	175.00	0.52	1.00	5.94	0
E6 /E3	65.50	68.00	2.50	97.50	207.50	0.47	1.10	4.71	-30	59.50	63.50	4.00	115.00	202.50	0.57	1.35	6.03	2
E6 /E28	69.00	71.50	2.50	85.00	195.00	0.44	1.10	6.17	-9	55.00	58.00	3.00	72.50	160.00	0.46	1.30	8.16	37
E6 /E37	61.50	64.00	2.50	75.00	185.00	0.41	1.40	4.46	-34	66.50	70.00	3.50	105.00	196.00	0.54	1.00	5.94	0
E6 /E7	68.00	71.00	3.00	85.00	201.00	0.43	0.95	4.20	-38	66.00	70.50	4.50	95.00	202.50	0.47	1.00	4.91	-17
E6 /E16	57.50	60.00	2.50	87.50	180.00	0.49	1.20	5.06	-25	55.50	59.00	3.50	92.50	185.00	0.50	1.15	5.93	0
E9 /E3	60.50	63.50	3.00	90.00	205.00	0.44	1.35	3.60	-47	58.00	60.50	2.50	90.00	190.00	0.48	1.10	3.53	-41
E9 /E28	63.50	66.50	3.00	102.50	202.50	0.51	1.40	4.72	-30	61.50	64.50	3.00	102.50	190.00	0.54	1.00	5.01	-16
E9 /E37	61.00	63.50	2.50	90.00	197.50	0.46	1.10	3.69	-46	60.50	63.50	3.00	102.50	187.50	0.55	1.05	5.01	-16
E9 /E7	58.00	60.50	2.50	75.00	171.50	0.44	1.40	6.34	-6	54.00	57.50	3.50	90.00	167.50	0.54	1.00	6.40	8

Table 10 continued

Crosses	Samaru									Kerawa								
	DA (day)	DS (day)	ASI	EH (cm)	PH (cm)	EPH	EPP	GY (t/ha)	GY* (%)	DA (day)	DS (day)	ASI	EH (cm)	PH (cm)	EPH	EPP	GY (t/ha)	GY* (%)
E9 /E16	65.00	67.00	2.00	77.50	180.00	0.43	1.00	4.20	-38	66.00	70.50	4.50	77.50	185.00	0.42	1.20	6.49	9
E3 /E28	65.50	68.50	3.00	87.50	191.00	0.46	1.00	7.20	6	59.50	61.50	2.00	105.0	190.00	0.56	1.10	6.31	6
E3 /E37	56.50	59.00	2.50	89.00	194.00	0.46	1.05	5.23	-23	60.00	63.50	3.50	97.50	202.50	0.48	1.20	6.12	3
E3 /E7	57.50	61.00	3.50	67.50	171.50	0.40	1.25	3.94	-42	60.50	63.00	2.50	105.0	182.50	0.57	1.15	6.31	6
E3 /E16	67.50	70.00	2.50	62.50	172.50	0.36	1.10	3.77	-44	64.00	67.00	3.00	95.00	192.50	0.50	1.20	4.36	-27
E28 /E37	61.00	63.50	2.50	97.50	200.00	0.49	1.35	3.43	-49	58.00	61.50	3.50	92.50	175.00	0.52	1.00	4.45	-25
E28 /E7	60.50	63.00	2.50	93.50	191.50	0.49	1.00	6.69	-1	55.00	58.00	3.00	80.00	180.00	0.45	1.15	6.58	11
E28 /E16	63.00	66.50	3.50	75.00	186.00	0.39	1.25	3.26	-52	65.00	67.00	2.00	92.50	202.50	0.46	1.15	5.47	-8
E37 /E7	61.00	63.00	2.00	82.50	183.50	0.45	1.35	8.14	20	61.50	64.00	2.50	87.50	175.00	0.50	1.10	5.10	-14
E37 /E16	60.00	62.50	2.50	65.00	161.50	0.40	1.10	4.46	-34	60.00	62.50	2.50	100.5	177.50	0.58	1.25	7.88	33
E7 /E16	62.00	64.50	2.50	55.00	167.50	0.33	1.30	5.40	-20	59.50	63.50	4.00	97.50	190.00	0.51	1.15	4.45	-25
Obasuper2	61.50	63.50	2.00	90.00	200.00	0.45	1.15	5.06	-25	71.00	74.50	3.50	92.50	195.00	0.48	1.00	4.36	-27
Obasuper4	64.50	66.50	2.00	81.50	179.00	0.46	1.10	6.77	0	58.00	61.50	3.50	105.0	197.50	0.53	1.10	5.94	0
Mean	61.30	63.80	2.50	82.49	186.76	0.44	1.16	4.85		59.37	62.53	3.16	92.86	184.37	0.50	1.12	5.52	
SE±	0.49	0.52	0.08	2.09	2.72	0.01	0.02	0.16		0.43	0.46	0.10	2.14	2.24	0.01	0.02	0.17	
CV (%)	5.80	5.70	24.20	17.20	10.40	10.80	10.10	12.50		2.70	2.80	24.80	18.50	9.20	13.80	19.00	19.60	

DA= Days to 50% anthesis; DS=Days to 50% silking; ASI= Anthesis Silking Interval; PH= Plant height; EH= Ear height; EPH= Ear to Plant height ratio; EPP= Ears per plant; GY= Grain yield; GY*=percentage GY increase over better check; Obasuper4=better check; SE= Standard Error of mean; CV= Coefficient of Variation.

The crosses which yielded higher than the better check at Samaru were E37/E7 and E3/E28 with GY of 8.14 tons/ha and 7.20 tons/ha, respectively. This amounted to 20% and 6% increases over the better check.

At Kerawa, mean DA ranged from 53 days for E8/E16 to 71 days for Obasuper2 (check1) with a mean of 59.4 days; DS ranges from 56 days for E8/E16 to 74.5 days for Check1 while ASI ranges from 2 days for E8/E3, E3/E28 and E28/E16 to 4.5 days for E6/E7 with a mean of 3.16 days (Table 10). EH ranged from 65.0 cm for E26/E9 to 120 cm for E8/E16; PH ranges from 150 cm for E26/E16 as the shortest cross to the tallest at 205 cm for E8/E16 with an average of 184.37cm; EPH from 0.37 for E26/E9 to 0.6 for E26/E3 with a mean of 0.50 (Table 10). Number of ears per plant (EPP) at Kerawa ranged from one for about 25% of the crosses to 1.35 with a mean of 1.12. The GY ranged from 2.97 tons/ha for E26/E16 (GY*=-50%) to 8.16 tons/ha for E6/E28 (GY*=37%) with a mean of 5.52 tons/ha. The higher mean GY at Kerawa was likely due to favourable environment of the farm which in addition to the optimum agronomic practices was a fallow for two years before the experiment. In Kerawa GY was 4.36 tons/ha for check1 and 5.94 tons/ha for check2. Crosses that out-yielded the better check were E26/E9 and E37/E7 by 33% (both yielding 7.88 tons/ha), E8/E9 by 17% (6.96 tons/ha), E8/E7and E9/E16 (6.49 tons/ha), E9/E7 (6.40 tons/ha), E3/E28 and E3/E7 (6.31 tons/ha). The only cross showing high GY at both locations was E3/E28. Lines E6, E8 and E9 appear in most of the high yielding crosses and mostly as the seed parent. Other lines involved in forming high yielding combinations were E28, E3, E26, E7, E37 and E16. Line E7 was a pollen parent in all its combinations of high GY crosses. Thus each of the nine lines formed at least one combination of high yielding cross.

The mean performance for the agronomic traits of the 36 crosses and the two commercial checks (Obasuper2 and Obasuper4) across the locations is presented in Table 11. The ANOVA showed highly significant differences for DA, DS and GY ($p<0.01$). Mean DA for the combined locations ranged from 56 days for E9/E3 to 67 days for E6/E28 with a mean of 60.3 days; DS ranged from 59 days for E9/E3 to 70.8 days for E6/E28 with a mean of 63.16 days while ASI across locations ranged from 2.3 days for E8/E9 and E8/E3 to 3.8 days for E6/E28 with a mean of 2.8 days. EH ranged from 84.5cm for E26/E9 to 106.7 cm for E8/E3 with a mean of 87.7 cm while PH ranged from 165 cm for E26/E9 to 211.8 cm for E8/E3 with a mean of 185.6 cm. The EPH from 0.4 to 0.5 with a mean of 0.47 (Table 11). The EPP combined across locations ranged from 0.98 for E6/E28 to 1.4 for E8/E26 with a mean of 1.14. GY for the combined locations ranged from 3 tons/ha for E3/E7 to 7.33 tons/ha for E26/E6 with a mean of 5.2 tons/ha. The crosses E6/E9 (7.12 tons/ha), E9/E28 (6.75 tons/ha), E3/E28 (6.63 tons/ha) and E37/E7 (6.62 tons/ha) yielded higher than the better check. E3/E28 and E37/E7 yielded more than 6.5 tons/ha across locations, notwithstanding the low yield of 5.17 tons/ha of E37/E7 at Kerawa. For the combined analysis, the two best performers over the better check for GY were E26/E6 (15.3%) and E6/E9 (12.7%).

Table 11. Mean performance of 36 crosses and two commercial checks for Agronomic traits across locations in 2013 wet season

Crosses/ Checks	DA (day)	DS (day)	ASI	EH (cm)	PH (cm)	EPH	EPP	GY (t/ha)	GY*(%)
E8 /E26	58.50	61.25	2.75	104.25	198.75	0.52	1.35	5.59	-12.0
E8 /E6	58.75	61.50	2.75	86.25	174.00	0.50	1.20	4.58	-27.9
E8 /E9	60.25	62.50	2.25	81.50	186.25	0.44	1.25	6.26	-1.4
E8 /E3	57.50	59.75	2.25	106.75	211.75	0.51	1.08	4.20	-34.0
E8 /E28	59.00	61.75	2.75	90.25	195.00	0.47	1.15	6.05	-4.8
E8 /E37	61.25	64.25	3.00	81.50	178.75	0.45	1.08	5.25	-17.3
E8 /E7	56.50	59.50	3.00	77.00	176.25	0.44	1.08	5.82	-8.5
E8 /E16	58.75	61.25	2.50	82.50	195.50	0.42	1.03	5.44	-14.4
E26 /E6	59.75	62.75	3.00	73.75	173.75	0.43	1.25	7.33	15.3
E26 /E9	58.50	61.00	2.50	84.50	165.00	0.50	1.05	3.67	-42.3
E26 /E3	57.25	60.00	2.75	93.25	178.75	0.52	1.05	4.19	-34.1
E26 /E28	59.25	62.50	3.25	70.75	166.25	0.42	1.05	3.50	-44.9
E26 /E37	60.00	63.25	3.25	78.75	176.25	0.45	1.15	4.05	-36.3
E26 /E7	58.00	60.50	2.50	83.25	170.75	0.48	1.03	4.81	-24.3
E26 /E16	62.50	65.75	3.25	106.25	205.00	0.52	1.23	5.37	-15.5
E6 /E9	62.00	64.75	2.75	78.75	177.50	0.45	1.20	7.16	12.7
E6 /E3	64.00	67.00	3.00	90.00	190.50	0.47	1.20	5.20	-18.2
E6 /E28	67.00	70.75	3.75	90.00	201.75	0.45	0.98	4.56	-28.3
E6 /E37	59.25	62.00	2.75	90.00	197.50	0.46	1.23	3.56	-43.9
E6 /E7	62.50	65.50	3.00	102.50	196.25	0.53	1.20	4.86	-23.5
E6 /E16	60.75	63.50	2.75	96.25	192.50	0.50	1.08	4.35	-31.6
E9 /E3	56.00	59.00	3.00	82.50	169.50	0.49	1.20	6.37	0.2
E9 /E28	62.50	65.00	2.50	96.25	190.50	0.51	1.05	6.75	6.3
E9 /E37	58.25	61.25	3.00	93.25	198.25	0.47	1.13	5.67	-10.7
E9 /E7	59.00	62.00	3.00	86.25	177.00	0.48	1.20	5.12	-19.4
E9 /E16	59.50	62.50	3.00	95.00	187.50	0.51	1.18	3.94	-38.0
E3 /E28	57.75	60.50	2.75	86.75	185.75	0.47	1.08	6.63	4.4
E3 /E37	56.75	59.50	2.75	108.75	205.00	0.53	1.15	5.74	-9.7
E3 /E7	60.50	63.00	2.50	76.25	175.00	0.43	1.08	2.98	-53.0
E3 /E16	56.50	59.50	3.00	90.00	182.50	0.49	1.18	5.49	-13.5
E28 /E37	65.50	68.75	3.25	77.50	182.50	0.43	1.10	5.35	-15.9
E28 /E7	65.75	68.50	2.75	78.75	182.50	0.43	1.15	4.06	-36.0

Table 11 continued

Crosses/ Checks	DA	DS	ASI	EH	PH	EPH	EPP	GY	GY*(%)
E28 /E16	64.00	66.75	2.75	83.75	194.25	0.42	1.20	4.36	-31.3
E37 /E7	61.25	63.50	2.25	85.00	179.25	0.47	1.23	6.62	4.2
E37 /E16	60.00	62.50	2.50	82.75	169.50	0.49	1.18	6.17	-2.9
E7 /E16	60.75	64.00	3.25	76.25	178.75	0.42	1.23	4.93	-22.5
Obasuper2	66.25	69.00	2.75	91.25	197.50	0.46	1.08	4.71	-25.9
*Obasuper4	61.25	64.00	2.75	93.25	188.25	0.49	1.10	6.35	0
Mean	60.34	63.16	2.83	87.67	185.57	0.47	1.14	5.18	
SE ±	0.34	0.35	0.07	1.55	1.76	0.01	0.01	0.12	
CV (%)	4.50	4.50	24.70	17.90	9.80	12.60	15.10	16.90	

DA= Days to 50% anthesis; DS=Days to 50% silking; ASI= Anthesis Silking Interval; PH= Plant height; EH= Ear height; EPH= Ear to Plant height ratio; EPP= Ears per plant; GY= Grain yield; GY*=percentage GY increase over better check; *Obasuper4=better check; SE= Standard Error of mean; CV= Coefficient of Variation.

4.2.2 Analysis of variance and mean performance of the diallel crosses for grain yield and carotenoids

Diallel analyses of variance for the 36 crosses (i.e. without checks), under individual locations and combined locations, are presented in Table 12 and Table 13, respectively. The traits considered in the diallel ANOVA were DA, PH and GY and the carotenoids (βCX, αC, βC, PVA, LUT and ZEA). For the crosses, the mean squares were significant ($p<0.05$) for DA, PH, GY, LUT, ZEA and βC at Samaru and for DA and GY at Kerawa. At Kerawa the crosses were significantly ($p<0.01$) different for only DA, PH and GY. The GCA mean squares at Samaru were highly significant ($p<0.01$) for DA, PH and GY and significant for LUT, ZEA and βCX ($p<0.05$). The GCA mean square at Kerawa was significant for only DA. For the SCA, LUT and ZEA were highly significantly ($p<0.01$) different at Samaru while DA was highly significantly different at Kerawa. GY had highly significant SCA at both locations.

The ratios of GCA:SCA mean squares were greater than unity (>1) for DA, PH, LUT, ZEA and βCX with DA being the highest (2.5 and 2.7) in both locations indicating preponderance of additive gene effect. The GY, βC, αC and PVA had GCA:SCA below unity (<1); the ratio for PH was also less than unity in Kerawa (Table 12) suggesting preponderance of non additive gene effects governing these traits. Heritability for agronomic traits at Samaru ranged from 26.73 for DA to 79.68% for GY and at Kerawa it ranged from 10.15 for PH to 80.46 for DA. For the carotenoids, heritability at Samaru ranged from 14.29 for αC to 41.63 for ZEA and at Kerawa it was from -10.77 for βC to 33.33 for αC.

