GENETIC ANALYSIS AND DROUGHT STRESS ASSESSMENT OF MARKER-BASED IMPROVED PROVITAMIN-A MAIZE SYNTHETICS

BY

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CERTIFICATION

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DEDICATION

‘Now to the King eternal, immortal, invisible, to God who alone is wise, be honor, and glory forever and ever, Amen (I Timothy 1:17). I dedicate this work to my mum and siblings who spent their little income on my early education, upon which I now build. To my lovely wife and daughter, for their supports and understanding. To the numerous Scientists, Researchers and Donors for making knowledge and resources available to embark on cutting edge research to combat nutrient deficiency, food insecurity and climate change effects.

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ABSTRACT

Maize is an important staple in Sub-Saharan Africa (SSA) but most varieties are low in Provitamin-A (PVA) carotenoids, and the performance adversely affected by drought stress. Development and adoption of PVA carotenoids-enriched drought-tolerant Maize Synthetics (MS) could help improve maize yields and reduce vitamin A deficiency in SSA. Marker Assisted Recurrent Selection (MARS) could be used to improve the nutritional quality and resilience of maize. However, the effects of MARS on carotenoid contents of MS and performance of the PVA carotenoids-enriched MS in hybrid combinations and under drought stress have not been adequately documented. The level of improvement of carotenoid content of MS using MARS, combining ability and effects of drought stress on yield of PVA carotenoids-enriched MS were evaluated.

Three selection cycles (C_0, C_1 and C_2) of two MS (PVASYNHGA and PVASYNHGB) each improved through MARS were crossed to generate nine Varietal-cross Hybrids (VH). The genotypes [selection cycles, VH and a check (PVASYN13)] were evaluated at Ikenne, Mokwa, Saminaka and Zaria using a 4×4 lattice design with four replicates. The genotypes were also evaluated under Managed Drought Stress (MDS) at Ikenne following standard procedures. Days to Silking (DS), Plant Height (PH, cm) and Ear Aspect (EA) were measured and Grain Yield (GY, t/ha) was estimated. The α-carotene, β-cryptoxanthin and β-carotene contents (μg/g) of grains were determined using HPLC, and PVA content (μg/g) estimated. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$. Genetic gain/cycle, Mid-parent Heterosis (MPH), Specific Combining Ability (SCA), General Combining Ability (GCA) and Drought Tolerance Index (DTI, where DTI of 0–0.49=low, 0.50–0.69=moderate and 0.70–1.0=high) were estimated.

Genotype and location effects were significant for GY, DS, PH, EA and PVA carotenoids, while genotype×location effect was significant for DS, EA, β-carotene and PVA. The GY, DS, PH, EA, α-carotene, β-cryptoxanthin, β-carotene and PVA ranged from 3.7±0.3 (Ikenne) to 6.4±0.4 (Mokwa), 54.4±0.8 (Mokwa) to 63.0±0.8 (Zaria), 206.4±5.9 (Ikenne) to 222.5±8.0 (Saminaka), 2.4±0.2 (Saminaka) to 2.7±0.2 (Ikenne), 0.8±0.1 (Ikenne) to 1.0±0.1 (Mokwa), 2.4±0.3 (Ikenne) to 3.3±0.4 (Mokwa), 5.1±0.3 (Ikenne) to 6.5±0.8 (Mokwa) and 6.8±0.3 (Ikenne) to 8.7±0.8 (Mokwa), respectively. The MARS increased β-carotene and PVA by 25.0% and 15.0%, respectively in PVASYNHGA, and α-carotene by 5.0% in PVASYNHGB. Four VH ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_2/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$ and $PVASYNHGBC_1/PVASYNHGAC_2$) had significant MPH and SCA for GY. Only $PVASYNHGBC_0/PVASYNHGAC_0$ (4.0%), $PVASYNHGBC_0/PVASYNHGAC_2$ (2.6%) and $PVASYNHGBC_0/PVASYNHGAC_1$ (2.3%) exhibited MPH for PVA. Three genotypes [$PVASYNHGAC_0$ (0.7), $PVASYNHGAC_1$ (0.3) and $PVASYNHGBC_2$ (0.1)] had significant GCA for GY, while $PVASYNHGAC_2$ (1.09) and $PVASYNHGBC_0$ (1.27) had significant GCA for PVA. Under MDS, significant genotypic differences were observed for GY and DTI. Drought stress reduced GY by 31.4% ($PVASYNHGBC_1/PVASYNHGAC_2$) to 69.8% ($PVASYNHGBC_1/PVASYNHGAC_0$). The four VH with MPH for GY out-yielded the check by 12.7% to 16.4% and exhibited moderate to high DTI.

Marker-assisted recurrent selection improved carotenoid contents of PVASYNHGA than PVASYNHGB. Genotypes $PVASYNHGAC_2$ and $PVASYNHGBC_0$ are suitable for provitamin-A inbred line development. Drought stress reduced grain yield of the

maize synthetics but four varietal-cross hybrids ($PVASYNHGBC_0$/$PVASYNHGAC_0$, $PVASYNHGBC_2$/$PVASYNHGAC_0$, $PVASYNHGBC_0$/$PVASYNHGAC_1$ and $PVASYNHGBC_1$/$PVASYNHGAC_2$) were drought tolerant.

Keywords: Marker-assisted recurrent selection, Drought stress, Varietal-cross hybrids

Word count: 496

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
AMD	Age-related Macular Degeneration
ASI	Anthesis-silking Interval
ASTD	Academic Staff Training Development
αC	α-carotene
βC	β-carotene
βCX	β-cryptoxanthin
BPH	Better-parent Heterosis
CIMMYT	International Maize and Wheat Improvement Center
crtRB1	β-carotene hydroxylase1
DA	Days to Anthesis
DArT	Diversity Arrays Technology
DNA	Deoxyribonucleic acid
DS	Days to Silking
DTI	Drought-stress Tolerance Index
EA	Ear Aspect
EH	Ear Height
EST	Express Sequence Tag
FAOSTAT	Food and Agriculture Organization Statistics
FRET	Fluorescence Resonance Energy Transfer
GBS	Genotyping by Sequencing
GCA	General Combining Ability
GD	Genetic Distance
GEI	Genotype by Environment Interaction
GGPP	Geranylgeranyl Pyrophosphate
GY	Grain Yield
HPLC	High Performance Liquid Chromatography
IAR	Institute of Agricultural Research
IITA	International Institute of Tropical Agriculture
IL	Inbred line
InDel	Insertion-Deletion
IPCC	Intergovernmental Panel on Climate Change
KASP	Kompetitive Allele Specific PCR
LCYB	Lycopene Beta Cyclase
LCYE	Lycopene Epsilon Cyclase
LGC	Laboratory of the Government Chemist
LSD	Least Significant Difference
LUT	Lutein
MABC	Marker-assisted Backcrossing
MARS	Marker-assisted Recurrent Selection
MAS	Marker-assisted Selection

MDS	Managed-drought Stress
MIP	Maize Improvement Programme
MNDC	Micronutrient Deficiency and Control
MPH	Mid-parent Heterosis
MS	Maize Synthetics
NGS	Next-Generation Sequencing
OPV	Open-pollinated Variety
PA	Plant Aspect
PCR	Polymerase Chain Reaction
PH	Plant Height
PSY	Phytoene Synthase
PVA	Provitamin A
QTL	Quantitative Trait Loci
RAE	Retinol activity equivalent
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
RRS	Reciprocal Recurrent Selection
SAS	Statistical Analysis System
SC	Selection Cycle
SCA	Specific Combining Ability
SNP	Single Nucleotide Polymorphism
SSA	Sub-Saharan Africa
SSR	Simple Sequence Repeat
STH	Standard Heterosis
TC	Total Carotenoid
UNSCN	United Nation Standing Committee on Nutrition
VAD	Vitamin A Deficiency
VH	Varietal-cross Hybrid
WAP	Weeks After Planting
WHO	World Health Organization
WWC	Well-Watered Condition
YSI	Yield Stability Index
ZXT	Zeaxanthin

CHAPTER 1

INTRODUCTION

Maize (*Zea mays* L.) is an important crop all over the world for food security, animal feeds, biofuel production and other industrial uses. The production of maize is approximately one billion tonnes each year (FAOSTAT, 2019), making it the second most important cereal crop in the world, after wheat. The products of maize make up 38%, 30% and 6.5% of source of food in Africa, the Americas and Asia, respectively (Prasanna *et al*., 2020). Maize is an essential crop usually consumed in sub-Saharan Africa (SSA) providing day-to-day protein, mineral nutrients and energy. Maize supplies the main calories in the domestic food intake of more than sixteen countries in Africa (Nuss and Tanumihardjo, 2011). Due to the high occurrence and untoward effects of Vitamin A Deficiency (VAD) in SSA, maize is earmarked among other crops for Provitamin-A (PVA) biofortification (Bouis and Welch, 2010).