For the combined analysis, location showed significant ($p<0.01$) differences for all the traits except PH, ZEA and βCX (Table 13). For the crosses, DA, PH, GY, LUT and ZEA were

significantly ($p<0.01$) different. The GCA and SCA variances were significantly ($p<0.01$) different for DA, PH, GY, LUT and ZEA. Crosses by location interactions were not significantly different for all the traits except DA and GY. However, both GCA by location and SCA by location interaction effects were significant for DA, PH and ZEA while SCA by location interaction effects were also significant for GY and LUT. The ratio of GCA:SCA ranged from 0.16 for αC to 3.97 for DA. The ratios were less than unity (<1) for PH and GY and all the PVA active carotenoids with exception of βCX. These indicate preponderance of non additive gene action for these traits while traits with ratios equal to or greater than one (i.e. DA, LUT, ZEA and βCX) indicate higher relative GCA effects to their corresponding SCA effects. Heritability across locations for agronomic traits ranged from 16.95% for PH to 69.67% for GY while for the carotenoids it was from 0 for αC to 49.20% for LUT.

The mean performance of the diallel crosses for the different carotenoid contents at Samaru, Kerawa and combined locations are presented in Table 14. Lutein (LUT) contents at Samaru ranged from 3.85 µg/g for E9/E7 to 13.69 µg/g for E8/E3 with an average of 6.73 µg/g. At Kerawa, LUT contents ranged from 3.25 µg/g for E8/E9 to 10.48 µg/g for E8/E6 with an average of 5.65 µg/g while across locations it ranged from 4.23 µg/g for E8/E9 and E9/E7 to 10.93 µg/g for E8/E3 with an average of 6.19 µg/g. LUT, thus, constituted 28.40% of total carotenoids content across locations.

Zeaxanthin (ZEA) contents at Samaru ranged from 4.91µg/g for E26/E16 to 16.14 µg/g for E26/E7 with a mean of 11.08 µg/g. ZEA contents at Kerawa ranged from 4.97 µg/g for E8/E26 to 14.70 µg/g for E8/E6 with a mean of 10.44 µg/g. Combined across locations, ZEA ranged from 6.04 µg/g for E26/E6 to 15.49 µg/g for E26/E7 with a mean of 10.76 µg/g. ZEA made up

49.56% of total carotenoids content combined across locations and therefore the highest carotenoid content in the crosses.

Table 12. Mean squares for days to anthesis (DA), plant height (PH), grain yield (GY), lutein (LUT), zeaxanthin (ZEA), β-cryptoxanthin (βCX), α-carotene (αC), β-carotene (βC) and total provitamin A (PVA) contents for 36 provitamin A maize crosses evaluated at Samaru and Kerawa in 2013

Source of variation	Df	Agronomic traits			Carotenoid contents					
		DA	PH	GY	LUT	ZEA	βCX	αC	βC	PVA
				Samaru						
Rep	1	55.13	107.56	0.24	3.95	2.97	0.51	0.00	0.33	0.84
Crosses	35	21.67*	707.43*	3.36**	8.47**	14.85**	0.48	0.04	0.44*	0.81
GCA	8	40.46**	1145.23**	1.92**	8.92*	15.56*	0.71*	0.01	0.43	0.59
SCA	27	16.1	577.71	3.79**	8.33**	14.64**	0.41	0.04	0.44	0.88
Error	35	12.53	366.5	0.38	3.62	6.12	0.33	0.03	0.26	0.54
GCA:SCA		2.51	1.98	0.51	1.07	1.06	1.73	0.17	0.98	0.67
Heritability (%)		26.73	31.75	79.68	40.12	41.63	18.52	14.29	25.71	20.00
				Kerawa						
Rep	1	24.5	2850.13	1.19	0.27	1.33	0.09	0.01	0.19	0.30
Crosses	35	24.1**	358.13	2.87**	5.66	14.97	0.29	0.02	0.29	0.55
GCA	8	46.88**	282.98	1.44	5.92	20.64	0.31	0.01	0.13	0.38
SCA	27	17.35**	380.39	3.29**	5.58	13.29	0.28	0.02	0.34	0.60
Error	35	2.61	292.13	1.14	4.03	10.43	0.50	0.01	0.36	0.86
GCA:SCA		2.7	0.74	0.44	1.06	1.55	1.11	0.41	0.37	0.64
Heritability (%)		80.46	10.15	43.14	16.82	17.87	-26.58	33.33	-10.77	-21.99

df= degrees of freedom; GCA= General combining ability; SCA= Specific combining ability; *significant at 0.05 level of probability; **significant at 0.01 level of probability.

Table 13. Mean squares for days to anthesis (DA), plant height (PH), grain yield (GY), lutein (LUT), zeaxanthin (ZEA), β-cryptoxanthin (βCX), α-carotene (αC), β-carotene (βC) and total provitamin A (PVA) contents for 36 provitamin A maize crosses across locations in 2013

Source of Variation	df	Agronomic traits			Carotenoids content					
		DA	PH	GY	LUT	ZEA	βCX	αC	βC	PVA
Location(L)	1	162.56**	303.34	19.93**	42.07**	14.87	0.82	0.12*	2.27**	4.55**
Rep(Loc)	2	39.81**	1478.84**	0.71	2.11	2.15	0.30	0.00	0.26	0.57
Crosses(C)	35	29.59**	581.56*	4.78**	9.37**	18.83**	0.30	0.03	0.42	0.69
GCA	8	69.96**	578.36**	2.30*	13.58**	28.52**	0.43	0.001	0.27	0.50
SCA	27	17.63**	582.51**	5.52**	8.12**	15.96**	0.27	0.03	0.47	0.74
CxL	35	16.18**	483.00	1.45*	4.76	11.00	0.46	0.03	0.31	0.68
GCAxL	8	17.38**	849.85**	1.06	1.27	7.68**	0.60	0.01	0.29	0.47
SCAxL	27	15.82**	375.60**	1.57*	5.79**	11.98**	0.42	0.03	0.31	0.74
Error	70	7.57	329.31	0.76	3.82	8.28	0.41	0.02	0.31	0.70
GCA:SCA		3.97	0.99	0.42	1.67	1.79	1.6	0.16	0.57	0.67
Heritability (%)		45.32	16.95	69.67	49.20	41.58	-53.33	0.00	26.19	1.45

df= degrees of freedom; GCA= General combining ability; SCA= Specific combining ability; *significant at 0.05 level of probability; **significant at 0.01 level of probability.

Table 14. Mean performance of 36 diallel crosses for Lutein (LUT), Zeaxanthin (ZEA), β-cryptoxanthin (βCX), α-Carotene (αC), β-Carotene (βC) and total Provitamin A (PVA) contents evaluated at Samaru, Kerawa and across locations in 2013 wet season

	Samaru						Kerawa						Combined					
Crosses	LUT*	ZEA	βCX	αC	βC	PVA	LUT	ZEA	βCX	αC	βC	PVA	LUT	ZEA	βCX	αC	βC	PVA
E8 /E26	8.19	10.24	2.94	0.4	2.41	4.08	3.36	4.97	1.41	0.34	1.66	2.53	5.77	7.61	2.04	0.37	2.17	3.31
E8 /E6	7.60	9.76	2.37	0.36	2.19	3.56	10.48	14.7	3.06	0.43	3.29	5.03	9.04	12.23	2.74	0.39	2.72	4.30
E8 /E9	5.21	7.94	2.72	0.48	2.41	4.02	3.25	5.63	2.29	0.35	1.83	3.15	4.23	6.78	2.12	0.42	2.50	3.58
E8 /E3	13.69	11.21	2.29	0.44	2.29	3.66	8.16	7.57	2.03	0.22	1.78	2.9	10.93	9.39	2.04	0.33	2.16	3.28
E8 /E28	5.62	8.95	2.00	0.44	1.52	2.74	3.84	9.37	2.50	0.23	1.64	3.00	4.73	9.16	1.58	0.33	2.25	2.87
E8 /E37	5.74	13.93	2.72	0.41	2.01	3.58	5.77	9.44	2.32	0.26	1.94	3.23	5.76	11.68	1.98	0.34	2.52	3.4
E8 /E7	9.05	13.03	2.47	0.45	2.32	3.78	6.97	11.83	3.20	0.34	1.92	3.69	8.01	12.43	2.12	0.40	2.83	3.74
E8 /E16	5.86	8.28	2.31	0.25	1.43	2.71	6.22	10.96	2.39	0.55	1.99	3.46	5.75	9.62	1.71	0.40	2.35	3.08
E26 /E6	5.54	5.50	1.95	0.41	2.11	3.29	7.02	6.57	2.27	0.46	2.4	3.77	6.44	6.04	2.25	0.44	2.11	3.53
E26 /E9	5.14	15.01	2.75	0.42	2.21	3.79	4.21	14.22	2.64	0.32	2.09	3.57	4.88	14.61	2.15	0.37	2.69	3.68
E26 /E3	6.81	9.99	3.48	0.38	2.23	4.16	5.55	11.48	2.33	0.39	2.05	3.41	5.35	10.74	2.14	0.38	2.91	3.78
E26 /E28	4.02	12.1	2.75	0.42	2.76	4.35	5.04	12.23	2.22	0.30	1.92	3.17	5.93	12.16	2.34	0.36	2.49	3.76
E26 /E37	6.66	8.38	2.28	0.36	1.80	3.12	7.65	10.21	1.96	0.50	1.56	2.79	5.84	9.30	1.68	0.43	2.12	2.95
E26 /E7	7.04	16.14	2.61	1.02	1.48	3.30	4.48	14.85	2.18	0.26	1.94	3.16	5.57	15.49	1.71	0.64	2.39	3.23
E26 /E16	5.37	4.91	1.39	0.32	1.36	2.22	8.04	13.94	2.48	0.37	1.94	3.36	7.32	9.43	1.65	0.34	1.93	2.79
E6 /E9	7.90	9.87	2.13	0.36	1.72	2.96	4.39	12.72	3.00	0.42	2.12	3.83	5.72	11.30	1.92	0.39	2.56	3.40
E6 /E3	7.93	9.02	1.89	0.43	1.78	2.94	5.18	10.56	2.47	0.32	2.32	3.72	5.27	9.79	2.05	0.38	2.18	3.33
E6 /E28	5.75	16.04	3.31	0.49	2.73	4.63	7.31	8.38	2.31	0.43	1.99	3.36	7.60	12.21	2.36	0.46	2.81	4.00
E6 /E37	5.23	11.58	1.92	0.35	1.98	3.12	4.83	8.23	1.99	0.30	1.33	2.47	6.38	9.90	1.66	0.32	1.96	2.80
E6 /E7	5.6	13.96	3.39	0.57	2.9	4.88	4.89	7.90	1.85	0.39	1.60	2.72	5.32	10.93	2.25	0.48	2.62	3.80
E6 /E16	5.66	15.23	2.52	0.66	3.40	4.99	4.42	14.52	2.69	0.55	2.22	3.84	4.43	14.87	2.81	0.61	2.61	4.42
E9 /E3	8.19	14.02	2.93	0.27	2.11	3.71	4.21	13.37	2.72	0.33	1.75	3.27	4.72	13.69	1.93	0.30	2.82	3.49
E9 /E28	7.6	14.7	2.91	0.48	3.12	4.82	9.43	14.09	2.39	0.49	2.18	3.62	7.51	14.39	2.65	0.48	2.65	4.22
E9 /E37	5.21	9.51	2.02	0.32	1.86	3.03	3.63	9.97	2.67	0.28	1.46	2.93	4.64	9.74	1.66	0.30	2.34	2.98

Table 14 continue

	Samaru						Kerawa						Combined					
Crosses	LUT	ZEA	βCX	αC	βC	PVA	LUT	ZEA	βCX	αC	βC	PVA	LUT	ZEA	βCX	αC	βC	PVA
E9 /E7	3.85	11.91	2.26	0.5	2.26	3.64	4.63	13.1	2.19	0.37	1.98	3.26	4.24	12.5	2.12	0.43	2.22	3.45
E9 /E16	7.42	8.82	1.86	0.44	2.44	3.59	4.71	8.72	1.83	0.5	2.22	3.38	4.84	8.77	2.33	0.47	1.84	3.49
E3 /E28	6.23	11.69	2.73	0.38	2.6	4.16	5.41	8.05	2.5	0.32	1.76	3.17	6.41	9.87	2.18	0.35	2.61	3.66
E3 /E37	8.24	11.54	3.22	0.37	1.64	3.43	5.91	9.86	1.9	0.29	1.62	2.72	6.07	10.7	1.63	0.33	2.56	3.08
E3 /E7	5.34	7.78	2.4	0.39	2.49	3.88	5.51	11.39	2.56	0.44	2.52	4.02	6.88	9.58	2.5	0.42	2.48	3.95
E3 /E16	7.28	10.46	1.72	0.4	2.38	3.44	6.47	12.95	2.73	0.49	2.71	4.32	8.27	11.7	2.55	0.44	2.23	3.88
E28 /E37	5.28	10.07	2.32	0.34	1.87	3.19	4.47	7.61	2.01	0.33	1.49	2.66	4.9	8.84	1.68	0.33	2.17	2.93
E28 /E7	6.58	10.09	2.92	0.37	2.62	4.27	5.82	8.29	1.92	0.29	1.6	2.7	6.55	9.19	2.11	0.33	2.42	3.48
E28 /E16	4.45	11.61	2.21	0.37	2.46	3.76	6.5	11.15	2.42	0.36	1.98	3.36	7.45	11.38	2.22	0.37	2.31	3.56
E37 /E7	4.98	11.91	2.16	0.37	2.1	3.37	6.63	9.74	2.07	0.27	1.77	2.94	9.21	10.83	1.94	0.32	2.11	3.15
E37 /E16	10.07	11.1	2.32	0.29	1.88	3.19	3.65	10.92	1.93	0.32	2.06	3.18	5.36	11.01	1.97	0.3	2.12	3.19
E7 /E16	8.41	12.71	2.88	0.57	2.42	4.15	5.41	6.4	2.18	0.37	1.69	2.96	5.6	9.56	2.06	0.47	2.53	3.55
Mean	6.73	11.08	2.47	0.42	2.2	3.65	5.65	10.44	2.32	0.37	1.95	3.3	6.19	10.76	2.4	0.4	2.08	3.47
SE±	0.29	0.38	0.08	0.02	0.07	0.10	0.26	0.42	0.07	0.01	0.07	0.10	0.20	0.28	0.05	0.01	0.05	0.07
CV (%)	*28.3*	22.3	23.3	42.8	22.9	20.1	*35.5*	30.9	30.4	28.8	30.9	28.1	*31.6*	26.7	26.9	37.6	26.7	24

* carotenoids content is in microgram per gram (µg/g); LUT= Lutein; ZEA= Zeaxanthin; βCX= β-cryptoxanthin; αC= α-Carotene; βC= β-Carotene; PVA= total Provitamin A; SE=Standard Error; CV= Coefficient of Variation..

Beta-cryptoxanthin (βCX) contents, which has Provitamin A activity, ranged from 1.39 µg/g for E26/E16 to 3.48 µg/g for E26/E3 with a mean of 2.47 µg/g at Samaru. At Kerawa βCX ranged from 1.41 µg/g for E8/E26 to 3.20 µg/g for E8/E7 with a mean of 2.32 µg/g while for the combined locations it ranged from 1.58 µg/g for E8/E28 to 2.81 µg/g for E6/E16 with a mean of 2.40 µg/g. βCX comprised 11% of the total carotenoids combined across locations. The best crosses for high βCX at Samaru were E26/E3, E6/E7, E6/E28 and E3/E37, (βCX greater than or equal to 3.0 µg/g) while at Kerawa they were E8/E7, E8/E6 and E6/E9. For the combined locations only E8/E6 and E6/E16 had βCX contents between 2.7 to 2.8 µg/g.

Alphα-carotene (αC) content which was the least relative to other carotenoids but also has provitamin A activity, ranged from 0.25 µg/g for E8/E16 to 1.02 µg/g for E26/E7 with a mean of 0.42 µg/g at Samaru. At Kerawa, αC ranged from 0.22 µg/g for E8/E3 and E8/E28 to 0.55 µg/g for E8/E16 and E6/E16 with a mean of 0.37 µg/g while for the combined locations, αC ranged from 0.3 µg/g for E9/E3, E9/E37 and E37/E16 to 0.64 µg/g for E26/E7 with a mean of 0.40 µg/g.

βeta-Carotene (βC) that contributes most to Provitamin A activity, ranged from 1.36 µg/g for E26/E16 to 3.40 µg/g for E6/E16 with a mean of 2.20 µg/g at Samaru (Table 14). The βC contents in Kerawa ranged from 1.33 µg/g for E6/E37 to 3.29 µg/g for E8/E6 with a mean of 1.95 µg/g. The βC contents combined across locations ranged from 1.84 µg/g for E9/E16 to 2.83 µg/g for E8/E7 with a mean of 2.08 µg/g. Though all the crosses tested had βC contents higher than the upper limits of normal yellow maize (0.5 to 1.5 µg/g according to Harjes *et al.* (2008)), crosses E6/E16, E9/E28, E6/E7, E26/E28, E6/E28, E28/E7, E3/E28 and E3/E7 had βC content between 2.5 and 3.4 µg/g at Samaru. At Kerawa, crosses having βC content between 2.5 and 3.3 µg/g were E8/E6, E3/E16 and E3/E7 while for the combined locations crosses E6/E16, E8/E6, E9/E28, E3/E16 and E3/E7 had βC above 2.5 µg/g.

The provitamin A (PVA) content, measured as sum of half βCX, half αC and βC content (0.5βCX+ 0.5αC + βC), ranged from 2.22 µg/g for E26/E16 to 4.99 µg/g for E6/E16 with an average of 3.65 µg/g at Samaru. PVA content at Kerawa ranged from 2.47 µg/g for E6/E37 to 5.03 µg/g for E8/E6 with a mean of 3.30 µg/g. Combined across locations, PVA contents of the crosses ranged from 2.79 µg/g for E26/E16 to 4.42 µg/g for E6/E16 with a mean of 3.39 µg/g. Cross E6/E16 ranked first at Samaru and combined across locations; but was the fourth ranking in PVA content at Kerawa. Twenty one crosses had PVA contents above 3.5 µg/g at Samaru with E6/E16, E6/E7, E9/E28, E6/E28, E26/E28 and E28/E7 being above 4.2 µg/g. Ten crosses had PVA content above 3.5 µg/g at Kerawa with E8/E6 (5.03 µg/g) being the highest. The combined locations had 16 crosses with PVA contents above 3.5 µg/g with E6/E16, E8/E6, E9/E28 and E6/E28 having PVA content above 4 µg/g. Thus, across locations, the non provitamin A carotenoids (LUT and ZEA) made up 77.74% of the total carotenoid content (ZEA = 49.57% and LUT 28.40%), while βCX was 11.01%, βC and PVA were 9.42% and 15.76%, respectively.

The best crosses based on PVA and grain yield (GY) across locations were: E6/E9 having PVA of 4.0 µg/g, βC 2.36 µg/g, βCX 2.81 and GY of 7.16t/ha.; E3/E16 had PVA 4.42 µg/g and GY 5.49 t/ha; E26/E6 had GY 7.33 t/ha and PVA 3.68 µg/g; E6/E9 GY was 7.16t/ha and PVA 3.998 µg/g; E9/E28 had GY of 6.75t/ha with PVA 3.663 µg/g; E3/E28 GY was 6.63t/ha and PVA 3.48 µg/g; E37/E7 GY was 6.62t/ha and PVA 3.15 µg/g and E9/E3 had GY 6.37 t/ha with PVA 3.45 µg/g. The lines E6, E7, E8 and E9 were the common parents in the best crosses.

4.3 Combining Ability

4.3.1 General combining ability effects

General combining ability (GCA) effects of the nine selected maize inbred lines at Samaru, Kerawa and for the combined locations are presented in Table 15. Line E8 (9450xKI 21-7-2-2-1-1) had significant negative GCA effects for DA and significant positive GCA for PH at Samaru. Line E8 had positive GCA effect for GY at Samaru and for GY and PH across the locations, though, the effects were not significant. GCA effects for E8 were negative and not significant for DA, PH and GY at Kerawa. For the carotenoids, E8 had negative GCA effects for ZEA, βCX, βC and PVA contents while it had positive GCA effects for LUT and zero (0) GCA for αC at Samaru (Table 15). The GCA effects for line E8 at Kerawa were negative for ZEA and αC but positive for LUT, βCX, βC and PVA. The GCA effects of E8 for the combined locations were also negative for ZEA and αC while they were positive for LUT, βCX, βC and PVA. Line E26 (KU1409 x MO17LPA x KU1409-11-4-1) had significant negative GCA effects for PH and GY at Samaru and for DA at Kerawa. Negative non significant GCA effects for DA, PH and GY were observed across the locations for line E26. Line E26 also had negative GCA effects for LUT, βC and PVA while it had positive GCA effects for ZEA, βCX and αC at Samaru. At Kerawa, Line E26 had negative GCA effects for LUT, βCX, αC and PVA while it had positive GCA effects for ZEA and βC. Across locations, GCA effects of line E26 were negative for LUT, βC and PVA while it was positive for the other carotenoids. Line E6 (SYN-Y-STR-34-1-1-1-1-2-1) had positive GCA effects for DA at both locations and across the locations being significant at Kerawa ($p<0.05$). Line E6 had positive GCA effects for PH at Samaru and across the locations.

Table 15. General combining ability (GCA) effects of 9 maize inbred lines for Days to anthesis (DA), Plant height (PH), grain yield (GY), Lutein (LUT), Zeaxanthin (ZEA), β-cryptoxanthin (βCX), α-Carotene (αC), β-Carotene (βC) and total Provitamin A (PVA) contents at Samaru, Kerawa and across locations in 2013

	Agronomic Traits			Carotenoids					
Lines	DA	PH	GY	LUT	ZEA	βCX	αC	βC	PVA
			Samaru						
E8	-2.74*	14.94*	0.42	1.02	-1.16	-0.05	0	-0.05	-0.08
E26	0.12	-15.56**	-0.69**	-0.73	0.3	0.15	0.06	-0.18	-0.07
E6	1.76	8.37	-0.01	-0.38	2.27*	0.26	-0.02	0.21	0.33
E9	-0.45	-4.27	0.41	-0.65	-0.25	0.38	-0.01	0.09	0.27
E3	-1.17	5.52	0.18	0.29	-0.48	-0.2	-0.01	0.07	-0.04
E28	3.26**	0.94	-0.01	-0.05	-0.67	-0.1	-0.01	0.16	0.11
E37	-0.45	-3.77	0.21	-0.13	1.01	0.05	0	-0.36	-0.33
E7	-0.02	-7.34	-0.08	1.47*	-0.47	-0.25	-0.01	0.04	-0.09
E16	-0.31	1.16	-0.44	-0.85	-0.56	-0.24	0	0.02	-0.1
SE±	1.34	7.24	0.23	0.72	0.94	0.22	0.07	0.19	0.28
			Kerawa						
	DA	PH	GY	LUT	ZEA	βCX	αC	βC	PVA
E8	-0.31	-4.95	-0.53	0.52	-1.92	0.07	-0.04	0.12	0.13
E26	-2.24**	2.19	0.11	-0.76	1.1	-0.05	-0.01	0.01	-0.03
E6	1.90*	-1.95	-0.12	0.54	1.04	0.15	0.01	0.01	0.09
E9	-1.02	-4.24	0.08	-0.45	-1.15	-0.07	0	-0.06	-0.09
E3	-3.10**	-0.52	0.63	0.28	0.76	-0.1	0	-0.12	-0.17
E28	2.33**	3.98	-0.33	0.01	-0.98	-0.12	0.02	-0.08	-0.13
E37	0.76	5.4	-0.01	-0.52	0.62	-0.18	-0.02	-0.05	-0.15
E7	0.9	-5.67	0.1	1.09	1.36	0.29	0.04	0.18	0.34
E16	0.76	5.76	0.07	-0.7	-0.83	0.01	0.01	0	0.01
SE±	0.61	6.46	0.4	0.76	1.22	0.27	0.04	0.23	0.35
			Combined						
	DA	PH	GY	LUT	ZEA	βCX	αC	βC	PVA
E8	-1.52	5.00	0.27	0.77*	-1.54*	0.01	-0.02	0.03	0.03
E26	-1.06	-6.68	-0.4	-0.74*	0.7	0.05	0.02	-0.09	-0.05
E6	1.83	3.21	0.04	0.08	1.66*	0.21	-0.01	0.11	0.21
E9	-0.74	-4.25	0.52	-0.55	-0.7	0.15	-0.01	0.02	0.09
E3	-2.13	2.5	-0.08	0.28	0.14	-0.15	-0.01	-0.03	-0.11
E28	2.80*	2.46	-0.01	-0.02	-0.83	-0.11	0.01	0.04	-0.01
E37	0.15	0.82	0.16	-0.32	0.81	-0.07	-0.01	-0.2	-0.24
E7	0.44	-6.5	-0.3	1.28**	0.45	0.02	0.01	0.11	0.13
E16	0.23	3.46	-0.18	-0.78*	-0.7	-0.12	0	0.01	-0.04
SE±	1.11	7.79	0.27	0.3	0.74	0.21	0.03	0.14	0.18

*significant at 0.05 level of probability; **significant at 0.01 ; SE±- Standard error for the difference between two GCA effects.

It also had non significant positive GCA for GY across the locations but had non significant negative GCA for GY at both locations. Line E6 had positive GCA effects for βC, PVA and ZEA at Samaru, Kerawa and across the locations. It had negative effects for LUT and αC at Samaru, and for βCX across locations. Line E9 ((9071 x 4058)-8-2-1-1) had negative GCA effects for DA and PH at both locations and across the locations. However, GCA effects for E9 were positive for GY at both locations and across the locations. Line E9 had negative GCA effects for LUT, ZEA and αC at both locations and across the locations. It also exhibited negative GCA effects for βCX, βC and PVA at Kerawa. Line E9 had positive GCA effects for βCX, βC and PVA at Samaru and across the locations. Line E3 (9450xKI 21-1-4-1-2-1) had negative GCA effects for DA at both locations and across the locations being highly significant at Kerawa. It also had negative GCA for PH at Kerawa and GY across the locations. However, E3 had positive GCA effects for PH and GY at Samaru and for GY at Kerawa. Line E3 had negative GCA effects for ZEA, βCX, αC and PVA at Samaru, for βCX, βC and PVA at Kerawa and for βCX, αC, βC and PVA across the locations. Line E28 (KU1414-SR/KVI11-7-1) had significant positive GCA effects for DA at both locations and across the locations. The GCA effects of E28 were also positive for PH but negative for GY at both locations and across the locations. Line E28 had negative GCA effects for LUT, ZEA, βCX and αC at Samaru while its GCA effects for βC and PVA were positive. At Kerawa, E28 had negative GCA effects for ZEA, βCX, βC and PVA. Line E28 had negative GCA effects for LUT, ZEA, βCX and PVA across locations. Line E37 (KU1409 x MO17LPA x KU1409-27-3-4-1) at Samaru showed negative GCA effects for DA and PH but positive GCA effect for GY. However, the GCA effects of Line E37 at

Kerawa and across the locations were positive for DA and PH while it was negative for GY at Kerawa. Line E37 had negative GCA effects for ZEA, βC and PVA at Samaru and for all the carotenoids at Kerawa and across the locations with exception of ZEA. For line E7 (9450xKI 21-7-3-1-2-5), the GCA effects were negative for DA, PH and GY at Samaru. At Kerawa, Line E7 had positive GCA effects for DA and GY but had negative GCA effects for PH. The GCA effects of E7 across the locations were positive for DA but negative for PH and GY. The GCA effects of E7 were negative for ZEA, βCX, αC and PVA at Samaru but positive at Kerawa and across the locations for all the measured traits with LUT being significant across the locations. Line E16 (KU1414-SR/KVI43-6-4) had negative GCA effects at Samaru for DA and GY but positive GCA for PH. Line E16 had positive GCA effects for DA, PH and GY at Kerawa and across the locations with the exception of GY across locations. Line E16 had negative GCA effects for all the carotenoids except αC and βC at Samaru and across locations, while it was negative for only LUT and ZEA at Kerawa.

Lines E7 and E8 showed significant positive GCA effects for LUT contents across locations (Table 15). Lines E9, E16 and E26, however, showed significantly negative general combining ability for LUT which was preferable to the positive values. Line E6 showed significant negative GCA for ZEA but had significant positive GCA effects for LUT. None of the lines showed significant general combining ability for carotenoids that have Provitamin A activity (i.e. βCX, αC, βC and PVA) at each location and across the locations (except E37 at Samaru having a significant negative GCA effect for βC content). Lines E6, E9, E28 and E7 have good GCA effect for βC and PVA contents. Generally,

these lines had significant positive GCA effects for almost all the traits at both locations, however, the values differed according to location.

4.3.2 Specific combining ability effects

Estimates of specific combining ability (SCA) effects for agronomic traits (DA, PH and GY) and the carotenoids at Samaru and Kerawa are presented in Table 16, and for the combined locations in Table 17. For DA, significant positive SCA effects were expressed by the crosses E26/E16, E6/E9 and E3/E7 at Samaru and E8/E9, E6/E3, E6/E28, E28/E37 and E28/E16 at Kerawa. At Samaru, none of the cross combinations had significant negative SCA effect which is the desirable effect for DA. However, at Kerawa, crosses E8/E28, E26/E28, E6/E9, E6/E37, E3/E28 and E3/E37 had significant negative SCA effects for DA. At Samaru, crosses E7/E16, E6/E3, E26/E28, E8/E37 and E26/E9 had significant negative SCA effects for PH which are desirable to reduce lodging and increase yields.

Significant negative SCA effects were also observed at Kerawa for E8/E6 and E3/E7 for PH. For GY, positive SCA effects are desirable indicating better performance of the cross over average performance of the parents. Thus, nine crosses: E8/E28, E26/E6, E26/E16, E6/E9, E9/E3, E9/E28, E3/E28, E37/E7 and E7/E16 had significant positive SCA effects for grain yield at Samaru, E37/E7 being the highest, followed by E26/E6 and E9/E28 (Table 16). At Kerawa, E3/E28, E37/E16, E6/E9, E26/E6 and E8/E7 had significant positive SCA effects for GY, E26/E6 and E37/E16 being the highest. Significant positive SCA effects for LUT were observed for E8/E3, E28/E16 and E37/E7 at Samaru and for

E8/E6 and E26/E28 at Kerawa. The negative SCA effects for LUT (desirable), were, however, expressed by 53% of the crosses at Samaru and by 56% at Kerawa. Among the crosses with negative SCA effects for LUT, only E9/E3 at Kerawa and E8/E9 at Samaru were significant.

For ZEA, crosses with significant positive SCA effects were E8/E7, E26/E37 and E3/E16 at Samaru and E8/E9 and E3/E16 at Kerawa. Thus, E3/E16 and E26/E37 had the highest positive SCA effects for ZEA at both locations. Significant negative SCA effects for ZEA were shown by the crosses E8/E16, E6/E16, E3/E37 and E3/E7 with E3/E7 being the highest at Samaru; the crosses E8/E26, E6/E3 and E7/E16 also showed significant negative SCA effects for ZEA at Kerawa. For carotenoids having Provitamin A activity (i.e. βCX, αC, βC and PVA), at Samaru, the crosses showing significant positive SCA effects for βCX were E6/E28, E3/E28 and E6/E28; for αC, E26/E37 and E3/E16; for βC, E26/E37, E3/E16, E26/E3 and E6/E7; and E6/E7 for PVA. The crosses that had significant positive SCA effects at Kerawa were E8/E16, E26/E28, E3/E37, E3/E16 and E28/E37 for αC; and E8/E6 for βC and PVA (Table 16).

For the combined locations (Table 17), DA had 50% of the crosses showing positive SCA effects (i.e 18 out of 36 crosses) with E6/E3 showing the only significantly positive effect. No crosses had significant negative SCA effect for DA. For PH, only crosses E8/E3 and E26/E16 had highly significant positive SCA effect.