The precursors of vitamin A are known as PVA carotenoids. Major PVA carotenoids in maize grain include α-carotene (αC), β-cryptoxanthin (βCX) and β-carotene (βC) (Howe and Tanumihardjo, 2006). Lutein (LUT) and zeaxanthin (ZXT) also known as xanthophylls are significant carotenoids in maize grain with antioxidant properties, although they lack PVA activities (Howe and Tanumihardjo, 2006). Vitamin A is organic compound group that cannot be produced naturally in humans but can be obtained from the consumption of PVA-enriched diets or food supplements (Gupta *et al*., 2019). It is essential for vision, growth and tissue differentiation, epithelial cellular maintenance, immune function and reproduction (Sommer and West, 1996). Vitamin A deficiency (VAD) is linked to several health maladies including xerophthalmia, inability to see at night (nyctalopia), delayed growth, low immune system and high infant death rate (Muthayya *et al*., 2013). In spite of numerous supplementation and food fortification programmes, VAD remains a main public health challenge, particularly among children and women (Rice *et al*., 2004). It has been reported that about 800,000 dealths of children and women globally are attributable to VAD (Rice *et al*., 2004) and more than four million children suffer from xerophthalmia annually, mostly in SSA and Southeast Asia (Rice *et al*., 2004; Muthayya *et al*., 2013). It is

against this backdrop, that PVA carotenoid enhancements have been undertaken in several crops including maize, wheat, cassava, orange, sweet potato, carrots and vegetables (Giuliano, 2017).

Among cereals, only yellow or orange maize can naturally produce a large amount of carotenoids in its kernel (Burt *et al*., 2011). Incidentally, high variability exists in grain carotenoids of different maize germplasm of temperate, tropical and subtropical origins (Pixley *et al*., 2013). However, most yellow maize cultivated and eaten worldwide have low PVA of 0.5 to 2.0 μg g^{-1}, while white maize commonly consumed in Africa lack PVA activities (Harjes *et al*., 2008; Pixley *et al*., 2013).

Substantial breeding efforts have been made to enhance the agronomic performance and PVA carotenoid content of maize and some staple crops (Menkir *et al*., 2008, 2014; Halilu *et al*., 2016; Muthasamy *et al*., 2016). The biosynthetic pathway of carotenoids has been well described and genes such as Lycopene Epsilon Cylase, (*LCYE*) and βC hydroxylase1 (*crtRB1*) associated with high carotenoid accumulations have been discovered (Harjes *et al*., 2008; Yan *et al*., 2010). Molecular markers and genomic regions linked to PVA carotenoids have been detected and validated (Babu *et al*., 2013; Azmach *et al*., 2013; Suwarno *et al*., 2015; Gebremeskel *et al*., 2018). These markers are normally used together with phenotypic quantification of carotenoids using tool such as High Performance Liquid Chromatography (HPLC) to select high PVA maize genotypes (Gupta *et al*. 2019).

Marker-assisted recurrent selection (MARS) is a breeding technique in which favourable alleles are accumulated using DNA markers tightly associated with specific traits of interests (Bernardo, 2008). Marker-assisted recurrent selection has been used in the enhancement of grain yield (GY), resistance to *Striga hermonthica* and drought-stress resistance in maize (Abdulmalik *et al*., 2017; Bankole *et al*., 2017). A two-fold increase in GY of some maize genotypes due to MARS relative to phenotypic selection was reported by Crosbie *et al*. (2006). Two maize synthetics [MS (PVASYNHGA and PVASYNHGB)] were developed by Breeders at IITA and subjected to two cycles of MARS using β-carotene hydroxylase1 Kompetitive Allele Specific PCR (*crtRB1*-KASP) markers to increase favourable alleles of PVA carotenoids. Assessment of the effects of such PVA markers on the enhancement of

GY and PVA content has not been studied extensively. Astatke (2018) reported an increase in the frequencies and fixation indices of four favourable alleles of *crtRB1*-KASP markers after two cycles of MARS used on the two synthetics.

The essence of the development and improvement of PVASYNHGA and PVASYNHGB through MARS at the IITA Maize Improvement Programme (MIP) was to create source populations for the extraction of diverse maize inbred lines with elevated PVA content and superior agronomic traits to derive PVA-enriched hybrids. Knowledge of the effects of MARS on the combining abilities of the two synthetics for GY and other desirable agronomic traits as well as PVA carotenoids could provide valuable information on the gene action of these traits. This will assist in the identification of suitable source populations for divergent parental lines with good General Combining Ability (GCA) and Specific Combining Abilities (SCA) to boost the expression of heterosis in hybrids. In maize breeding, few studies have reported combining abilities for GY and PVA carotenoids, mainly in maize inbred lines (Senete *et al*., 2011; Suwarno *et al*., 2014; Muthusamy *et al*., 2016; Obed-Bio *et al*., 2019). However, the combining ability and heterosis for GY, agronomic traits and PVA content in MS improved using high throughput MARS has not been reported. Egesel *et al*. (2003) attributed the gene action controlling βC, βCX, LUT and ZXT to additive effects, while Suwarno *et al*. (2014) and Obeng-Bio *et al*. (2019) reported that both additive and non-additive gene effects control maize carotenoids. In contrast, Halilu *et al*. (2016) reported the preponderance of non-additive gene effects on all measured carotenoids in some tropical maize inbred lines. The conflicting results in these studies underpin the basis for more research into the mode of gene action of carotenoids in maize.

Heterosis has been explored for GY and agronomic performance in commercial maize hybrids production (Hallauer *et al*., 2010). However, heterosis for grain carotenoids is reported to be unusual and irregular in yellow and dent maize (Burt *et al*., 2011). Genetically divergent parents with significant SCA effects are important for assessing the potential heterosis that could be attained in maize germplasm (Hallauer *et al*., 2010). Consequently, Suwarno *et al*. (2014) proposed exploitation of heterotic groups to maximize heterosis for PVA content.

Drought stress and its associated effects arising from climate change have become serious threats to grain yield of maize and other crops. Of the maize field cultivated globally, about one hundred and sixty million hectares is rain-fed (Edmeades, 2013), thus subject to random drought stress. Losses of grain yield of maize to drought annually are reported to average around 15% of well-watered yield potential on a global scale (Edmeades, 2013). Climate change has modified the weather patterns globally and the corn belt of Nigeria has become prone to recurrent drought stress (FAO, 2013). The development of climate-resilient crop varieties has become adaptive strategies in mitigating emerging threats of drought stress. One of the most effective strategies for developing drought tolerant maize is to manage stress in experimental trials, partly or entirely, in dry season through irrigation system (Bänziger *et al*., 2000). Exposure of genetic materials to moisture stress at flowering and grain filling stages with resultant yield losses of 40–90% has been used for the identification of drought tolerant maize varieties (Heisey and Edmeades 1999). However, limited studies have been reported on the effects of drought stress on PVA-biofortified maize. Assessment of PVA-enriched maize synthetics, their selection cycles and varietal-cross hybrids under managed-drought stress will provide information on their responses, stability and heterosis of the hybrids under moisture deficit conditions. This study was thus conducted to:

(i) assess the effect of marker-assisted recurrent selection on the agronomic performance and provitamin-A carotenoids of two maize synthetics and their selection cycles;

(ii) assess the combining ability and heterosis for grain yield and provitamin-A carotenoids of the maize synthetics and their selection cycles;

(iii) evaluate the effects of drought stress on grain yield and agronomic performance of maize synthetics, selection cycles and varietal-cross hybrids;

(iv) assess the effects of drought stress on the stability of the maize genotypes and heterosis of the varietal-cross hybrids and

(v) investigate the relationships between grain yield and yield-associated traits under drought stress.

CHAPTER 2
LITERATURE REVIEW

2.1 General biology of maize

Maize (*Zea mays*) is the only species in the genus Zea and belongs to the grass family, Poaceae. It is diploid with chromosome number 2n=20. Maize is a monoecious, determinate annual crop and has large, narrow, opposing leaves, borne alternately along the length of a solid stem. Maize is one of the most efficient plants in terms of energy capturing from the sun and conversion to food. The leaf axis of the plant bears the shoot which arises from axillary bud apices and develops into female inflorescence, known as the ear. Several leaves called husks cover the ear (Purseglove, 1972). The husks serve as protection for the developing ear against birds, rodents, insects and inclement weather conditions. The thick axis of the ear, the cob bears between 4 to 30 rows of ovaries, each containing an ovule. Ears with mature ovaries have silks which serve as canals of pollination. About 300 – 1,000 ovules develop into kernnels, depending on the genotype and environmental factors in which they are grown (Purseglove, 1972).