Table 16. Specific Combining Ability (SCA) effects of 36 crosses of 9 maize inbred lines for agronomic traits and carotenoids at Samaru and Kerawa in 2013

	Samaru									Kerawa								
Crosses	DA	PH	GY	LUT	ZEA	βCX	αC	βC	PVA	DA	PH	GY	LUT	ZEA	βCX	αC	βC	PVA
E8 /E26	2.41	19.00	0.53	1.17	0.02	0.36	-0.08	0.44	0.58	-0.54	11.55	0.59	-2.05	-4.65	-0.93	0.03	-0.42	-0.87
E8 /E6	-3.23	-14.43	-0.49	0.23	-2.44	-0.31	-0.05	-0.17	-0.35	-0.18	-24.3	-1.28	3.77	5.13	0.51	0.09	1.21	1.52*
E8 /E9	0.98	0.21	-0.05	-1.89	-1.75	-0.09	0.07	0.18	0.17	3.75	0.48	0.68	-2.47	-1.74	-0.04	0.03	-0.19	-0.19
E8 /E3	2.20	21.43	-1.45	5.66	1.76	0.07	0.03	0.07	0.12	-0.18	16.77	-0.87	1.71	-1.71	-0.26	-0.11	-0.17	-0.35
E8 /E28	-2.23	20.00	1.04	-2.09	-0.3	-0.33	0.02	-0.79	-0.94*	-2.61	-15.23	0.21	-2.35	1.83	0.23	-0.12	-0.35	-0.29
E8 /E37	3.98	-27.79	-0.12	-1.88	2.99	0.25	-0.02	0.22	0.34	0.96	3.34	-0.55	0.12	0.30	0.10	-0.04	-0.09	-0.06
E8 /E7	-3.45	-16.71	0.01	-0.17	3.58	0.29	0.04	0.12	0.29	-1.68	1.91	1.37	-0.30	1.95	0.52	-0.02	-0.33	-0.08
E8 /E16	-0.66	-1.71	0.53	-1.04	-3.87	-0.24	-0.01	-0.07	-0.19	0.46	5.48	-0.15	1.56	-1.12	-0.13	0.13	0.33	0.33
E26 /E6	-1.59	-9.43	2.68**	-0.08	1.36	-0.13	-0.04	-0.03	-0.12	-0.75	-6.45	2.38	-1.21	1.63	0.22	-0.04	0.13	0.21
E26 /E9	-0.88	-24.29	-1.17*	-0.22	-1.15	0.48	-0.09	0.12	0.31	1.18	5.84	-2.06	1.11	1.09	0.13	0.04	0.15	0.23
E26 /E3	-0.66	-6.57	-0.08	0.52	1.20	0.34	-0.05	0.66	0.81	1.25	2.13	-0.92	-0.12	-0.07	0.04	-0.06	0.08	0.07
E26 /E28	-1.09	-17.00	-1.35*	-1.94	-2.33	-0.25	-0.11	-0.39	-0.57	-4.18	-12.38	-1.15	2.75	-0.34	-0.18	0.12	-0.32	-0.35
E26 /E37	0.13	0.21	-1.32*	0.78	3.75	-0.06	0.54	-0.18	0.05	1.39	-6.30	-0.42	0.11	2.69	0.09	-0.07	0.03	0.04
E26 /E7	-2.80	2.79	-0.34	-0.44	-1.04	-0.24	-0.12	-0.35	-0.53	-0.25	-5.23	1.04	-1.59	-0.18	0.44	0.03	-0.01	0.22
E26 /E16	4.48	35.29	1.05*	0.21	-1.8	-0.49	-0.05	-0.27	-0.54	1.89	10.84	0.54	0.99	-0.15	0.19	-0.04	0.36	0.44
E6 /E9	6.48	4.29	0.97*	2.19	2.93	0.20	0.10	0.23	0.38	-4.96	-17.52	1.91	1.57	-1.96	-0.09	0.06	0.08	0.07
E6 /E3	-0.30	-15.5	-0.51	1.28	-1.3	-0.61	-0.05	-0.50	-0.83	8.61	14.77	0.65	-1.64	-4.02	-0.39	-0.07	-0.51	-0.74
E6 /E28	1.77	5.07	-0.58	-0.56	1.27	0.75	0.18	0.33	0.79	2.68	16.77	-0.70	-1.31	-2.6	-0.49	0.00	-0.28	-0.53
E6 /E37	-2.02	13.79	-1.40**	-1.00	-0.35	0.15	-0.14	0.05	0.06	-3.75	2.84	-2.19	-1.46	1.26	0.42	-0.02	-0.17	0.04
E6 /E7	0.55	14.86	0.01	-2.22	1.81	0.43	0.08	0.67*	0.92*	-0.39	13.91	-0.09	2.14	1.24	-0.37	0.08	0.04	-0.10
E6 /E16	-1.66	1.36	-0.67	0.15	-3.29	-0.47	-0.09	-0.58	-0.86	-1.25	-0.02	-0.68	-1.85	-0.69	0.18	-0.10	-0.50	-0.46
E9 /E3	-1.59	-16.36	0.96*	-2.52	1.55	-0.38	0.10	-0.10	-0.24	-0.96	-11.45	0.56	-0.85	3.04	0.03	0.00	0.20	0.22

Table 16 continued

	Samaru									Kerawa								
Crosses	DA	PH	GY	LUT	ZEA	βCX	αC	βC	PVA	DA	PH	GY	LUT	ZEA	βCX	αC	βC	PVA
E9 /E28	1.48	7.71	2.00**	1.38	1.52	-0.02	-0.03	0.16	0.13	-0.89	6.55	0.15	0.20	-0.26	0.37	-0.07	-0.06	0.09
E9 /E37	-3.80	15.43	-0.19	0.27	-0.31	0.32	-0.05	-0.29	-0.15	1.18	17.63	-0.14	1.23	-0.05	-0.17	-0.06	-0.23	-0.34
E9 /E7	-3.23	-3.50	-1.18*	0.69	-2.59	-0.21	-0.02	0.16	0.05	1.54	8.70	0.67	-0.78	0.73	0.02	0.04	0.45	0.48
E9 /E16	0.55	16.5	-1.34*	0.10	-0.21	-0.29	-0.08	-0.45	-0.63	-0.82	-10.23	-1.78	-0.02	-0.85	-0.25	-0.04	-0.41	-0.56
E3 /E28	-2.80	-1.57	1.72*	0.31	0.16	0.75	-0.03	0.19	0.55	-3.32	-7.16	1.38	-0.12	-1.93	-0.18	-0.10	-0.15	-0.29
E3 /E37	0.91	16.64	0.73	-1.61	-3.33	-0.01	-0.16	-0.48	-0.57	-3.75	16.41	0.26	0.81	-0.85	0.34	0.20	0.21	0.48
E3 /E7	4.48	15.21	-1.89*	-1.91	-5.22	-0.64	-0.08	-0.95	-1.31	-0.39	-27.52	-1.71	1.02	1.38	-0.04	-0.04	-0.07	-0.10
E3 /E16	-2.23	-13.29	0.52	-1.72	5.19	0.49	0.25	1.11	1.48	-1.25	-3.95	0.65	-0.81	4.15	0.46	0.17	0.40	0.71
E28 /E37	0.98	-3.79	-0.80*	-1.59	-2.6	-0.57	0.02	0.44	0.17	3.82	-8.09	0.87	-0.44	-1.36	-0.19	0.13	0.39	0.36
E28 /E7	3.05	-7.71	-0.94*	1.91	0.52	-0.41	-0.01	-0.02	-0.23	1.68	10.48	-0.64	-0.28	2.13	0.25	0.06	0.67	0.82
E28 /E16	-1.16	-2.71	-1.07	2.57	1.76	0.08	-0.04	0.08	0.10	2.82	9.05	-0.12	1.54	2.53	0.21	-0.04	0.10	0.19
E37 /E7	0.27	8.00	3.21**	3.71	0.29	-0.12	-0.06	0.22	0.13	0.75	-8.45	-0.01	0.41	-2.68	-0.37	-0.11	-0.31	-0.55
E37 /E16	-0.45	-22.5	-0.12	1.31	-0.44	0.04	-0.14	0.02	-0.03	-0.61	-17.38	2.18	-0.78	0.70	-0.23	-0.04	0.16	0.03
E7 /E16	1.13	-12.93	1.12*	-1.57	2.66	0.89	0.16	0.16	0.68	-1.25	6.20	-0.63	-0.63	-4.57	-0.44	-0.05	-0.44	-0.68
SE±	2.17	11.72	0.38	1.17	1.51	0.35	0.11	0.31	0.45	0.99	10.47	0.65	1.23	1.98	0.43	0.06	0.37	0.57

*significant at 0.05 level of probability; **significant at 0.01 level of probability; DA= Days to 50% anthesis; PH= Plant height; GY= Grain yield ; LUT= Lutein; ZEA= Zeaxanthin; βCX= β-cryptoxanthin; αC= α-Carotene; βC= β-Carotene; PVA= total Provitamin A; SE±= Standard error for the difference between two SCA effects.

The other crosses that possessed high positive SCA effects for PH though not significant were E8/E26, E6/E28, E6/E7, E9/E37 and E3/E37. Desirable significant negative SCA effects for PH was observed for E8/E6 and E37/E16. Other crosses with high negative SCA effect for PH were E8/E37, E26/E28 and E9/E3. For GY, 19 crosses (53% of the crosses) had positive SCA effect. Crosses E26/E6, E6/E9, E3/E28 and E37/E7 had significant positive SCA effect; E26/E6 showing the highest (2.53) followed E37/E7 (1.60). The remaining 47% of the crosses showing negative SCA effect for GY were E8/E3, E26/E9, E6/E37, E9/E16, and E3/E7 being significant.

Highly significant positive SCA effect for LUT was observed for E8/E3 across locations. Negative SCA effect for LUT was, however, observed by 53% of the crosses across locations with only effects for E8/E9 and E8/E28 being significant. For ZEA, crosses with significant positive SCA effects were E26/E37 and E3/E16 across locations. However, none of the crosses had significant negative SCA effect for ZEA across locations. The cross E3/E16 had significant positive SCA effects for αC, βC and PVA, while E8/E6 had significant positive SCA effect for only βC. Crosses E8/E28, E6/E3, E6/E16 and E3/E7 had significant negative SCA effects for βC with E6/E3 also exhibiting significantly negative SCA effects for PVA. Though, many of the crosses had high positive SCA effects for carotenoids having PVA activities, effects were not significant.

Table 17. Specific combining ability (SCA) effects of 36 crosses of 9 maize inbred lines for agronomic traits and carotenoids across locations in 2013

Crosses	DA	PH	GY	LUT	ZEA	βCX	αC	βC	PVA
E8 /E26	0.94	15.28	0.56	-0.44	-2.32	-0.29	-0.03	0.01	-0.15
E8 /E6	-1.71	-19.37*	-0.89	2	1.35	0.1	0.02	0.52*	0.58
E8 /E9	2.37	0.35	0.31	-2.18*	-1.74	-0.06	0.05	-0.01	-0.01
E8 /E3	1.01	19.1*	-1.16*	3.68**	0.03	-0.1	-0.04	-0.05	-0.12
E8 /E28	-2.42	2.38	0.63	-2.22*	0.77	-0.05	-0.05	-0.57*	-0.62
E8 /E37	2.47	-12.22	-0.33	-0.88	1.65	0.18	-0.03	0.07	0.14
E8 /E7	-2.56	-7.4	0.69	-0.23	2.76	0.4	0.01	-0.1	0.11
E8 /E16	-0.1	1.88	0.19	0.26	-2.49	-0.18	0.06	0.13	0.07
E26 /E6	-1.17	-7.94	2.53**	-0.65	1.49	0.04	-0.04	0.05	0.05
E26 /E9	0.15	-9.22	-1.61**	0.45	-0.03	0.31	-0.03	0.13	0.27
E26 /E3	0.29	-2.22	-0.5	0.2	0.56	0.19	-0.05	0.37	0.44
E26 /E28	-2.63	-14.69	-1.25*	0.41	-1.34	-0.22	0.01	-0.35	-0.46
E26 /E37	0.76	-3.04	-0.87	0.45	3.22*	0.01	0.23*	-0.08	0.05
E26 /E7	-1.53	-1.22	0.35	-1.01	-0.61	0.1	-0.04	-0.18	-0.15
E26 /E16	3.19	23.06*	0.79	0.6	-0.98	-0.15	-0.04	0.05	-0.05
E6 /E9	0.76	-6.62	1.44*	1.88	0.48	0.05	0.08	0.16	0.22
E6 /E3	4.15*	-0.37	0.07	-0.18	-2.66	-0.5	-0.06	-0.51*	-0.78*
E6 /E28	2.22	10.92	-0.64	-0.93	-0.66	0.13	0.09	0.02	0.13
E6 /E37	-2.88	8.31	-1.80**	-1.23	0.46	0.29	-0.08	-0.06	0.05
E6 /E7	0.08	14.38	-0.04	-0.04	1.52	0.03	0.08	0.35	0.41
E6 /E16	-1.46	0.67	-0.67	-0.85	-1.99	-0.14	-0.09	-0.54*	-0.66
E9 /E3	-1.28	-13.9	0.76	-1.68	2.3	-0.18	0.05	0.05	-0.01
E9 /E28	0.29	7.13	1.07	0.79	0.63	0.17	-0.05	0.05	0.11
E9 /E37	-1.31	16.53	-0.16	0.75	-0.18	0.07	-0.05	-0.26	-0.25
E9 /E7	-0.85	2.6	-0.26	-0.05	-0.93	-0.09	0.01	0.3	0.26
E9 /E16	-0.13	3.13	-1.56*	0.04	-0.53	-0.27	-0.06	-0.43	-0.59
E3 /E28	-3.06	-4.37	1.55*	0.09	-0.89	0.28	-0.07	0.02	0.13
E3 /E37	-1.42	16.53	0.49	-0.4	-2.09	0.16	0.02	-0.14	-0.04
E3 /E7	2.04	-6.15	-1.80**	-0.44	-1.92	-0.34	-0.06	-0.51*	-0.71
E3 /E16	-1.74	-8.62	0.59	-1.27	4.67**	0.47	0.21*	0.75**	1.09**
E28 /E37	2.4	-5.94	0.03	-1.01	-1.98	-0.38	0.08	0.42	0.27
E28 /E7	2.37	1.38	-0.79	0.82	1.32	-0.08	0.03	0.32	0.3
E28 /E16	0.83	3.17	-0.61	2.05	2.14	0.14	-0.04	0.09	0.14
E37 /E7	0.51	-0.22	1.60*	2.06	-1.19	-0.24	-0.08	-0.05	-0.21
E37 /E16	-0.53	-19.94**	1.03	0.27	0.13	-0.09	-0.09	0.09	0
E7 /E16	-0.06	-3.37	0.25	-1.1	-0.95	0.22	0.06	-0.14	0
SE±	1.72	8.39	0.54	1.04	1.5	0.28	0.08	0.24	0.37

DA= Days to 50% anthesis; PH= Plant height; GY= Grain yield; SE± = Standard error for the difference between two SCA effects.

4.4 Correlations among Grain yield, Days to 50% anthesis, Plant height and the Carotenoids

Grain yield (GY) indicated positive phenotypic correlation (rP) with PH, PVA, βC, βCX, αC and ZEA being significant with βC, βCX and αC (Table 20). GY was non-significantly negatively correlated (rP) with DA and LUT. However, the genotypic correlation coefficients (rG) showed marked reduction in magnitude and change in directions for all the corresponding phenotypic correlations (rP) between GY and each of the studied variables. The genotypic correlation between GY and βC, βCX and αC were -0.14, -0.19 and -0.32, respectively (Table 18), while it was also low and positive with DA (rG=0.05) and LUT (rG= 0.06). The DA was phenotypically negatively correlated with all the carotenoids but genotypically positively correlated with them**,** except βCX and ZEA which were negative **(**rG= -0.05 and -0.16, respectively**)**. The phenotypic correlation (rP) between DA and αC was significantly negative (rP= -0.45) but the genotypic correlation was non-significant and positive (rG= 0.21). However, the phenotypic and genotypic correlations between DA and PH were both positive and had the same magnitude (rP = rG = 0.18).