The apical meristem of the stem develops into the male inflorescence known as the tassel. The tassel is prominent at the top of the plant consisting of a central spike and a variable number of lateral spikelets. Spikelets bear anthers which enclose pollen grains. The amount of pollen produced by a tassel is estimated at 18 million pollen grains (Kiesselbach, 1980). An average of 42,500 pollen grains is provided for each square inch of the field. Considering that maize in the field sheds pollen for an average of 13 days, each silk receives an average of 13 pollen grains per day (Kiesselbach, 1980). Pollen is carried mainly by wind, thus pollination can occur, although rarely, over long distances measured in kilometers.

2.2 Maize production

Maize is one of the world's leading cereals grown annually across over 166 countries with varying soil types, climate, biodiversity and management practices. In 2021, the global maize production was over one billion tonnes (Table 2.1) on approximately two

Table 2.1. The top maize producing nations in the world, area harvested and average grain yield in 2021

S/N	Country	Production (tonnes)	Area harvested (ha)	Average yield (t/ha)
1	United States of America	383,943,000	34,555,670	11.11
2	China	272,552,000	43,324,100	6.29
3	Brazil	88,461,943	19,024,538	4.65
4	Argentina	60,525,805	81,465,96	7.43
5	Ukraine	42,109,850	5,481,800	7.68
6	India	31,650,000	9,860,000	3.21
7	Mexico	27,503,477.82	7,139,621	3.85
8	Indonesia	20,010,000	3,495,981	5.72
9	South Africa	16,870,705	3,118,300	5.41
10	France	15,358,300	1,549,520	9.91
11	Russia	15,239,865.1	2,901,612	5.25
12	Romania	14,820,690	2,554,680	5.80
13	Canada	13,983,859	1,390,500	10.06
14	Nigeria	12,745,000	6,000,000	2.12
15	Ethiopia	10,722,000	2,530,000	4.24

Source: FAOSTAT (2021)

hundred million hectares (FAOSTAT, 2021). The United States of America is the highest producer of maize with over 347 million tonnes, contributing nearly 35% to the world production annually. This is followed by China with more than 20% of the world's production.

Yields of over 10 t/ha are usually obtained in the US, higher than the average global yield of 5.3 t/ha (Kumar *et al*., 2012). Nigeria is the fourteenth highest producer of maize in the world. According to FAOSTAT (2021), Nigeria currently ranks second behind South Africa in maize production in Africa, with an estimated twelve and half million tonnes on 6.8 million hectares of land. However, the average yield of maize in Nigeria is low (2.12 tonnes/ha) in spite of the research efforts geared towards improving maize varieties. Maize production constraints in Nigeria include poor soil fertility, pests and diseases, drought stress and poor management practices.

2.3 Importance and utilization of maize

All parts of maize plants are useful and can be utilized for various purposes depending on the region. In developing nations, especially in Africa and Latin America, maize is used mainly for food. In the green state, maize can be roasted, boiled or baked and used as salad. Maize contains about 4% fat, 10% protein, 72% starch, and trace amount of vitamins and minerals, furnishing energy density of approximately 365 Kcal/100 g (Ranum *et al*., 2014). Freshly harvest or dried grains of maize may be used or processed traditionally or in combination with other food materials as staple food or snacks. In Nigeria, maize grains is usually processed and transformed into food products such as pap (*ogi*), *tuwo, donkunnu, maasa, donkwa, kokoro*, etc. (Abdulrahaman and Kolawole, 2006). Maize is also a basic ingredient of some indigenous drinks, soft drinks, wines and malt liquor and confectionaries. It is the most essential cereal crop in the economy of African countries because is cheaper than other cereals like oat, barley, wheat, sorhum, millet and rice, thus is more affordable by majority of the populace.

In the developed nations, a larger amount of maize is utilized for livestock feeding and as raw materials for industrial uses. As livestock feed, maize grains are either fed directly to animals or used as supplements. It is a key source of calories in animal feeding and feed formulation. Maize gives the highest conversion of dry substance to meat, milk and egg compared to other cereals (Okoruwa, 1997). It is high in starch

content which is rich in energy and low in fiber content. Maize stover which contains 30 to 40% of total nitrogen, 75% of potassium, sulphur, magnesium and calcium is used by many farmers in unindustrialized nations as roughage feed for livestock (Olaniyan, 2015).

Industrial use of maize is categorized into wet and dry milling processes (Okoruwa 1997). Products from wet milling include corn starch, corn syrup, high fructose syrup, dextrose and corn oil. Corn starch is modified into baking powders, candies, and puddings. Paper and textile industries also make use of corn starch. Corn syrups are used in confectionaries, bakery and dairy products. Produces obtained from dry milling of maize include maize meal, flour, grits, oil and by-products for animal feed, all of which are important industrial products. In the last one and half decades, the use of maize for biofuel production significantly increased, accounting for approximately 40% of the maize production in the United States (Ranum *et al*., 2014). Countries such as Brazil, Germany, France, Italy, and Japan use significant amount of maize for biofuel. However, biofuel initiatives in Africa is still at the conceptualization or preliminary stages, therefore, most maize produced on the continent are consumed as food and feedstock for livestock. Maize is one of the primary feedstuff used to produce ethanol due to its high starch. Corn-based ethanol production in 2009 was 10.6 billion gallons with an estimated potential of 12.5 billion gallons per year (Ranum *et al*., 2014). Maize Stover and its biomass are also used for the production of biogas for industrial uses (Olaniyan, 2015).

2.4 Micronutrient and its deficiency

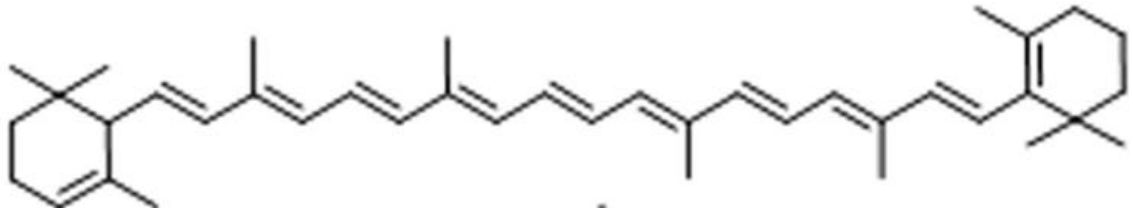
Micronutrients have assumed great public health importance over the past four decades. As a result, considerable researches have been carried out to elucidate the physiological functions of micronutrients in the body and the attendant effects of consuming diets deficient of them (FAO/WHO, 2004). Micronutrients are needed in small quantity in the body for the production of enzymes, hormones, regulation of growth activities, development and functioning of immune and reproductive systems (Ekweagwu *et al*., 2008). In addition, micronutrients are required for energy production, synthesis of RNA and DNA, promotion of physical growth and sexual maturation (Singh, 2004). Although major emphasis has been on protein-energy malnutrition, FAO/WHO (2004) data show that deficiency of micronutrients is far

more severe. The most prevalent micronutrient deficiencies in humans include those of vitamin A (beta-carotene), zinc (Zn), iron (Fe), folic acid, selenium (Se), and iodine (I). About two billion people are reported to suffer from micronutrient deficiency while 815 million people are undernourished (Global Nutrition Report, 2017). Micronutrient deficiency refers to inadequate levels of vitamins and minerals in the human body. It is also known as 'hidden hunger'. Micronutrient deficiency causes enormous ill-health, retards physical and mental growth, results in poor quality of life and declined economic output (Steven *et al*., 2020). Vitamin A and zinc deficiencies are likely the most prevalent micronutrient deficiencies causing substantial illness among children and mothers (FAO/WHO, 2006).

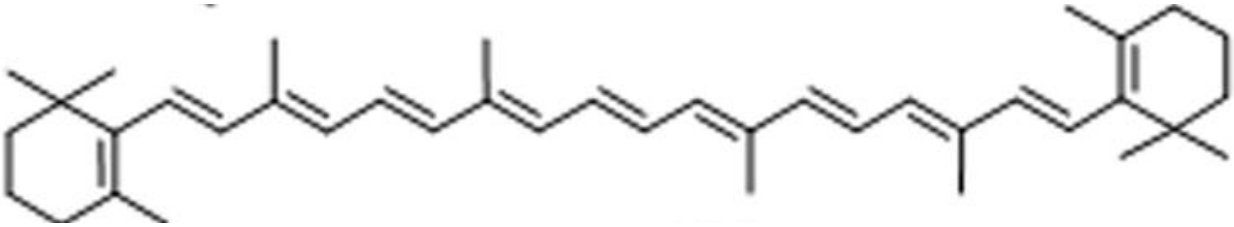
Sub-Saharan Africa and indeed Nigeria have alarming prevalence of micronutrient deficiencies that have persisted over decades (Anjorin *et al*., 2019). Available data shows that 71% of children under the age of 5, 47% of non-pregnant women and 58% of pregnant women in Nigeria had anemia in 2011 which was attributed mainly to iron deficiency (WHO, 2015). Forty two percent of this children population is vitamin A deficient while 21% is estimated to be at risk of insufficient zinc intake (Wessells and Brown, 2012). Interventions such as supplementation of children food and fortifications of staples have been undertaken both at national and international levels to mitigate micronutrient deficiencies. Given the seriousness of micronutrient deficiency, the Federal Ministry of Health in Nigeria has developed guidelines on Micronutrient Deficiency and Control (MNDC) with the aim of achieving high impact in short, medium and long term (Anjorin *et al*., 2019).

2.5 Structure of Vitamin A and important dietary sources

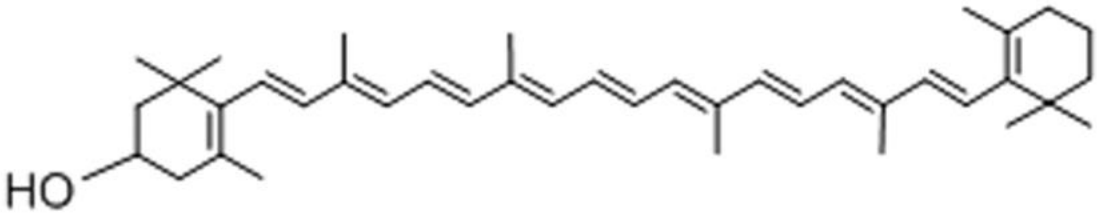
Vitamin A is one of the most significant micronutrients earmarked for research and enhancement. Vitamin A is a group of unsaturated nutritional organic compounds that includes retinol, retinal, retinoic acid, and several PVA carotenoids mostly βC (Fennema, 2008). According to West *et al.* (2008) and US Institute of Medicine (2001), the molecule of vitamin A contains the -ionone ring and chain moiety (Figure 2.1) and among the carotenoids, the βC is the most important because it contains two molecules of retinols, while αC and βCX contain one molecule each of vitamin A. Retinol Activity Equivalent (RAE) is a quantitative measurement of retinol in relation



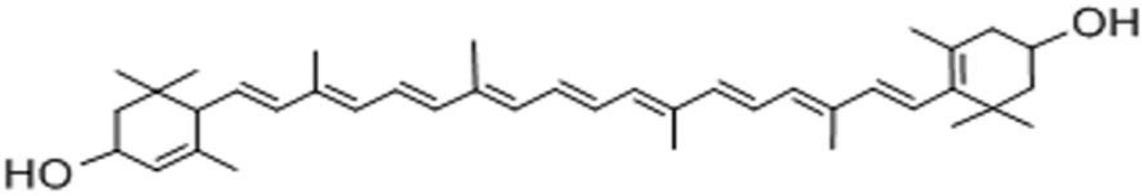
(a) αcarotene



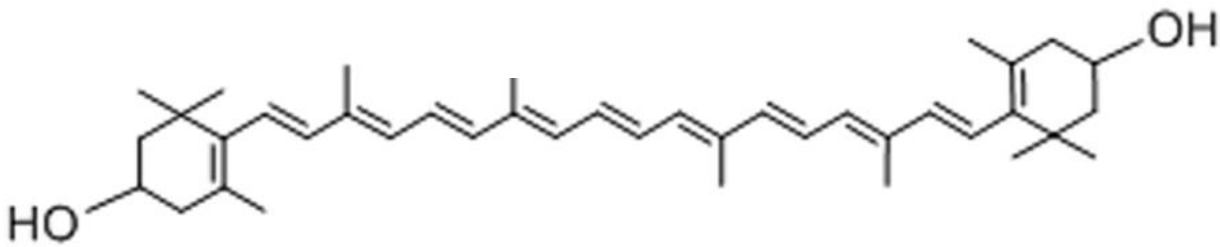
(b) βcarotene



(c) βcryptoxanthin



(d) Lutein



(e) Zeaxanthin

Figure 2.1. Chemical structure of PVA carotenoids (a, b, c) and xanthophylls (d and e)

to other sources of vitamin A in the diet (Otten *et al*., 2006); 1 μg RAE is equivalent to 1 μg all-transretinol (vitamin A), 12 μg βC, and 24 μg αC or βCX. Naturally, vitamin A is not synthesized by higher animals and humans, and thus can only be obtained from animal-based dietary sources, such as milk, eggs and liver or as precursors in the form of PVA carotenoids from crops. Some crops from which it can be obtained include orange-fleshed sweet potato and cassava, coloured vegetables, fruits like pawpaw, carrots, oranges and dark green leaves (West *et al*., 2008). Life-long vital biological processes in the human body such as organogenesis, morphogenesis, visual cycle, immune responses, cellular differentiation and proliferation are regulated by the nutritional function of vitamin A (FAO/WHO, 2004; West *et al*., 2008). A form of vitamin A called eleven-cis retinal helps the eyes capture light and improve vision when there is little light available (West *et al*., 2008).

2.6 Vitamin A deficiency

Vitamin A deficiency (VAD) affects an estimated 42.2% of children below the age of 5 and 15.3% of pregnant women in SSA (WHO, 2009). This is primarily because millions of people in the region feed on crops deficient in nutrient. The UN Standing Committee on Nutrition, UNSCN (2010) reported a 22.4 to 45.8% VAD prevalence among children in Africa with a slight improvement in the last two decades. Deficiency in Vitamin A slows down cognitive ability and physical growth, decreases iron mobilization, weakens immune function thus heightening one's predisposition to diseases and reducing the likelihood of one's survival from severe illness (Wurtzel *et al*., 2012). Visual conditions characterized by loss of vision associated with ulcer (Keratomalacia), xerophthalmia and night blindness are the symptoms that are characteristic of VAD (Biesalski, 2013). Pregnant and lactating women, and preschool children require high level of vitamin A (WHO, 2009); therefore, VAD is thought to be an incapacitating and possibly a deadly public health issue for them (FAO/WHO, 2004).

There had been improvements in the interventions against VAD and a decline in the cases globally; but a rather slow advancement has been made in SSA and Southeast Asia, as these regions encumbered with severe burden of VAD (UNSCN, 2010). Based on the study of Sherwin *et al*. (2012), over 500,000 children lose their sights yearly to xerophthalmia caused by VAD making it the commonest cause of avoidable blindness.

Furthermore, Biesalski (2013) reported that VAD and other malnutrition problems are the reason of death of over three million children yearly.

The main cause of VAD in SSA is as a result of inaccessibility of micronutrient densed foods due to widespread poverty arising from economic crises, high cost of nutritious food and the effects of climate change (Bloem *et al*., 2010). According to the study of FAO/WHO (2004), most staple foods in SSA are mainly root or tuber crops which are high in starch and cereals which are low in vitamin A and thus cannot meet the recommended daily allowance of the nutrient. Many families with limited resources cannot afford animal-based and dairy products and do not prioritize fruits that can supply trace amount of vitamin A, but which availabilities are also constrained by season (Biesalski, 2013).

Adequate and regular intake of carotenoid-rich food has been associated with low risk of cancer and cardiac arrest (McDermott, 2000) and high immune functions (Watzl *et al*., 2003). Furthermore, its consumption averts night blindness and helps maintain good health by neutralizing free radicals (Sen and Chakraborty, 2011). In recent years, carotenoid content in staple foods has been the subject of much research due to their nutritional and health benefits (Hefferon, 2015). Therefore, breeding for elevated carotenoid contents in common crop varieties would not only reduce vitamin A deficiency but would also provide more antioxidant-enriched meals.

2.7 Lutein and zeaxanthin carotenoids, functions and important dietary sources

Lutein (LUT) and zeaxanthin (ZXT) are fat-soluble antioxidants which are categorized as oxygenated carotenoids or xanthophylls. Structurally, the difference between LUT and ZXT is in the type of ionone ring. The LUT contains a β-ionone ring and a ε-ionone ring, whereas ZXT has two β-ionone rings (Figure 2.1d and 2.1e). The LUT and ZXT are isomers and have no PVA activities but play important role in eye health. The percentage constituent of foremost carotenoids in human serum is 20% (LUT), 20% (lycopene), 10% (βC), 8% (βCX), 6% (αC) and 3% (ZXT) (Khachik *et al*., 1997; Abdel-Aal *et al*., 2013). Lutein, ZXT, together with their isomer, meso-ZXT are the major components of the Macular Pigment (MP), a compound concentrated in the macula region of the retina that is responsible for fine-feature vision (Eisenhauer *et al.*, 2017). Xanthophylls and their isomers shield the macula pigment from blue light damage, improve sharpness of vision and sift destructive reactive oxygen.