PH was negatively correlated with PVA, βC, αC and ZEA at both the genotypic and phenotypic levels. The correlation between PH and ZEA was highly significant and negative at the genotypic level (rG= -0.67) while at the phenotypic level, though in the same direction, it was non significant (rP= -0.08) (Table 18). PH was positively correlated with LUT at both the genotypic and phenotypic levels with the genotypic value being higher than the phenotypic (rP= 0.03; rG= 0.11).

Table 18. Estimates of genotypic correlation coefficients (upper diagonal) and phenotypic correlation coefficients (lower diagonal) among agronomic and carotenoid traits in 36 Provitamin A maize crosses tested across two locations in 2013 wet season

	GY	AD	PH	PVA	βC	βCX	αC	ZEA	LUT
GY	1	0.05	-0.20	-0.06	-0.14	-0.19	-0.32	-0.07	0.06
AD	-0.20	1	0.18	0.05	0.16	-0.05	0.21	-0.16	0.12
PH	0.11	0.18	1	-0.03	-0.04	0.10	-0.02	-0.67**	0.11
PVA	0.38	-0.05	-0.15	1	0.59**	0.26	0.27	-0.06	0.04
BC	0.54**	-0.17	-0.09	0.91**	1	-0.31	-0.31	-0.05	0.10
BCX	0.48*	-0.11	-0.21	0.84**	0.69**	1	-0.36	-0.02	0.04
AC	0.79**	-0.45*	-0.03	0.40	0.54**	0.57**	1	0.03	0.07
ZEA	0.05	0.06	-0.08	0.75**	0.58**	0.56**	0.11	1	-0.29
LUT	-0.25	-0.12	0.03	0.04	-0.03	-0.17	-0.28	0.35	1

DA= Days to anthesis; PH= Plant height; GY= Grain yield ; LUT= Lutein; ZEA= Zeaxanthin; βCX= β-cryptoxanthin; αC= α-Carotene; βC= β-Carotene; PVA= total ProvitaminA.

* and ** indicate significant at 5% and 1% levels of probability, respectively.

PVA was genotypically and phenotypically positively correlated (rP and rG) with the carotenoids βC, βCX, αC, ZEA and LUT. The genotypic correlation with ZEA was negative (rG = -0.06). The phenotypic correlation between PVA and βC and βCX were highly significant (rP= 0.58 and 0.56, respectively). βC was phenotypically highly significantly positively correlated with βCX, αC and ZEA but genotypically negatively non-significantly correlated (rG) with them. βCX was highly significantly positively correlated (rP) with αC and ZEA but negatively correlated at the genotypic level (rG= -0.36 and -0.02, respectively). LUT was phenotypically negatively correlated with αC, βCX and βC but positively correlated with PVA and ZEA. LUT was, however, positively correlated with all the carotenoids except ZEA at the genotypic level (rG) (Table 18).

4.5 Cause and Effect Relationships Among the Carotenoids

The path coefficient analysis of genotypic correlations between GY as the dependent variable, with the independent variables, DA, PH and the carotenoids, PVA, βC, βCX, αC, ZEA and LUT is presented in Table 19. PVA had high positive direct effect on GY (44.08%) but had negative genotypic correlation (rG) with it. This was influenced by the high negative indirect effects via βC and αC at -0.535 (36.01%) and -0.238 (16.02%), respectively. Similarly, βC and αC that were highly significantly positively correlated (rP) with GY at 0.54 and 0.79, respectively, had lower non-significantly negative correlation with GY at the genotypic level (rG = -0.14 and -0.32, respectively).

Table 19. Direct (diagonal, bold) and indirect effects (off-diagonal) of Path analysis of genotypic correlation coefficient (rG) between Grain Yield (GY) of Provitamin A maize crosses with DA, PH and all the carotenoids traits

		DA	PH	PVA	βC	βCX	αC	ZEA	LUT	rG with GY
DA	P	**0.374**	-0.094	0.034	-0.145	0.000	-0.181	0.049	0.013	0.050
	%	42.02	10.49	3.81	16.32	0.01	20.36	5.51	1.48	
PH	P	0.066	**-0.527**	-0.018	0.040	0.000	0.021	0.211	0.012	-0.195
	%	7.41	58.81	2.05	4.46	0.02	2.34	23.57	1.34	
PVA	P	0.019	0.015	**0.655**	-0.535	0.000	-0.238	0.019	0.004	-0.060
	%	1.30	1.00	44.08	36.01	0.03	16.02	1.26	0.30	
βC	P	0.060	0.023	0.387	**-0.907**	0.001	0.274	0.016	0.011	-0.136
	%	3.58	1.38	23.04	54.04	0.08	16.31	0.94	0.64	
βCX	P	-0.017	-0.052	0.170	-0.624	**0.002**	0.316	0.006	0.004	-0.194
	%	1.42	4.36	14.29	52.36	0.16	26.56	0.48	0.37	
αC	P	0.078	0.013	0.179	0.285	-0.001	**-0.871**	-0.008	0.007	-0.318
	%	5.41	0.88	12.41	19.77	0.05	60.40	0.59	0.50	
ZEA	P	-0.058	0.352	-0.039	0.045	0.000	-0.023	**-0.317**	-0.031	-0.071
	%	6.71	40.68	4.47	5.22	0.00	2.70	36.62	3.60	
LUT	P	0.046	-0.065	0.027	-0.090	0.000	-0.058	0.092	**0.107**	0.059
	%	9.47	13.33	5.56	18.63	0.02	11.98	18.90	22.12	
Residual effect										**0.859**

Residual effect = √-0.241; Bold figures in diagonal line indicate direct effects; P= Path coefficient; % = Percentage direct and indirect effects.DA= Days to anthesis; PH= Plant height; LUT=Lutein; ZEA= Zeaxanthin;βCX= β-cryptoxanthin; αC= α-Carotene; βC= β-Carotene; PVA= total ProvitaminA.

CHAPTER 5: DISCUSSION

5.1 Genetic Diversity

Genetic diversity of 31 inbred lines of medium to high PVA maize was determined by use of 14 polymorphic SSR markers. Allelic variations over 33 loci were uncovered with an average of 2.36 alleles per locus using the 14 SSR markers. This value was lower than previously reported alleles per locus of 5.21 (Senior *et al.*, 1998) for 94 elite U.S. maize inbreds, 4.9 for 40 U.S maize inbreds (Lu and Bernardo, 2001) and 21.7 for 260 inbreds encompassing a greater portion of maize gene pool (Liu *et al.,* 2003). The observed mean alleles per locus was, however, close to 3.96 reported by Adeyemo *et al.* (2011) using larger number (74) of SSR markers for 38 tropical-adapted yellow maize inbred lines of similar genetic background. The differences are probably due to differences in number of lines examined, more diverse selection of inbreds (tropical, subtropical, and temperate), and larger number of SSR markers (40-94). The PIC range of 0.20 to 0.53 for the 14 SSR markers used were within the range of that reported by Adeyemo *et al.* (2011) who observed PIC mean of 0.56 for 74 core set of highly informative SSR markers. This indicates that the 14 SSR markers used in this research are adequate to discriminate maize inbred lines. This corroborated finding of Liu *et al.* (2003) that 9-12 SSR markers are sufficient to fingerprint maize inbred lines. Thus, the 14 SSR markers used in this research effectively separated the 31 tropical-adapted yellow maize inbred lines into two main groups and different clusters and subclusters consistent with their pedigrees.

Gene diversity for the 14 SSRs and 31 inbred lines was 0.45 indicating moderate gene diversity compared to 0.56 (Senior *et al*., 1998) and 0.82 (Liu *et al*., 2003). Since estimates of gene diversity are not affected by differences in sample size (Liu *et al.,* 2003), the higher gene diversity values

reported by Senior *et al.* (1998) and Liu *et al*. (2003) are likely a function of the use of a greater portion of dinucleotide repeat SSRs, labeled markers and the more diverse set of inbred lines. The Euclidean genetic similarity distance (Appendix Table 4) used for constructing the relationship tree showed that only 5% (22 out of 434 pairs) of the lines were closely related with distances between 0.10 and 0.29 while 41% (180 out of 434 pairs) had large dissimilarity with distances between 0.7 and 1 indicating moderate to high genetic diversity among the inbred lines. The effectiveness of the 14 SSR markers used in determining relationships among the studied inbred lines was in agreement with their pedigree relationship. Gerdes and Tracy (1994) pointed out that pedigree relationship can be used as a benchmark to test markers effectiveness. The result was similar to other findings (Liu *et al.,* 2003; Adeyemo *et al.,* 2011), indicating that SSRs are efficient markers to classify closely related lines. PCoA based on Euclidean distance clearly showed the variability between groups of the inbred lines and within each group. Thus, the inbred lines in subgroup 1 (E14, E19, E20, E26 and E28) and subgroup 2 (E37, E23 and E36), both belonging to group I, and all the other lines in group II (i.e. subgroups 3 and 4), can be utilized as potential parents for hybridization among clusters within groups to exploit heterosis.

The nine selected inbred lines expressed at least 3-4 alleles favouring increased endosperm provitamin A (βC, βCX and PVA) levels as presented and predicted by Harjes *et al.* (2008) and Yan *et al.* (2010). Thus, genotypes with desirable alleles can be selected at seedlings stage by marker assisted selection to restrict resources from managing non desirable genotypes. Marker based screening is also time saving; it can be accomplished with high throughput (hundreds of samples) in 24 hours using leaves from seedlings or matured plants contrary to HPLC analysis that may take weeks. Thus, these molecular markers (*lcyE* and *crtRB1)* hold great potential for accelerated and

resource efficient introgression and selections of medium to high provitamin A maize germplasm (Mhike *et. al*., 2010).

5.2 Variability and Mean Performance

The analysis of variance (ANOVA) showed significant differences amongst the crosses and the checks for all the agronomic traits (DA, DS, ASI, EH, EPH, EPP and GY) indicating existence of variations in these traits and scope for selection. Mean performance showed promising crosses with higher yields than the commercial hybrid checks and desirable agronomic traits at both locations and across the locations. Differences in performance for the agronomic traits at the different locations indicate heterogeneity in experimental fields, differences in soil fertility, moisture and even land leveling. These are similar to findings of Dickert and Tracy (2002) and Duraes *et al.* (2002).

Similarly, ANOVA for the crosses (without checks) showed significant differences for DA, PH, GY, LUT, ZEA and βC at Samaru location but no significant differences were observed at Kerawa location for all the carotenoids indicating that Samaru is a better test location for the carotenoid traits where the crosses can be phenotypically distinguished. The results also indicate existence of variability and scope for improvement of LUT, ZEA and βC. These results are in agreement with reports of Egesel *et al.* (2003a), Menkir and Maziya-Dixon (2004), Chander *et al.* (2008a) and Menkir *et al.* (2008) who also reported existence of variability in carotenoids in other maize populations. The GCA mean squares were significant for DA, PH, GY, LUT, ZEA and βCX. The SCA mean squares were also significant for DA, GY, LUT and ZEA. These suggest both additive and non additive gene effects control these traits. The relative importance of these gene effects as

estimated using GCA:SCA indicates preponderance of additive gene action for DA, PH, LUT, ZEA and βCX but preponderance of non additive gene action for GY, βC, αC and PVA. Egesel *et al.* (2003a), Chander *et al.* (2008a) and Suwarno *et al.* (2014) also reported presence of both additive and non additive gene actions in control of the carotenoids. The across locations ANOVA revealed that location differences were significant for DA, GY, LUT, βC, αC and PVA but not for PH and ZEA. The effects of location on βC, αC and PVA could probably be indicating differences in soil nutrients required for metabolites and/or enzymes co-factors needed for carotenoids biosynthesis. The lack of significant location effect on ZEA for hybrid maize was reported by Egesel (2003a). This could be due to the fact that ZEA is lower down in the biosynthetic pathways of βC and βCX, and therefore depends on βCX as its precursor (Vallabhaneni *et al.,* 2009 and Rodriquez-Amaya, 2010). Genotype (Crosses) differences were not significant for βCX, βC, αC and PVA but significant for LUT and ZEA. This is contrary to the report by Menkir *et al.* (2008) that significant differences existed for βCX, βC, αC and PVA in addition to LUT and ZEA for tropical-adapted maize inbred lines. The difference could probably be due to difference in the nature of materials studied being homozygous but heterogenous and thus more genetically diverse in the other studies compared to the F_1 crosses of inbred lines fixed for PVA used in this study. Significant GCA and SCA effects were also observed for DA, PH, GY, LUT and ZEA but not for βCX, βC, αC and PVA across locations suggesting use of recurrent selection to improve these traits. The observed non significant crosses by location interaction for all the carotenoids was similar to the report by Menkir *et al.* (2008) that carotenoids concentrations were not strongly affected by replications or locations or genotype by environment interactions. It thus, implied that screening for variability in carotenoids contents can be conducted with samples obtained from a single location. However, GCA x Location and SCA x Location interactions were both significant for DA, PH and ZEA while

SCAxL was also significant for GY and LUT. This agrees with report of Egesel *et al.* (2003a) and indicates location specific additive and non additive gene effects for control of DA, PH and ZEA and for non additive gene effects for GY and LUT. These results and those of Egesel *et al.* (2003a), Menkir and Maziya-Dixon (2004), Pfeiffer and McClafferty (2007), Menkir *et al.* (2008) and Suwarno *et al.* (2014) indicate provitamin A expression is more influenced by genotype or by environment than by genotype x environment interactions.

The combined across location GCA:SCA also indicate preponderance of additive gene action for DA, PH, LUT, ZEA and βCX but preponderance of non additive gene action for GY, βC, αC and PVA. This suggests development of hybrids for high GY, βC, αC and PVA will be more appropriate. Using many parents in synthetic varieties development will enable better exploitation of the additive and non additive gene actions for DA, PH, LUT, ZEA and βCX. Synthetics are developed from recombination of elite inbred lines (usually 10 – 24 lines) and are therefore hybrids of many parents with yield potential greater than open pollinated varieties (OPV) and composite but less than double (DX), three way (TX) and single (SX) cross hybrids. It has the advantage of been broad based like OPV and recyclable by farmer. Though, unlike OPV it has shorter lifespan to retain its variability but retained it hybrid vigor longer than DX, TX and SX (Hallauer *et al.,* 2010).

All the crosses evaluated in this research have provitamin A contents within the range for first generation medium to high PVA maize genotypes (3-8µg/g) with 15µg/g PVA being the final target (HarvestPlus, 2008). The crosses, however, indicated dominance of the lower scoring parent since non additive gene action seems to be predominant for the carotenoids expressions. Although Burt (2010) reported lack of heterotic effect on carotenoids contents for most of the crosses he examined, it should be noted that the lack of heterosis is not always an indication of lack of genetic divergence

between populations as earlier reported by Cress (1966). Another likely reason for the lower than better parents performance of the crosses for the carotenoids could be the source of the inbred lines as opined by Chahal and Gosal (2006), that inbred lines developed through pedigree method do not produce crosses that show substantial superiority over the crosses of the original lines used for developing the inbred lines. Crosses of original lines refer to the crosses from which the inbred lines were derived, usually from segregating population of productive single cross hybrids developed for better *per se* performance for desirable traits (in this case for PVA). The non PVA carotenoids (LUT and ZEA) content of 77.74% of the total carotenoids agree with the results of Egesel *et al.* (2003a) for hybrids and that of Menkir *et al.* (2008) for inbred lines. ZEA was the major carotenoid with a value of about 50%. This result agrees with earlier works of Wong (1999) and Egesel *et al.* (2003a). It also agrees with Suwarno *et al.* (2014) who reported ZEA as the major carotenoid, though it made up only 20.4%. The level of LUT was the second highest (28.40%) and this result concurs with that of Egesel *et al.* (2003a), but differs with that of Suwarno *et al.* (2014) who reported LUT as the least (12.21%) and βCX as the second highest carotenoid (16.85%). The results differed probably owing to the differences in the germplasm used in the studies. As for βC, it made up 9.42% which is greater than what was obtained by Egesel *et al.* (2003a) (4.7%) but lower than what Suwarno *et al.* (2014) reported (13.43%). Egesel *et al.* (2003a) used medium PVA maize (βC=1.22-2.42 µg/g); this research used medium to high PVA maize (βC=1.8 – 8.3 µg/g; PVA = 4.0-9.8 µg/g), while Suwarno *et al.* (2014) also used medium to high PVA maize lines (PVA= 4.4-18.2 µg/g). Also, these differences probably resulted from using inbred lines derived from introgressing PVA enhancing alleles discovered in some maize variants (Harjes *et al.,* 2008 and Yan *et al.,* 2010). The maize mutants/variants cause decreased cyclization of βC to βCX and βCX to ZEA (Harjes *et al.,* 2008). It also reduced conversion of αC to LUT resulting in accumulation of

βC, βCX and αC (Yan *et al.,* 2010). The least carotenoid with PVA activity was αC in this research which is similar to the results of Menkir *et al.* (2008). The results from this study, thus, suggest scope for improvement of GY, βC, αC and PVA.