Xanthophylls are key carotenoids in minimizing and inhibitimg cataracts and Age-related Macular Degeneration (AMD), which is the foremost cause of blindness in elderly people in industrialized nations. Age-related Macular Degeneration (AMD) surges with advancement in age and it seems to occur more in men than in women (Park *et al*., 2015). A study in the US forecasts that the number of patients with AMD is likely to double between 2010 and 2050; and this disease is becoming a crucial public health issue (Rein *et al*., 2009). A study revealed that, a higher consumption of carotenoids, particularly LUT and ZXT is concomitant with a decreased AMD risk due to the antioxidant properties and ability to filter light (Bernstein *et al*., 2016).

Lutein and ZXT are the commonest xanthophylls found in leaf green vegetables, such as lettuce, peas, spinach and broccoli. The carotenoids are also found in egg yolks (Perry *et al*., 2009) and cereals including durum wheat, maize grain and their products (Abdel-Aal *et al*., 2010). The content of LUT ranges from 5.4 to 7.4 μg/g in high-LUT wheat species and about 21.9 μg/g in maize. LUT and ZXT are the major carotenoids in corn milled fractions and account for about 70% of the total carotenoids (Kean *et al*., 2008). Therefore, maize is a suitable ingredient in functional foods requiring high LUT. Chicken egg yolk contains high amount of LUT and ZXT. Yolk of average weight of 18 g contains LUT and ZXT concentrations of 292 ± 117 μg/yolk and 213 ± 85 μg/yolk, respectively (Abdel-Aal *et al*., 2013).

Lutein and ZXT do not have any official recommended dietary intake levels. However, an intake of 6 mg/day has been suggested for adults to decrease the likelihood of AMD (Eisenhauer *et al*., 2017). Studies show that the required intake of LUT and ZXT differs from time to time in diverse populations (Scott *et al*., 1996). Evidence suggests that intake of major carotenoids including LUT and ZXT range between 1 to 5.33 mg/day across Europe, America and Australia (Olmedilla *et al*., 1997; Tucker *et al*., 1999; Mares-Perlman *et al*., 2002), which is below the optimum. The past decade has seen efforts to develop foods rich in carotenoids so that the elderly population can consume more of them, especially in the form of dietary supplements. However, most biofortification programmes of maize are targeted mainly towards improving PVA. Breeding programmes that simultaneously improves PVA and the xanthophyll components are important in mitigating micronutrient deficiency in infants and the elderlies.

2.8 Maize as a model crop for PVA enrichment

Maize is the second most cultivated cereal after wheat worldwide with an annual production of over 1 billion tonnes (FAOSTAT, 2021). The crop is majorly used for animal feed in the advance countries (Prasanna *et al*., 2020), but serves as food and source of calorie to over one billion people in Latin America and Africa.There had been a decline in production and yield of maize in SSA between 2012 and 2016 (FAOSTAT, 2016). This may have been due to drought stress arising from climate change and social crisis in some production areas of the region. However, over three hundred million people survive on maize in Africa (Prasanna *et al*., 2020).

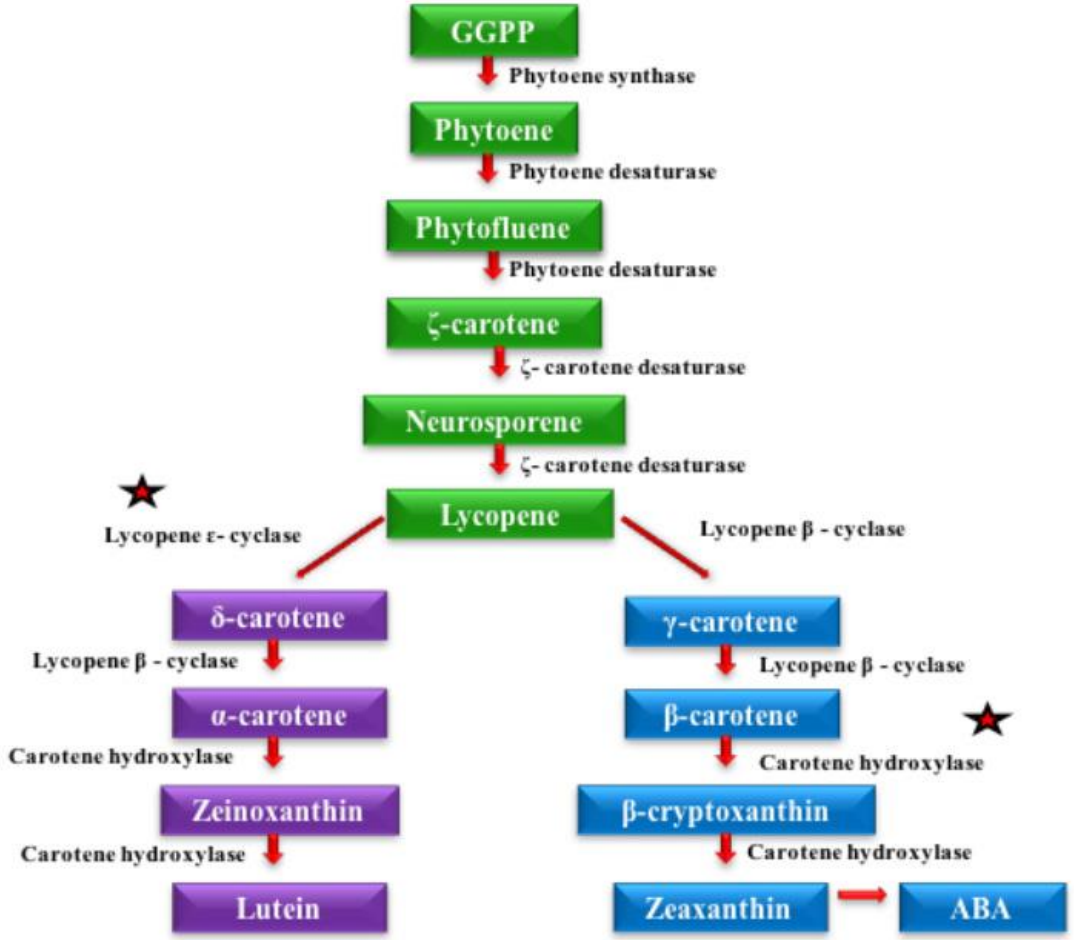
Maize is one of the major staples that have been selected for PVA biofortification to drive nutrient enrichment in rural areas of SSA by the HarvestPlus programme. This is because maize is a cheap and common source of food for many people, especially among low income earners whom animal protein source and fruits are out of reach (Bouis and Welch, 2010). The daily consumption of maize by most people in Africa ranges from 52 to 328 g/person/day (Ranum *et al*., 2014). White maize is more common in the East and Southern parts of Africa mainly because of cultural acceptability, while yellow and orange kernel maize is mostly found in West and Central Africa. In Nigeria, the yellow and orange maize is more common in the southern part of Nigeria while the white is popularly grown in the northern part of the country. The grains of white, yellow and orange kernel maize are processed into popular local delicacies, drinks, snacks and infant weaning pastes and powdery food. White maize generally lack PVA activities and are not used for PVA-enrichment programmes. However, yellow and orange maize have the capacity to naturally accumulate PVA and thus serves as an ideal type for PVA-enrichment (Menkir *et al*., 2017). Furthermore, maize is adapted to various production ecologies and diverse nutritious, high yielding and attractive cultivar can be developed on sustainable basis (Menkir *et al*., 2017). Maize is an ideal model for other grass species due to historical collections of carotenoid mutants, genome sequence, and other molecular resources. In addition, high genetic variability for carotenoids exists in diverse maize panels for continuous scientific investigation for PVA enhancement (Liu *et al*., 2003; Harjes *et al*., 2008).

2.9 Provitamin-A carotenoids in maize

Yellow and orange maize naturally accrues PVA carotenoids which include αC, βCX and βC. The PVA carotenoids are also the substances for the biochemical production of LUT and ZXT (Wurtzel *et al*., 2012). Beta-carotene is the most abundant PVA carotenoid in foods derived from plants constituting 80% of vitamin A consumed (Biesalski, 2013). Lutein and ZXT are generally known as xanthophyll and have no PVA activities (Meyers *et al*., 2014). They are however known to diminish the risk of cataracts, blindness associated with old age and act as photo-protection in plants. The suitability of PVA carotenoids of maize as important source of vitamin A in humans is due to their ability to release retinol stored in the liver through oxidative cleavage (Combs, 2012). Hence, maize is ideal for PVA enhancement. However, most maize varieties commonly grown in SSA and other parts of the world contain low (1.5 – 2.0 µg/g) in PVA content, a range that is a far cry from the global target of 15 µg g^{-1} set by the Harvest Plus challenge programme while half of that was considered as intermediate target for the breeding programme (Pixley *et al*., 2013). Consequently, considerable efforts are being made to elevate PVA level in maize germplasm through classical and molecular breeding techniques to minimize the prevalence and burden of VAD (Pfeiffer and McClafferty, 2007).

2.10 The biosynthetic pathway of carotenoids

Wurtzel *et al*. (2012) described the carotenoid biosynthetic pathway of plant as one that has been well characterized after decades of molecular genetic analyses (Figure 2.2). Phytoene is the first carotenoid produced in the biosynthetic pathway from the condensation of two geranylgeranyl pyrophosphate (GGPP) molecules; and the reaction is catalyzed by phytoene synthase (*PSY*) (Wurtzel *et al*., 2012). The first point of branching of the pathway ensues at cyclization of lycopene with the enzyme lycopene beta cyclase (*LCYB*) producing a molecule with two β rings at both ends of linear lycopene. Plants that contain *phytoene synthase* gene (*PSY*) produce carotenoid in both endosperm and leaves and it is the main determinant of kernel colour variation from white to yellow and intense orange (Buckner *et al*., 1996). The co-actions of *LCYB* and lycopene epsilon cyclase (*LCYE*) generate β, ε-carotene which is a precursor of LUT.



GGPP**:** Geranylgeranyl Pyrophosphate Synthase; ABA: Abscisic Acid

Figure 2.2. Carotenoid biosynthetic pathway in maize (Adapted from Sagare *et al*., 2018)

The quantity of carotenes directed to each branch of the biosynthetic pathway is controlled by the relative activities of *LCYB* and *LYCE* (Cunningham and Gantt, 2001). Beta carotene hydroxylase 1 (*crtRB1*; also known as, *HYD3*) is another important gene in the biosynthetic pathway. It causes hydroxylation of alpha and beta carotenes into non-PVA carotenoids, LUT and ZXT thereby reducing PVA content. Reduced hydroxylation will result in increased PVA content; therefore, the branching of the pathway and hydroxylation process are the causal factors regulating PVA levels in maize kernel (Yan *et al*., 2010).

2.11 Variations in alleles linked to carotenoid accumulation

Different studies have shown that variations in the carotenogenic genes have resulted in the variations of PVA and total carotenoid accumulations of maize endosperms of diverse germplasm (Harjes *et al*., 2008; Yan *et al*., 201; Fu *et al.*, 2013). Harjes *et al*. (2008) on one hand reported four important useful polymorphic sites in *LCYE* which varied the proportion of carotenoids in the alpha and beta branches of the carotenoid biosynthetic pathway and tripled PVA content of the maize genotypes while Yan *et al*. (2010) in a different study detected three functional polymorphic sites in the downstream of *crtRB1* and was associated with 40% of the variability in βC content in the endosperm of maize. Three PCR-based functional markers were developed by the experimenters for the detection of polymorphisms at different loci of the genes. These markers are being used in genotyping and selection for enhanced PVA content in maize breeding. Fu *et al*. (2013) detected two polymorphisms in phytoene synthase (*PSY1)***,** which was associated with 7 to 8% of the variability in total carotenoids in maize grain. In a study by Kandianis *et al*. (2013), *LCYE* and *crtRB1* genes were found to express pleiotropic effects on alpha- and beta-branch chains, suggesting that carotenoids could be influenced by many QTLs.

Different studies have assessed the effects of the functional markers of *LCYE* and *crtRB1* on carotenoids, either as individual gene or the combined effects in the genetic backgrounds of different maize germplasm (Burt *et al*., 2011; Vignesh *et al*., 2012; Babu *et al.*, 2013; Azmach *et al*., 2013). Burt *et al*. (2011) noted that though some maize inbred lines harboured undesirable alleles of *crtRB1* at the two loci 3'TE and 5'TE but had high level of βC content. Similar inconsistencies were observed by Vignesh *et al*. (2012) in some maize line which exhibited low level of βC though they

harboured desirable allele of *crtRB1* at 3'TE locus. Two of the three polymorphic loci of *LCYE* (3' indel and 5'TE) and one site of *crtRB1* (3'TE) was confirmed by Babu *et al*. (2013) using 26 diverse segregating tropical maize populations. The *LCYE* effect on the ratio of alpha-beta branch carotenoids and PVA content were not consistent in the diverse maize populations. On the contrary, sites that were polymorphic in *crtRB1* had significantly effect on βC and PVA contents. Knowing that the germplasm used for determining and authenticating the functional markers are largely of temperate origin having low frequencies of favourable allele, Azmach *et al*. (2013) investigated the potential of the DNA markers in IITA germplasm panel comprising materials of temperate and tropical origins. The results showed that the functional DNA markers *crtRB1*-5'TE and *crtRB1*-3'TE were consistently and strongly associated with PVA content across the tropical maize inbred lines tested. Recent Genome-Wide Association Studies on diverse panels of maize inbred lines show that in addition to *PSY*, *LYCE* and *CrtRB1* genes, there are other key genes, genomic regions, QTLs, enzymes and transcription factors that contribute to enhanced PVA accumulation in maize kernels (Suwarno *et al*., 2015; Azmach *et al*., 2018, Owens *et al*., 2019).

2.12 Provitamin-A carotenoid extraction and quantification in maize kernel

Provitamin A carotenoids are highly degradable, therefore, extraction and quantification procedures that will optimize the process is important. Analyzing kernels at same physiological maturity and moisture level, short duration of sample storage in -20^{o}C after harvest and protection of samples from light, heat, acid and oxygen have been recommended as basic precautionary measures to obtaining good PVA (Pixley *et al*., 2013). In general, there is no standard method for carotenoid extraction in laboratories (Rivera and Canela-Garayoa, 2012). Nevertheless, most extraction methods follow a common process of milling and freeze-drying followed by liquid-liquid or liquid-solid extraction procedure using organic solvents (Ishida and Chapman, 2012). The organic solvents include hexane, tetrahydrofuran, methanol, ethanol or ethyl acetate (Pixley *et al*., 2013). The common recent procedure for PVA carotenoid extraction in maize breeding is Galicia *et al*. (2012) which is based on the protocol developed by Kurilich and Juvik (1999).

Some methods of screening PVA content in maize include visual scoring, colorimetric procedure, Near Infrared Reflectance Spectrophotometry (NIRS) and liquid-based chromatography. The methods apart from the liquid chromatography are limited in

usage because of the wide range of carotenoids in maize kernel. Breeders use visual scoring on the basis of colour variation of the maize kernel. They often select for deep-orange grain in preference to pale yellow as it has been reported that deep-orange kernel contain higher PVA content than the yellow kernel maize (Pixley *et al*., 2013). This method has not proven to be reliable for a wide variety of germplasm, perhaps because of the confounding effects of other phenolics in maize kernel. It is however, inexpensive and useful in early generation selection. The NIRS has been successfully used in quantifying LUT, ZXT and total carotenoid but not for PVA carotenoids.

High Performance Liquid Chromatography (HPLC) is currently the commonest and most efficient method of PVA carotenoid determination. The process is time-consuming and exhorbitant, especially for large number of samples. The results of HPLC are usually consistent and highly reproducible. The Ultra Performance Liquid Chromatography usually serves as an excellent substitute to HPLC as the reagents are cheaper. Carotenoid analysis is quite complex, therefore, the use of laboratory replicates is recommended. Inter-laboratory comparisons of results should be conducted where possible to ensure data accuracy.

2.13 Some advances in breeding carotenoid-rich tropical maize

New and conscious determinations have been put in place to raise the PVA content in tropical maize from 3.0- 4.7 µg/g to 15 µg/g, which is a goal set by the HarvestPlus Challenge Programme (Bouis and Welch, 2010). This has been a key breeding priority of the IITA maize programme which prompted scientists to bring together well-characterized temperate maize inbred lines with tropical origin from the University of Illinois (Liu *et al*., 2003) to increase PVA level in tropical maize. These inbred lines contain 5.2 to 15 µg/g of βC. Numerous tropical-adapted backcross maize inbred lines with moderate PVA content as recipients and twelve elite ILs introduced as donors were crossed to recover new useful alleles of βC in the tropical maize without diminishing the effects of favourable alleles for yield and resilient traits found in the tropical maize (Dudley, 1982). The maize inbred lines were developed through many generations of selfing and visual selection of lines with bright yellow, orange, flint and semi-flint kernels up to S_3 inbreeding stage and subsequent selection based on phenotypic quantification using HPLC (Menkir *et al*., 2015). Chandler *et al*. (2013) reported that colour intensity of maize kernel has high heritability and that genotypes

with bright yellow and deep orange kernels would likely have higher PVA concentration than the genotypes with flint or slightly orange colour kernels.