5.3 Combining Ability

The parents (i.e. the nine maize inbred lines) varied significantly for GCA effects. Significant differences were obtained for different traits for the lines indicating possibility of obtaining good hybrids from these parents for the targeted traits. Lines with high positive GCA estimates for DA, PH and GY were E8, E9, E3 and E37. Similar GCA estimates of maize inbred lines for agronomic traits were reported by Dickert and Tracy (2002), Mhike *et al.* (2010) and Halilu *et al.* (2012). Lines E6, E7, E9 and E28 have good general combining ability for ZEA, βC and PVA contents. High GCA estimates of 1.47 for LUT for E7, 1.66 and -1.54 for ZEA for E6 and E8, respectively, 0.38 for βCX for E9, 0.21 for βC for E6 and -0.36 for βC for E37 were in the upper ranges of the results reported for these carotenoids by Egesel *et al.* (2003a) and Suwarno *et al.* (2014). These findings indicate moderate to high additive genetic effects for DA, GY, ZEA, βCX, βC, αC and PVA which are therefore, fixable even though the GCA:SCA showed preponderance of non additive gene action for GY, βC, αC and PVA. The significant positive GCA estimates for E28 and E6 for DA, E8 (+ve) and E26 (-ve) for PH, E8 and E9 for GY, E8, E7 for LUT, E6 for ZEA, E6 and E7 for βC suggest that through breeding programmes these parents can generate variable materials for improving carotenoids contents in maize genotypes adapted to the tropics.

Significant SCA effects were observed for DA, PH, GY and carotenoids contents (both positive and negative) in desired directions for some of the combinations. Significant SCA mean squares

observed for GY (3.21), 1.11 for βC and 0.75 for βCX were higher than the highest values reported by Egesel *et al.* (2003a) for these traits, respectively, but are within the range reported by Suwarno *et al.* (2014). The performance of E8/E28, E26/E6, E26/E16, E6/E9, E9/E3, E9/E28, E3/E28, E37/E7 and E7/E16 crosses for GY, and E3/E16 and E8/E6 for αC, βC and PVA indicate the possibility of improvement for GY, βC, αC and PVA. This was confirmed from the results that crosses with high SCA effects had parents with high GCA effects for the particular traits (Tables 18 and 19). Though, many of the crosses had positive SCA effects for carotenoids having PVA activities, they were not significant. According to Griffing (1956), GCA estimates close to zero indicate that the genotypes do not differ from the general mean of all crosses. Positive or negative values indicate that the particular parent is better or worse than the other parent included in a diallel as compared to the mean of the cross, and therefore can be selected for improvement.

The observed preponderance of non-additive gene effect for GY, PH, βC, αC and PVA could probably be due to the use of inbred lines in this research as suggested by Crossa *et al.* (1990) who indicated that non-additive effects increase in importance with increase of inbreeding effects because more homozygotic loci exist. Vasal *et al.* (1992) observed similar non-additive genetic effects for PH, while Egesel *et al.* (2003a) and Burt (2010) reported similar results for βC and βCX. This result agrees with report of Babu *et al.* (2012) who showed that gene actions for *LcyE*-5´TE and *CrtRB1*-3´TE, associated with total provitamin A concentration at molecular levels, were partially dominant and partially recessive (i.e., not purely additive). Thus, since dominance or overdominance predominates the highest yielding genotypes must be heterozygotes (Sprague and Eberhart, 1977). Therefore, hybrids like synthetic, single, three way and double cross hybrids will be more appropriate for higher PVA maize varieties. The presence of higher SCA than GCA effects further suggest use of recurrent selection to increase the additive gene actions (Chahal and Gosal,

2006) and to harness both the additive and non additive gene actions for the PVA carotenoids. Recurrent selection using the inbred lines as testers would accumulate the additive genes and exploit heterosis in order to increase GY, other agronomic traits and carotenoids contents. It will also enable recycling of the inbred lines and improve the population mean for short term and long term goals. This will be necessary to develop higher PVA inbred lines since they seem to indicate lower parent overdominance.

The heritability observed in this study are in agreement with results of Chander *et al.* (2008b) who reported high broad sense heritability estimates for non PVA carotenoids and that of Wong *et al.* (2004) who reported medium heritability for all the carotenoids. However, the PVA contributing carotenoids for which the population under study was developed showed low H^2 indicating higher non genetic effects for these traits in the population. This indicates that genetic factors were predominantly responsible for the expression of the non-PVA carotenoids and for GY, DA and PH. Hence, selection for these traits should be initiated at S_1 using families as selection units. The broad sense heritability estimates in this finding are in good agreement with previous investigations in maize (Wong *et al.,* 2004*;* Chander *et al.*, 2008b; Chander *et al.,* 2008a). These findings, therefore, imply that mass selection in the breeding programme will be effective to improve DA, LUT and ZEA which have high heritability. For the other traits, PH, GY, βCX, βC and PVA, hybrid development will be most effective as they indicate prevalence of non additive gene action. This will, thus, involve inbreeding and recombination as in the recurrent selections to improve βCX, βC and PVA in the population. Recurrent selection, as the name implies, is a breeding method that is conducted repetitively. The time frame of recurrent selection methods is not predetermined. They are conducted for the lifetime of a breeding program to provide systematic genetic improvement of

the germplasm resources (Hallauer, 1985). The base population for the recurrent selection should be formulated using the 31 inbred lines which are genetically diverse as indicated by the molecular markers and owing to the fact that 30 to 45 maize inbred lines are most adequate for short and long term use of recurrent selection (Baker and Curnow, 1969 and Rawlings, 1970). This will also improve the population mean for higher βCX, βC and PVA contents than what would be obtainable with pedigree method of inbred lines development (Chahal and Gosal, 2006).

5.4 Correlations and Path Analysis

Grain yield (GY) showed low correlations with DA and PH in agreement with the observed low associations of yield with days to flowering and plant height, averaged across numerous studies (Hallauer *et al.,* 2010). The DA and PH have the same positive and non-significant correlation coefficients at both phenotypic and genotypic levels (rP=rG=0.18) which is also consistent with results of genotypic correlations of PH and DA averaged across studies (rG=0.27) (Hallauer *et al.,* 2010). The phenotypic correlations among GY, DA, PH and all the measured carotenoids, however, were higher than the genotypic correlations and were in different directions (sign). This indicates that phenotypic correlations are influenced by environmental factors pointing to the fact that micronutrients uptake characteristics are largely affected by environmental effects on plant growth and nutrient availability (Gelof and Gabelman, 1983; Stevens, 2008). The phenotypic correlations among the carotenoids were in agreement with the findings of Menkir *et al.* (2008) in which they reported different correlations among the carotenoids for different trials with most of the correlations between pairs of carotenoids being non-significant with the exception of ZEA that showed significantly positive correlations with βC and βCX across the trials. These differences

might be explained on basis of the fact that correlations are defined by the evaluated genotypes and the test environments (Ado *et al.,* 1988).

The phenotypic correlations between GY and the carotenoids were significantly positive with βC, βCX and αC. However, the genotypic correlations were non-significant and negative between GY and all the carotenoids, indicating a masking by environmental effects. The phenotypic correlations between GY and LUT (rP=-0.25) was negative and rather reduced and reversed at the genotypic level (rG =0.06). The results for the genotypic correlations were similar to those of the phenotypic correlations for GY and the carotenoids like reported by Suwarno *et al.* (2014). This is likely due to the large number of entries (df=154) considered in Suwarno *et al.* (2014) as compared to 36 entries (df=34) in this study, causing the phenotypic correlations to approach the genotypic correlations (Hallauer *et al.,* 2010). The reduction in magnitude and change in direction of the correlations were caused by the relatively high negative direct effects of βC at -0.907 (54.04%) and αC at -0.871 (60.40%) on GY. High negative indirect effect of βCX at -0.624 (52.36%) via βC was also responsible for the reduction in magnitude and change in direction of the correlations between GY and βCX (rP = 0.48 and rG = -0.19), even though the supporting indirect positive effects via PVA (14.29%) and αC (26.56%) were relatively high. The residual effect for the path analysis of rG between GY with DA, PH, PVA, βC, βCX, αC, ZEA and LUT was 86% indicating that the independent variables (traits) considered in this study accounted for only 14% causal factors of GY. αC, which is considered as the least carotenoid in maize (Menkir *et al.,* 2008), and therefore not considered in Suwarno *etal.* (2014), showed the highest significantly positive phenotypic correlation and the highest negative genotypic correlation among the carotenoids with GY (rP= 0.79; rG= -0.32). It contributed the highest proportion of negative direct effect on GY at 60.40% which is in the

same direction as the rG. Thus, αC presented the most reliable relationship with grain yield. To use this relationship, a linear equation based on the use of direct and indirect effects was suggested. According to Omokhafe and Alika (2003), the direct effect of the primary independent traits and the indirect effects of the secondary independent traits will serve as weighting factors. The equation is given as:

$-0.32GY = -0.87\alpha C + 0.18PVA + 0.29\beta C$

$GY = 2.72\alpha C - 0.56PVA - 0.91\beta C$

The negative sign on GY shows that the correlation is negative. This implies that a selection of 2.72 units lower αC content should be accompanied by an increase of 0.5 units of PVA and 0.91 units of βC and an increase in mean grain yield. Separate model of cause and effects will need to be developed for any primary trait of interest, since direct and indirect effects are not reversible (Omokhafe and Alika, 2003). Thus to utilise those traits with high indirect causes but low genotypic correlation (rG), they should be selected as secondary independent traits with appropriate model through primary traits whose rG and direct contributions are in the same direction. Secondary independent traits whose indirect contributions are in the same direction with the direct and genotypic correlation are more reliable and useful for indirect selections (Omokhafe and Alika, 2003). The significantly positive phenotypic correlation (rp) between ZEA and all the other carotenoids (PVA, βC, βCX, αC) coupled with the relatively high heritability of ZEA suggest that ZEA can be used as a primary independent trait for indirect selection of PVA, βC and βCX. This also has the advantage for easy screening by spectrophotometric techniques (Mishra and Singh, 2010), because ZEA is abundant and more stable to light exposure. These findings concur with results of Menkir *et al.* (2003) and Burt *et al.* (2010) who reported significantly positive phenotypic correlations between ZEA with both βC and βCX. The case of αC, having one β and one ε ring,

correlated with the β, β-ring provitamin A carotenoids; βCX and βC. The involvement of two different enzymes, lycopene β-cyclase and lycopene ε-cyclase might explain the observed correlations Sandmann (1994) as it is noted in Santos and Simon (2006) who reported that αC is produced after the synthesis of β-carotene in carrot, hence the significant correlations observed for αC with the βC and βCX. ZEA, which is down the β, β ring carotenoid pathway, was also significantly correlated with βCX and βC. This was consistent with the relationships identified among the carotenoids in their biosynthetic pathways (Rodriguez-Amaya, 2010 and Bartley and Scolink, 1995).

Days to 50% anthesis (DA) was significantly negatively correlated with αC while it was phenotypically non-significantly negatively correlated with the other carotenoids except LUT. But DA genotypic correlations with all the carotenoids were positive with exception of βCX. These findings suggest a possible link between the induction of carotenoids during flower development and anthesis (Bartley and Scolink, 1995); possibly a case of pleitropic effects of gene(s) in carotenoids pathways that influence anthesis in maize.

The DA was phenotypically negatively correlated, with GY but was genotypically positively correlated. This shows masking of the positive genotypic correlation, though very low (rG=0.05), by environment. This can be explained on the basis of the fact that inbreeding which produces a delay in flowering and in seed set, was reduced in the crosses used in this study resulting in the positive rG between DA and GY. This result corroborates reports of Hallauer *et al.* (2010) for negative genotypic correlations between DA and GY of inbred lines and reports of Odongo *et al.* (2001) for positive phenotypic correlations rP between DA and GY for a maize population. Plant height (PH) was phenotypically positively correlated with GY while it was genotypically negatively correlated

with it. This indicates that taller plants may have higher photosynthetic capacity and therefore more assimilates to sink for higher GY. The negative genotypic correlation, however, shows that PH is governed by different genetic system from GY and not by pleiotropism. The negative direct effect of PH on GY was contrary to the report of Odongo *et al.*, (2001) who observed positive direct effect of PH on GY. PH was negatively correlated with PVA, βC, αC and ZEA at both the genotypic and phenotypic levels which indicate that taller plants tend to use up more of the carotenoids in the β, β-ring provitamin A pathway that are precursors of abscisic acid (ABA), a phytohormone that modulates developmental and stress processes (Koornneet, 1986), such that the taller the plant the lower the carotenoids that will be available for storage in yellow seeds.

Since GY is not significantly genotypically correlated with DA, PH, αC, βC, βCX, PVA, ZEA and LUT, GY and provitamin A can be improved concurrently with provitamin A. Thus, as opined by Hallauer *et al.* (2010) that "indirect selection for a complex trait, such as yield, is not plausible. Yield is an expression of fitness and drastic changes in one component of yield are usually accompanied by adjustments in other component(s), implying the existence of correlated changes of gene frequencies. It seems that the most effective method for yield improvement is direct selection for yield itself; there may be correlated changes among yield components, but these correlated changes will be in concert with development of the most physiologically efficient genotype for expression of yield." However, during selection, independent culling method should be applied to reduce undesirable traits having high negative indirect effects (e.g. DA,PH, ZEA and LUT).

CHAPTER 6: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The genetics of thirty one yellow maize inbred lines adapted to the tropics were studied. Nine parents were selected from the 31 inbred lines and used to develop 36 F_1 progenies using half diallel crossing method. The main objectives were to analyse the inbred lines for genetic diversity using molecular markers and to evaluate developed crosses for agronomic traits and carotenoids contents in two locations. In the addition, the study is to identify lines with good general and specific combining abilities. Statistical analyses were performed with PowerMarker, PAST, Statistica, MEGA5, SAS and Microsoft excel. Correlations among characters and heritability of the traits were also obtained. The results showed that:

1. Fourteen polymorphic SSR markers used for genetic diversity evaluation of 31 tropical-adapted yellow maize inbred lines separated the lines into two main groups, four clusters and several subclusters consistent with their pedigrees. In another analysis using *lcyE* and *crtRB1* functional markers, nine of the inbred lines randomly selected showed presence of 3-4 alleles that favour increased endosperm provitamin A contents (β-carotene, β-cryptoxanthin and total provitamin A) accumulation.
2. There were significant variations for agronomic traits and some carotenoids contents traits among the 31F_1 crosses across locations. The variability for carotenoids contents was significant for β-carotene, lutein and zeaxanthin, though, lutein and zeaxanthin do not have Provitamin A activity but they have other health benefits. Location effects were significantly different for days to anthesis, grain yield, lutein, β-carotene, α-carotene and total provitamin

A but not for plant height and zeaxanthin while Crosses by Location effects were not significant for β-cryptoxanthin, β-carotene, α-carotene and total provitamin A. The lack of significant genotype x environment interaction for β-cryptoxanthin, β-carotene, α-carotene and total provitamin A indicates stability of the genotypes for these traits.