The result from HPLC showed that 23 lines derived from backcross amassed more βC and PVA than the recurrent parents by 23 – 313% and 32 – 190%, respectively and the top four lines had PVA contents that exceeded the breeding goal of 15 µg/g (Menkir *et al.,* 2015). Many of the lines derived from the backcrosses harboured favourable alleles of *LCYE* and *crtRB1* (Azmach *et al*., 2013). The best performing line has been regularly crossed with elite tropical maize lines to generate crosses for the development of inbred lines with superior agronomic performance and elevated PVA content (Menkir *et al*., 2017). To broaden the genetic base and elevate both the PVA and xanthophyll contents of tropical maize inbred lines, six broad-based populations brought from Thailand were crossed as donors with the backcross-derived inbred lines. The result revealed the range of the carotenoids in the crosses as 7.1–13.8 µg/g PVA, 4.4–8.2 µg/g βCX, 23.2–46.4 µg/g ZXT and 9.0–23.8 µg/g LUT, respectively (Menkir *et al*., 2017).

The breeding strategies involving the introduction of temperate inbred lines and crossing with tropical-adapted lines and subsequent selection through visual selection and phenotypic quantification using HPLC has proven to be effective (Menkir *et al*., 2017). The maize improvement programme of IITA has used this strategy in quantifying carotenoids and identifying lines harbouring favourable alleles for carotenoid content (Menkir *et al*., 2017). Numerous inbred lines and hybrids developed through conventional breeding have surpassed the breeding target of the HarvestPlus Challenge Program, confirming the efficacy of the conventional breeding strategies (Bouis and Welch, 2010).

Several studies by other researchers also revealed that the selection of PVA-biofortified maize ILs was efficacious in developing hybrids with heightened PVA concentration, high yield and excellent agronomic performance (Suwarno *et al*., 2015; Obeng-Bio *et al*., 2019; Azmach *et al*., 2021). In these studies, it has been demonstrated that PVA-enriched maize ILs can be developed not only for hybrid maize, but also for maize synthetics to deliver cheap maize varieties to poor rural farmers through the informal seed system (Iseghohi *et al*., 2020).

2.14 Development of PVA-enriched maize synthetics

Development of hybrids is usually very expensive and tedious. Most poor rural farmers in sub-Saharan Africa cannot afford hybrid seeds. Developments of synthetics fill this gap and make it possible to deliver improved seeds to rural farmers at lower cost. According to Lonnquist (1949), synthetics are open-pollinated populations formed from the intercross of genotypes of good combining ability and are maintained in isolation through open pollination, mass selection or recurrent selection. In maize breeding, the term synthetic variety has become synanimous with open-pollinated varieties developed from ILs that have demonstrated excellent combining ability in all possible combinations. The components of a synthetic variety could be inbred (usually) or mass selected populations in context of maize. The components are maintained so that the synthetic variety could be reconstituted when required. In synthetics, productivity loss is minimal in each generation of seed advancement (Katepa-Mupondwa *et al.,* 2002). Farmers can use their saved seed up to four years and thereafter replace them.

Synthetic varieties are developed by selecting genotypes that enhance hybrid performance in cross combinations. It is therefore essential to select male and female parents from diverse origins that can increase heterosis. The number of parents used, their GCA and SCA determine the overall productivity of the synthetics and the magnitude of heterosis of their crosses (Seif and Link, 2007). Synthetics serve as cheaper source of seeds for poor farmers and produce competitive yields for commercialization compared to hybrids. Breeders also use synthetics as source populations for extractions of new ILs (Pandey *et al*., 1984). It has become of great value in maize improvement and other crops such as pearl millet, sunflower, alfafa, sugarbeet and those in which pollination control is difficult.

The genetic potential of any maize genotype depends on its *per se* performance and combining ability in hybrid combinations. Numerous studies have reported the *per se* performance of maize synthetics in some key traits, such as grain yield, earliness, resistance to diseases (Velasco *et al*., 1999) and protein and amino acid content, but not much studies have been reported for PVA carotenoids (Iseghohi *et al*., 2020). The first generation of PVA-rich orange open-pollinated maize varieties was released by the IITA Maize Improvement Programme (MIP) in conjuction with a national institute, the Institute of Agricultural Research (IAR) in Nigeria in June, 2016 (IITA, 2016).

IITA in partnership with IAR developed these varieties using conventional breeding as part of strategies to prevent the prevalence of VAD. The varieties released by National Variety Release Committee of Nigeria as Sammaz 38 and Sammaz 39 are IITA synthetic PVASYN2 and PVASYN8 (IITA, 2016). Others later released are Sammaz 43 and Sammaz 44 (Mengesha and Maru, 2017).

2.15 Synthetic cross hybrid

The conventional and common maize hybrids are those derived from the cross of known inbred lines. This includes single cross hybrid (A × B), double-cross (A × B) × (C × D) and three-way cross (A × B) × C. Although these hybrids are characterized by uniformity and high yield, they are tedious to develop and expensive for poor rural farmers. Affordable and alternative form of hybrids include variety cross (variety A × variety B), synthetic cross (synthetic A × synthetic B) and population cross (population A × population B).

2.16 Applications of molecular markers to crop improvement

Molecular markers have been deployed in breeding programmes to accelerate genetic gains over the years. The use of molecular markers is more advantageous than the use of morphological markers because molecular markers are not subjective to plant's physiological stage or environmental conditions (Chen *et al*., 2021). Molecular markers are used in breeding for DNA finger printing of parental lines, hybrid verification, genetic diversity assessment, variety identification, MARS and genomics-assisted breeding. Over the last thirty years, diverse molecular markers comprising RAPDs, RFLPs, AFLPs and SSRs, InDel markers and SNPs have been developed and deployed effectively in diverse maize studies (Lynch and Milligan, 1994; Qu and Liu, 2013; Liu *et al*., 2015).

Single nucleotide polymorphism markers is presently the most common form of genetic variation among individuals, and are the most recently developed DNA marker technology. They have become markers of choice because they are high-density and highly polymorphic within genomes. In addition, SNPs are co-dominant markers; the documentation system is simple and is cost-effective (Elshire *et al*., 2011). They are amenable to various high- and medium- throughput genotyping platforms that take into consideration diverse breeding needs for variable marker densities and cost per sample. The availability of high quality multiplex platforms for genotyping, such as Genotyping by Sequencing (GBS), Next-Generation Sequencing (NGS) technologies

(Elshire *et al*., 2011), Diversity Arrays Technology, DArTseq, and Sequenom (Sansaloni *et al*., 2011), and chip-based Illumina GoldenGate technologies, have made the applications of SNPs widespread. However, these high-throughput SNP platforms are not appropriate for small-scale genotyping system often applicable to studies involving QTL mapping, MABC, and MARS, because of a high cost per sample (Chen *et al*., 2021). Therefore, uniplex genotyping assays such as KASP, Taqman, and Amplifluor have become useful alternatives because of their flexibilities in the design of assays, ease of run, and affordability (Neelam *et al*., 2013). Therefore, breeders are able to use smaller subgroup of SNPs that are informative, thereby removing the unintended data points when using fixed array SNPs. The most competitive of the uniplex system in crop improvement is the KASP assay (Chen *et al*., 2021).

2.17 Kompetitive allele-specific PCR

Kompetitive allele-specific PCR (KASP) is a PCR-based SNP genotyping method initially developed by Laboratory of the Government Chemist (LGC) or KBioscience, United Kingdom, for their in-house genotyping. It has since evolved into a global benchmark technology. The technology is based on allele-specific oligonucleotide extension and fluorescence resonance energy transfer (FRET) for signal generation (Semagn *et al*. 2012). The KASP assay technology is flexible and its chemistry functions well in 96-, 384-, and 1536-well plate format (He *et al*., 2014). As a result of this, small data can be generated on few samples of about 22 over 1 SNP to as high as thousands of samples over many SNPS in a single day (He *et al*., 2014). KASP genotyping method has a shorter turnaround time and lower genotyping error rate of 0.7-1.6% when compared to the Illumina Goldengate patform (2.0-2.4%) (Semagn *et al*., 2013). In addition, KASP genotyping costs for MARS were 7.9-46.1% cheaper than those of the BeadXpress and GoldenGate platforms (Semagn *et al*., 2013).