3. Significant GCA and SCA effects were observed for days to anthesis, plant height, grain yield, lutein, zeaxanthin, β-cryptoxanthin, β-carotene, α-carotene and total provitamin A suggesting the traits were governed by both additive and non additive gene effects. Use of recurrent selection method is therefore suggested. The GCA:SCA ratio indicates preponderance of additive gene effect for days to anthesis, plant height, lutein, zeaxanthin and β-cryptoxanthin but preponderance of non additive gene effect for grain yield, β-carotene, α-carotene and total provitamin A. This suggests that development of hybrids will be more appropriate for grain yield and biofortification of the inbreds for provitamin A content.
4. Mean performance showed promising crosses with higher yields than the commercial hybrid checks at Samaru, Kerawa and across the locations. The crosses E6/E9 (7.12 tons/ha), E9/E28 (6.75 tons/ha), E3/E28 (6.63 tons/ha) and E37/E7 (6.62 tons/ha) yielded higher than the better check, Obasuper 4 (6.35 t/ha), with E3/E28 yielding more than 6.5 tons/ha in both locations and combined across the locations. The non provitamin A carotenoids (lutein and zeaxanthin) made up 77.74% of the total carotenoids content (zeaxanthin = 49.57% and lutein = 28.40%). While β-cryptoxanthin was 11.01%, β-carotene and total provitamin A were 9.42% and 15.76%, respectively. The least of the carotenoids with provitamin A activity was α-carotene. All the crosses evaluated have provitamin A contents within the target range for first generation PVA maize varieties (3-8µg/g). Sixteen crosses had total

provitamin content above 3.5 µg/g PVA. The crosses E6/E16, E8/E6, E9/E28 and E6/E28 have more than 4 µg/g total provitamin A.

5. The parents (i.e. the nine inbred lines) varied significantly for GCA indicating possibility of obtaining improved hybrids and varieties for the target traits. Lines with high positive GCA estimates for grain yield, days to anthesis and plant height are E8, E9, E3 and E37. Lines E6, E9, E28 and E7 have good general combining ability for β-carotene and total provitamin A contents. Other parents with good general combining ability for high lutein and zeaxanthin are E8, E6, and E7. These results indicate that through breeding programmes these parents will generate variable materials for improving carotenoids contents. Significant SCA variances were observed for grain yield and other agronomic traits and carotenoids contents (both positive and negative) in desired directions for some of the combinations. The performance of E8/E28, E26/E6, E26/E16, E6/E9, E9/E3, E9/E28, E3/E28, E37/E7 and E7/E16 crosses for GY, and E3/E16 and E8/E6 for α-carotene, β-carotene and total provitamin A indicate the possibility of improvement for grain yield, β-carotene, α-carotene and total provitamin A.
6. Heritability (H^2) was high for grain yield (H^2>60%), moderate for days to anthesis, lutein and zeaxanthin (30-60%), and low for plant height, β-cryptoxanthin, β-carotene, α-carotene and total provitamin A (H^2<30%). This indicates that genetic factors were predominantly responsible for the expression of the non-provitamin A carotenoids and for days to anthesis, and grain yield. However, the provitamin A contributing carotenoids showed low heritability indicating higher non genetic effects for these traits.
7. The correlation analysis showed both negative and positive phenotypic and genotypic correlation coefficients among traits. The phenotypic correlations among grain yield, days to

anthesis, plant height and all the measured carotenoids, however, were higher than the genotypic correlations and were in opposite directions (sign). This indicated that observed phenotypic correlations were influenced by environmental factors pointing to the fact that micronutrients uptake characteristics are largely affected by environment on plant growth and nutrient availability. The phenotypic correlations between grain yield and the carotenoids were significantly positive with β-carotene, β-cryptoxanthin and α-carotene. However, the genotypic correlations between grain yield with all the carotenoids were non-significantly negative, indicating a masking by environmental effects suggesting that grain yield and provitamin A can be improved concurrently. Thus, the most effective method for yield improvement will be direct selection for yield itself. However, during selection, independent culling method should be applied on the traits having negative indirect contributions.

6.2 Conclusions

Combating vitamin A deficiency in people depending on maize as staple diet requires that maize should be biofortified to provide Provitamin A endogenously. This would require maize germplasm that have substantial quantity of carotenoids and heritable traits of interest. After studying the genetics of carotenoids in tropical-adapted medium to high provitamin A maize inbred lines it was concluded that:

1. Genetic diversity exists among the 31 provitamin A maize inbred lines adapted to the tropics and this presents scope to exploit heterosis and create variability.
2. Fourteen polymorphic SSR markers were sufficient to genotype as many as 31 inbred lines.

3. The research showed that functional markers of the carotenoids metabolic pathways revealed polymorphism of alleles that favor Provitamin A carotenoids accumulation in the studied lines. This is an indication that there exists variability in the alleles which presents scope for improvement. The markers can be used to track introgressions of such favorable alleles for PVA accumulation in the tropical-adapted maize during breeding e.g backcrossing.
4. Significant variation exists among the crosses for agronomic traits and carotenoids content. Crosses that yielded higher than the commercial hybrid check Obasuper 4 (6.35 t/ha) were E6/E9 (7.12 tons/ha), E9/E28 (6.75 tons/ha), E3/E28 (6.63 tons/ha) and E37/E7 (6.62 tons/ha). Four crosses, E6/E16, E8/E6, E9/E28 and E6/E28 had more than 4 µg/g PVA.
5. Among parents with positive GCA and SCA effects for grain yield, total provitamin A, β-carotene, β-cryptoxanthin and zeaxanthin, E3, E6, E8, E9 and E37 were identified as the most promising, with the crosses E8/E28, E26/E6, E26/E16 and E6/E9 being most desirable for grain yield, and E3/E16 and E8/E6 for α-carotene, β-carotene and total provitamin A.
6. Both additive and non additive gene effects governed the inheritance of provitamin A carotenoids with preponderance of non-additive gene effect in the inheritance of total provitamin A, β-carotene, β-cryptoxanthin, grain yield, and plant height. Use of recurrent selection method is therefore suggested with the development of hybrids being more appropriate for grain yield and biofortification of the inbreds for provitamin A content.
7. Correlation studies among traits indicated both positive and negative associations. The phenotypic correlations among grain yield, days to anthesis, plant height and all the measured carotenoids, however, were higher than the genotypic correlations and were in opposite directions (+ve, -ve). This indicates that observed phenotypic correlations were

influenced by environment pointing to the fact that micronutrients uptake characteristics are largely affected by environmental effects on plant growth and nutrient availability.

8. αC presented the most reliable relationship with grain yield for indirect selection. A linear equation based on the use of direct and indirect contributions to GY was suggested.
9. GY is genotypically not significantly correlated with days to anthesis, plant height, α-carotene, β-carotene, β-cryptoxanthin, total provitamin A, zeaxanthin and lutein. Therefore, grain yield and provitamin A can be improved concurrently. The most effective method for yield improvement will be direct selection for yield itself. However, during selection, independent culling method should be applied on the traits having negative indirect contributions.

6.3 Recommendations

1. The functional markers for detecting alleles of provitamin A carotenoids *lycE* and *crtRB1* should be utilized for Marker Assisted Selection (MAS) to save resources and fast track development and delivery of PVA maize varieties for production and consumption for low income population of Sub-Saharan Africa.
2. The following crosses: E8/E28, E26/E6, E26/E16 and E6/E9 that gave high grain yield and E3/E16 and E8/E6 that had high provitamin A content are recommended for further utilization in hybrids development e.g. double cross hybrid varieties.
3. Lines with high GCA for grain yield were E8, E9, E3 and E37 and those for provitamin A were E6, E9, E28 and E7. These lines can be used for further breeding of high yielding provitamin A maize varieties.

4. On the basis of results obtained from this research, recurrent selections method using inbred lines as testers and a Multi-Stage Selection Scheme are recommended as appropriate breeding strategies to develop high Provitamin A maize (biofortification) with high grain yields, appropriate maturity, plant height, standability and high prolificacy adapted to Sub-Saharan tropics. Mass selection is recommended in the S_o generation. This offers the first opportunity to eliminate undesirable materials. Selection for reduced height, early maturity and resistance to lodging should be done first followed by selection for grain yield in each recurrent selection cycle. Since plant and ear heights, and days to flowering are highly heritable, recurrent selection will be very effective in reducing height and days to flowering. Since grain yield tends to be negatively correlated with height, selection for yield should follow selection for reduced height and resistance to lodging in each cycle. Whenever possible, selection should be made prior to anthesis to reduce the number of self-pollinations. Selection from the S_1 families will follow and provide the prospect to select for less heritable traits such as the provitamin A carotenoids contents, ear to plant heights ratio for resistance to lodging, ears per plant harvested for prolificacy etc.
5. Base population for the recurrent selection should be formulated using the 31 genetically diverse inbred lines, as indicated by the molecular markers, being adequate for short and long term response to selection.
6. A parallel pedigree breeding program involving F_2, three-way, and backcross progenies, where elite inbred lines or commercial varieties within the basic heterosis pattern are crossed (and often backcrossed) to the best line from the last cycle of recurrent selection, is recommended.

7. More exotic germplasm of higher provitamin A contents should be introduced from other breeding programs like IITA and CIMMYT to increase variability in the provitamin A maize population.

Finally, timely planting, optimum planting rate, good weed control, and rotation with a legume crop are important factors in maize yields that can be obtained with no cash expenditure and are therefore recommended.

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APPENDICES

Appendix 1. Maturity and quantity of tropical-adapted medium to high provitamin A maize inbred lines increased by selfing and sib-mating in 2010 rainy season at Samaru

Entry	Pedigree	Maturity (days)	Quantity of seeds obtained (g)
E1	(9450Xcm116x9450)-3-3-1-2-1-B-B-B-B-B-B-B	125.5	142.5
E2	9450xKI21-1-4-1-2-1-B-B-B-B-2-B-B-B	125	55
E3	9450xKI21-1-4-1-2-1-B-B-B-B-1-B-B-	124.75	170.62
E5	TZMI214xA619LPAxTZMI214-10-3-B-B-B-B-B-B	127	52.7
E6	SYN-Y-STR-34-1-1-1-1-2-1-B-B-B-B-B-B-B-B-B	126.67	68.8
E7	9450xKI21-7-3-1-2-5-B-B-B-B-B-B	121	258.8
E8	9450xKI21-7-2-2-1-1-B-B-B-B-B-B	120.5	426.7
E9	(9071x4058)-8-2-1-1-B-B-B-B-B-B	120.25	383
E10	KU1409/KU1414-SR/CI187-B-B-B-B-B-B	130	1
E11	KU1414-SR/KVI43-4-1-B-B-B-B-B-B	128	8.9
E12	KU1414-SR/KVI43-6-1-B-B-B-B-B-B	130	37.2
E14	(9450xKI28)-5-1-1-1-B-B-B-B-B-B-B	128	19
E16	KU1414-SR/KVI43-6-4-B-B-B-B-B-B	127	117.6
E18	KU1409/KU1414-SR/SC55-B-B-B-B-B-B	128	4.3
E19	ACR97TZL-CCOMP1-Y-S3-33-5-B-B-B-B-B-B-B-B	126.33	42.8
E20	ACR97TZL-CCOMP1-Y-S3-13-1-B-B-B-B-B-B-B-B	130.5	16.3
E21	9450xKI21-1-4-1-1-2-B-B-B-B-B-B-B	124	53.9
E22	POP61-SR-11-2-3-3-1-B-B-B-B-B-B-B-B	128	38.7
E23	(MP420x4001xMP420)-3-1-3-1-B-B-B-B-B-B-B-B	129.33	55.7
E24	9450xKI21-1-5-3-2-1-B-B-B-B-B-B-B	122	15.8
E25	9450xKI21-1-5-3-2-2-B-B-B-B-B-B-B	130	1.3
E26	KU1409xMO17LPAxKU1409-11-4-1-B-B-B-B-B-B	121.33	68.9
E27	9450xKI21-3-1-1-2-1-B-B-B-B-B-B-B	120.33	391.1
E28	SYN-Y-STR-34-1-1-1-1-2-1-B-B-B-B-B-B-B-B-B-B-B-B	127.33	122.9
E30	ACR97SYN-Y-S1-27-B-B-B-B-B-B-B-B	121	24.6
E31	ACR97SYN-Y-S1-38-B-B-B-B-B-B-B-B	126.5	30.3
E32	KU1409xMO17LPAxKU1409-27-3-1-1-B-B-B-B-B	124	59.7
E33	KU1409xMO17LPAxKU1409-27-3-1-1-B-B-B-B-B	124.75	138.6
E35	(GT-MAS:Gk x BABANGOYO x GT-MAS:Gk)-1-1-1	128	31.4
E36	(MP420x4001xMP420)-3-1-3-1-B-B-B-B-B-B-B-B	124	24.8
E37	KU1490xMO17LPAxKU1409-27-3-4-1-B-B-B-B-B	124	126.6

Appendix 2. Quantity of tropical-adapted medium to high provitamin A maize inbred lines increased by selfing and sib-mating in 2011 rainy season at Samaru

Entry	Pedigree	Quantity of seeds obtained (g)
E1	(9450Xcm116x9450)-3-3-1-2-1-B-B-B-B-B-B-B	200
E2	9450xKI21-1-4-1-2-1-B-B-B-B-2-B-B-B	1300
E3	9450xKI21-1-4-1-2-1-B-B-B-B-1-B-B-	1200
E5	TZMI214xA619LPAxTZMI214-10-3-B-B-B-B-B-B	100
E6	SYN-Y-STR-34-1-1-1-1-2-1-B-B-B-B-B-B-B-B-B	500
E7	9450xKI21-7-3-1-2-5-B-B-B-B-B-B	800
E8	9450xKI21-7-2-2-1-1-B-B-B-B-B-B	200
E9	(9071x4058)-8-2-1-1-B-B-B-B-B-B	600
E11	KU1414-SR/KVI43-4-1-B-B-B-B-B-B	0
E16	KU1414-SR/KVI43-6-4-B-B-B-B-B-B	1100
E19	ACR97TZL-CCOMP1-Y-S3-33-5-B-B-B-B-B-B-B-B	600
E20	ACR97TZL-CCOMP1-Y-S3-13-1-B-B-B-B-B-B-B-B	700
E21	9450xKI21-1-4-1-1-2-B-B-B-B-B-B-B	2940
E26	KU1409xMO17LPAxKU1409-11-4-1-B-B-B-B-B-B	1300
E27	9450xKI21-3-1-1-2-1-B-B-B-B-B-B-B	1000
E28	SYN-Y-STR-34-1-1-1-1-2-1-B-B-B-B-B-B-B-B-B-B-B	1000
E30	ACR97SYN-Y-S1-27-B-B-B-B-B-B-B-B	300
E31	ACR97SYN-Y-S1-38-B-B-B-B-B-B-B-B	200
E32	KU1409xMO17LPAxKU1409-27-3-1-1-B-B-B-B-B	200
E33	KU1409xMO17LPAxKU1409-27-3-1-1-B-B-B-B-B	700
E35	(GT-MAS:Gk x BABANGOYO x GT-MAS:Gk)-1-1-1	100
E36	(MP420x4001xMP420)-3-1-3-1-B-B-B-B-B-B-B-B	800
E37	KU1490xMO17LPAxKU1409-27-3-4-1-B-B-B-B-B	800

Appendix 3. Mean performance of tropical adapted medium to high PVA maize inbred lines evaluated at Samaru under controlled pollination during the 2011 wet season

Entry	DA	DS	PH	EH	RL	SL	PA	EA	Husk	Pharv	Eharv	FW (kg/plot)	GY (kg/ha)
E1	63.75	67.13	95.53	34.28	1.42	1.55	3.00	4.00	1.53	3.12	1.17	0.17	233.08
E11	61.26	64.36	48.42	20.75	2.88	3.02	3.93	2.98	3.04	2.73	0.16	0.01	39.58
E16	50.50	53.25	116.25	47.50	1.00	1.00	1.25	2.75	2.00	12.00	9.50	0.45	785.71
E19	52.75	59.50	116.25	35.00	1.50	1.50	3.00	3.00	2.00	4.50	3.75	0.25	428.57
E2	49.25	51.50	85.00	41.25	2.75	1.50	2.50	2.75	1.75	7.75	5.00	0.50	928.57
E20	56.50	57.00	140.00	48.75	1.00	1.00	2.50	2.75	1.75	6.75	6.50	0.35	500.00
E21	45.00	47.50	152.50	67.50	3.25	1.25	1.00	2.25	1.00	21.50	16.25	1.03	2100.00
E26	54.00	56.50	111.25	43.75	2.75	1.50	1.75	2.50	1.50	9.25	8.75	0.48	928.57
E27	48.25	49.00	108.75	42.50	3.50	2.00	2.00	2.75	1.25	12.75	8.25	0.38	714.29
E28	52.50	55.00	126.25	57.50	1.50	1.50	1.25	3.00	1.50	11.00	9.00	0.38	714.29
E3	46.75	50.50	118.75	41.25	2.50	1.50	2.25	3.00	1.00	12.00	10.25	0.50	857.14
E30	56.75	59.38	80.53	28.03	1.67	1.55	3.50	2.75	1.53	2.12	1.42	0.25	304.51
E31	53.00	55.38	99.28	35.53	1.42	1.55	2.50	3.50	1.78	5.62	1.67	0.20	233.08
E32	59.25	62.25	72.50	28.75	2.00	2.00	3.75	3.00	2.00	2.50	0.50	0.10	142.86
E33	55.25	58.00	101.25	36.25	1.50	1.25	2.00	3.00	1.25	7.25	4.75	0.33	500.00
E35	58.00	61.00	102.50	35.00	3.00	3.00	3.00	3.50	3.00	5.50	0.00	0.05	71.43
E36	50.25	51.00	87.50	37.50	1.25	1.00	3.00	3.50	2.00	6.50	3.75	0.38	571.43
E37	62.75	65.75	98.75	36.25	1.25	1.00	2.50	3.75	1.00	3.25	2.50	0.33	571.43
E5	55.25	58.25	90.00	38.75	2.50	2.50	2.75	3.50	2.50	2.25	0.25	0.05	71.43
E6	56.75	59.75	108.75	47.50	2.50	2.25	2.25	3.00	2.00	15.50	7.25	0.25	357.14
E7	47.50	50.50	105.00	43.75	1.00	1.00	1.75	2.75	1.25	11.50	9.00	0.35	571.43
E8	48.50	51.50	90.00	31.25	2.25	2.00	2.75	3.50	2.50	3.50	2.00	0.15	142.86
E9	49.25	53.25	88.75	33.75	1.25	1.00	2.75	3.00	1.75	5.00	5.00	0.33	428.57
Mean	53.61	56.40	101.90	39.67	1.98	1.63	2.48	3.06	1.78	7.56	5.07	0.31	530.26
SE	5.24	5.48	22.03	9.96	0.79	0.61	0.77	0.42	0.58	4.96	4.19	0.21	440.47
CV(%)	6.09	5.31	17.85	25.98	52.04	47.69	23.62	31.83	55.18	32.76	51.33	61.11	61.04

DA= Days to 50% anthesis; DS=Days to 50% silking; ASI= Anthesis Silking Interval; PH= Plant height; EH= Ear height; EPH= Ear to Plant height ratio; EPP= Ears per plant; GY= Grain yield; SE= Standard Error of mean; CV= Coefficient of Variation.

Appendix 4. Euclidian Similarity distance for 31 tropical-adapted yellow maize inbred lines containing medium to high Provitamin A contents

OTU	E1	E10	E11	E12	E14	E16	E18	E19	E2	E20	E21	E22	E23	E24	E25	E26	E27	E28	E3	E30	E31	E32	E33	E35	E36	E37	E5	E6	E7	E8
E1	0.00																													
E10	0.74	0.00																												
E11	0.86	0.35	0.00																											
E12	0.83	0.40	0.25	0.00																										
E14	0.71	0.74	0.66	0.53	0.00																									
E16	0.95	0.20	0.15	0.20	0.54	0.00																								
E18	0.79	0.45	0.20	0.35	0.69	0.25	0.00																							
E19	0.61	0.81	0.76	0.71	0.51	0.81	0.76	0.00																						
E2	0.76	0.76	0.51	0.74	0.56	0.66	0.40	0.66	0.00																					
E20	0.86	0.90	0.64	0.51	0.35	0.69	0.64	0.29	0.61	0.00																				
E21	0.71	0.61	0.45	0.69	0.61	0.61	0.35	0.61	0.15	0.66	0.00																			
E22	0.74	0.30	0.25	0.40	0.74	0.30	0.35	0.61	0.56	0.69	0.51	0.00																		
E23	1.06	0.96	0.81	0.71	0.66	0.76	0.61	0.66	0.61	0.51	0.66	0.76	0.00																	
E24	0.66	0.96	0.81	0.95	0.76	0.96	0.71	0.56	0.51	0.81	0.35	0.76	0.81	0.00																
E25	0.56	0.96	0.91	0.95	0.66	0.96	0.71	0.35	0.51	0.61	0.45	0.76	0.71	0.30	0.00															
E26	0.91	0.51	0.56	0.51	0.51	0.40	0.66	0.61	0.69	0.59	0.71	0.51	0.56	0.96	0.66	0.00														
E27	0.81	0.40	0.45	0.61	0.61	0.40	0.35	0.71	0.35	0.79	0.30	0.40	0.66	0.66	0.66	0.61	0.00													
E28	0.96	0.86	0.81	0.66	0.45	0.66	0.81	0.45	0.91	0.54	0.86	0.86	0.61	0.61	0.51	0.45	0.86	0.00												
E3	0.76	0.76	0.51	0.64	0.56	0.66	0.40	0.66	0.10	0.61	0.15	0.66	0.71	0.40	0.51	0.79	0.45	0.81	0.00											
E30	1.00	0.56	0.40	0.44	0.69	0.35	0.51	0.96	0.71	0.81	0.76	0.35	0.61	0.91	0.81	0.45	0.66	0.71	0.81	0.00										
E31	0.95	0.71	0.45	0.49	0.74	0.51	0.56	0.81	0.76	0.66	0.81	0.40	0.56	0.96	0.86	0.51	0.81	0.76	0.86	0.15	0.00									
E32	0.59	0.35	0.30	0.45	0.79	0.35	0.20	0.86	0.51	0.74	0.56	0.35	0.71	0.91	0.81	0.66	0.45	1.01	0.61	0.51	0.56	0.00								
E33	0.51	0.54	0.39	0.54	0.71	0.54	0.39	0.81	0.56	0.69	0.51	0.64	0.86	0.66	0.76	0.81	0.64	0.86	0.45	0.79	0.85	0.29	0.00							
E35	0.79	0.35	0.10	0.24	0.59	0.15	0.20	0.76	0.51	0.61	0.45	0.25	0.81	0.81	0.81	0.56	0.56	0.81	0.51	0.30	0.35	0.30	0.49	0.00						
E36	1.11	1.05	1.00	0.76	0.61	0.85	0.79	0.74	0.86	0.45	0.81	1.05	0.35	0.66	0.76	0.74	0.85	0.49	0.76	0.86	0.81	1.00	0.85	0.96	0.00					
E37	0.69	0.73	0.68	0.64	0.90	0.73	0.58	0.69	0.61	0.61	0.56	0.43	0.51	0.81	0.81	0.69	0.63	1.05	0.71	0.64	0.49	0.58	0.69	0.64	0.66	0.00				
E5	0.59	0.45	0.40	0.56	0.69	0.45	0.51	0.35	0.51	0.64	0.45	0.25	0.81	0.61	0.51	0.45	0.56	0.71	0.61	0.61	0.66	0.51	0.66	0.40	1.10	0.54	0.00			
E6	0.90	0.81	0.56	0.51	0.69	0.61	0.56	0.51	0.85	0.51	0.79	0.61	0.81	0.54	0.64	0.91	0.81	0.45	0.74	0.74	0.79	0.76	0.74	0.54	0.66	0.95	0.66	0.00		
E7	0.81	0.71	0.56	0.76	1.01	0.71	0.45	0.71	0.56	0.96	0.40	0.51	0.76	0.35	0.66	1.01	0.40	0.86	0.56	0.86	0.91	0.66	0.74	0.66	0.91	0.69	0.56	0.45	0.00	
E8	0.86	0.66	0.51	0.71	0.86	0.66	0.40	0.76	0.40	0.81	0.35	0.35	0.51	0.61	0.71	0.86	0.25	1.01	0.51	0.61	0.66	0.51	0.69	0.61	0.86	0.44	0.61	0.71	0.25	0.00
E9	1.16	0.73	0.48	0.54	0.76	0.53	0.48	0.79	0.71	0.51	0.69	0.49	0.61	0.91	0.91	0.79	0.69	0.78	0.74	0.51	0.56	0.64	0.87	0.44	0.66	0.64	0.74	0.40	0.66	0.51

Appendix 5. PCR conditions for the SSR, *lycE* and *crtRB1* markers

SSR	*lycE* 5'TE	*lycE* SNP216 and *lycE* 3'TE	*crtRB1* 5'TE	*crtRB1* 3'TE
Method : TDSSR1	Method : TD 64-54 L5TE	Method : TD 68-58z	Method : TD64-54c5pt	Method : TD68-58d4c
Reaction Volume : 25ul	Reaction Volume : 25ul	Reaction Volume : 25ul	Reaction Volume : 25ul	Reaction Volume : 25ul
Cover Heating : On	Cover Heating : On	Cover Heating : On	Cover Heating : On	Cover Heating : On
Cover Temperature : 105(C)	Cover Temperature : 105(C)	Cover Temperature : 105(C)	Cover Temperature : 105(C)	Cover Temperature : 105(C)
Notes : maize ssr markers	Notes : LYCE 5`TE	Notes : LYCE SNP216	Notes :	Notes :
Stage 1	Stage 1	Stage 1	Stage 1	Stage 1
Step 1	Step 1	Step 1	Step 1	Step 1
TEMP(C) : 94.0	TEMP(C) : 94.0	TEMP(C) : 94.0	TEMP(C) : 95.0	TEMP(C) : 95.0
TIME(MM:SS) : 2:00	TIME(MM:SS) : 4:00	TIME(MM:SS) : 3:00	TIME(MM:SS) : 4:00	TIME(MM:SS) : 4:00
RAMP RATE(%) : 100%	RAMP RATE(%) : 100%	RAMP RATE(%) : 100%	RAMP RATE(%) : 100%	RAMP RATE(%) : 100%
AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set
PAUSE : None	PAUSE : None	PAUSE : None	PAUSE : None	PAUSE : None
Stage 2 - For 9 cycles	Stage 2 - For 10 cycles	Stage 2 - For 19 cycles	Stage 2 - For 19 cycles	Stage 2 - For 10 cycles
Step 1	Step 1	Step 1	Step 1	Step 1
TEMP(C) : 93.0	TEMP(C) : 94.0	TEMP(C) : 94.0	TEMP(C) : 94.0	TEMP(C) : 94.0
TIME(MM:SS) : 0:15	TIME(MM:SS) : 1:00	TIME(MM:SS) : 1:00	TIME(MM:SS) : 1:00	TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%	RAMP RATE(%) : 100%	RAMP RATE(%) : 100%	RAMP RATE(%) : 100%	RAMP RATE(%) : 100%
AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set
PAUSE : None	PAUSE : None	PAUSE : None	PAUSE : None	PAUSE : None
Step 2	Step 2	Step 2	Step 2	Step 2
TEMP(C) : 65.0	TEMP(C) : 64.0	TEMP(C) : 66.0, 66.0, 68.0, 66.0, 66.0, 66.0	TEMP(C) : 64.0	TEMP(C) : 68.0
TIME(MM:SS) : 0:20	TIME(MM:SS) : 1:00	TIME(MM:SS) : 1:00	TIME(MM:SS) : 1:00	TIME(MM:SS) : 0:45

RAMP RATE(%) : 100%
AUTO-DELTA : Delta Temp: -1,
Start Cycle: 2
PAUSE : None

Step 3
TEMP(C) : 72.0
TIME(MM:SS) : 0:30
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Stage 3 - For 24 cycles

Step 1
TEMP(C) : 93.0
TIME(MM:SS) : 0:15
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Step 2
TEMP(C) : 55.0
TIME(MM:SS) : 0:20
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Step 3
TEMP(C) : 72.0
TIME(MM:SS) : 0:30
RAMP RATE(%) : 100%

RAMP RATE(%) : 100%
AUTO-DELTA : Delta Temp: -1,
Start Cycle: 2
PAUSE : None

Step 3
TEMP(C) : 72.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Stage 3 - For 28 cycles

Step 1
TEMP(C) : 94.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Step 2
TEMP(C) : 54.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Step 3
TEMP(C) : 72.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%

RAMP RATE(%) : 100%
AUTO-DELTA : Delta Temp: -0.5,
Start Cycle: 2
PAUSE : None

Step 3
TEMP(C) : 72.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Stage 3 - For 19 cycles

Step 1
TEMP(C) : 94.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Step 2
TEMP(C) : 58.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Step 3
TEMP(C) : 72.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%

RAMP RATE(%) : 100%
AUTO-DELTA : Delta Temp: -0.5,
Start Cycle: 2
PAUSE : None

Step 3
TEMP(C) : 72.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Stage 3 - For 19 cycles

Step 1
TEMP(C) : 94.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Step 2
TEMP(C) : 54.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Step 3
TEMP(C) : 72.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%

RAMP RATE(%) : 100%
AUTO-DELTA : Delta Temp: -1,
Start Cycle: 2
PAUSE : None

Step 3
TEMP(C) : 72.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Stage 3 - For 28 cycles

Step 1
TEMP(C) : 95.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Step 2
TEMP(C) : 58.0
TIME(MM:SS) : 0:45
RAMP RATE(%) : 100%
AUTO-DELTA : Not Set
PAUSE : None

Step 3
TEMP(C) : 72.0
TIME(MM:SS) : 1:00
RAMP RATE(%) : 100%

AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set
PAUSE : None	PAUSE : None	PAUSE : None	PAUSE : None	PAUSE : None
Stage 4	Stage 4	Stage 4	Stage 4	Stage 4
Step 1	Step 1	Step 1	Step 1	Step 1
TEMP(C) : 72.0	TEMP(C) : 72.0	TEMP(C) : 72.0	TEMP(C) : 72.0	TEMP(C) : 72.0
TIME(MM:SS) : 5:00	TIME(MM:SS) : 15:00	TIME(MM:SS) : 10:00	TIME(MM:SS) : 15:00	TIME(MM:SS) : 10:00
RAMP RATE(%) : 100%	RAMP RATE(%) : 100%	RAMP RATE(%) : 100%	RAMP RATE(%) : 100%	RAMP RATE(%) : 100%
AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set
PAUSE : None	PAUSE : None	PAUSE : None	PAUSE : None	PAUSE : None
Step 2	Step 2	Step 2	Step 2	Step 2
TEMP(C) : 4.0	TEMP(C) : 4.0	TEMP(C) : 10.0	TEMP(C) : 10.0	TEMP(C) : 4.0
TIME(MM:SS) : Infinity	TIME(MM:SS) : Infinity	TIME(MM:SS) : Infinity	TIME(MM:SS) : Infinity	TIME(MM:SS) : Infinity
RAMP RATE(%) : 100%	RAMP RATE(%) : 100%	RAMP RATE(%) : 100%	RAMP RATE(%) : 100%	RAMP RATE(%) : 100%
AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set	AUTO-DELTA : Not Set
PAUSE : None	PAUSE : None	PAUSE : None	PAUSE : None	PAUSE : None