2.18 How KASP genotyping works

According to LGC (2015), to run a KASP assay reaction, four components are important: (1) The assay components (2) denaturation of template DNA and annealing components, (3) generation of complement of allele-specific tail sequence, and (4) signal generation

1. Assay components

The KASP assay entails the template DNA, SNP of interest, the KASP-assay mix containing two competing allele-specific forward primers with unique tail sequences,

and the KASP master mix containing FRET cassette (universal FAM and HEX labeled cassettes) plus the *Taq* polymerase, dNTPs and $MgCl_2$ in an optimized buffer solution.

2. Denaturation of template DNA and annealing components (PCR round one)

A hot start activation of the KASP *taq* is required for the first round of the protocol. The template DNA is denaturated in this round and using the common reverse primer, one of the allele-specific primers amplifies the target SNP.

3. Generation of complement of allele-specific tail sequence (PCR round two)

A complementary copy of the tail of allele-1 is produced by the reverse primer after it binds to the allele-1 and extends it

4. Signal generation (PCR round three)

There is an increase in allele-specific tails in this round of PCR. Upon binding with the new tail sequences, the fluorescent-labeled part of the cassette releases the fluorescent-labeled oligonucleotide from the quencher to produce a fluorescent signal.

Following the completion of the KASP-PCR, reaction plates are read, and data analyzed using any cluster viewing software. Signals detected are plotted as graphs. Samples of the same genotype cluster together. Samples of homozygous alleles fluoresce red colour (i.e. HEX alleles), green colour represents heterozygous alleles (i.e. one HEX allele and one FAM allele), whereas, blue colour is homozygous for FAM allele.

2.19 Application of KASP-SNPs to maize breeding

Recently, KASP markers have been extensively used in various maize studies, including diversity analyses; QTL analyses, fine mapping, Marker-assisted Selection (MAS) and Genomic Selection (GS) (Semagn *et al*., 2013; Bojikoba and Cokojiob, 2017). At The International Maize and Wheat Improvement Center (CIMMYT), KASP is used to generate in excess of one million data points annually. The KASP maize library was developed from the mapping data against the reference genome of B73 (Jones *et al.*, 2009) and a SNP mining study from Express Sequence Tag (EST) sequences (Bately *et al.*, 2003). The work done using the KASP maize library illustrates the power of SNP markers to accelerate genetic gain in crop improvement. For instance, 71,311 KASP-SNP markers were developed from RNA-Sequence datasets generated from genotyping 368 maize ILs, of which 46 of the loci were 100% polymorphic and relevant in discrimating among the ILs (Chen *et al*., 2021). The KASP-SNP marker (snpZM0015) found in the *crtRB1* gene influencing the content of

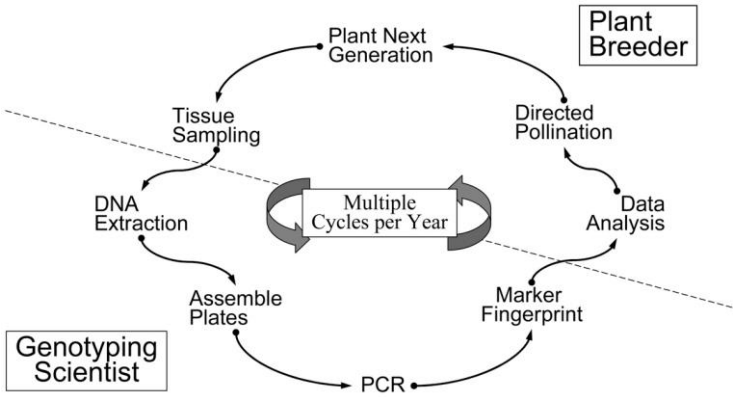
PVA effectually differentiated desirable and undesirable alleles among seventy maize ILs (Obeng-Bio *et al*., 2020). Using KASP, the IITA-MIP has generated over 17,000 data points for diverse maize germplasm (Gedil and Menkir, 2019). However, the effects of these KASP-SNP-based improvements on the carotenoid contents, agronomic traits and combining ability of biofortified maize synthetics have not been examined.

2.20 Marker-assisted Recurrent Selection (MARS)

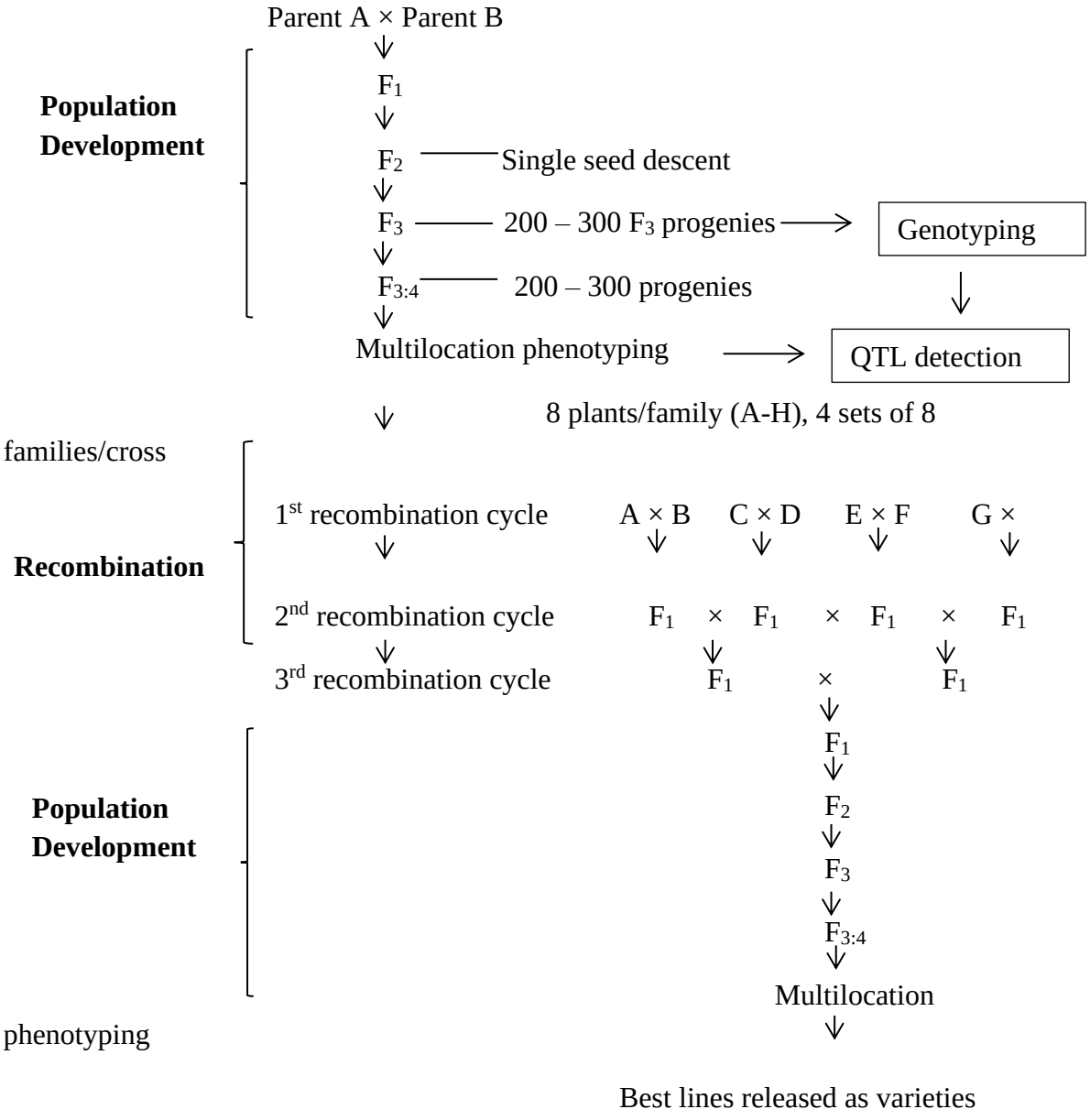
Selection is an important breeding strategy practiced to improve important traits. Phenotypic recurrent selection is usually influenced by environmental effects and takes at least 2-3 cropping seasons to complete one cycle (Gokidi *et al*., 2016). Marker-based selection offers the possibility of expediting this process, making it more efficient as is not influenced by environment and is plant stage-insensitive. Marker-assisted recurrent selection (MARS) is a scheme that allows the use of molecular markers for the identification and selection of multiple genomic regions involved in the expression of complex traits in order to assemble the best-performing genotypes within a single or across related populations (Ribaut *et al*., 2010) (Figure 2.3a). Performing genotypes are selected and intercrossed in the same cropping season to make one cycle of selection. MARS enhances the efficiency of recurrent selection and helps to integrate multiple favourable genes or QTLs from different sources based on multi-parent populations (Gazal *et al.*, 2015). MARS has been mostly used to improve F_2 populations before extraction of inbred lines from them (Singh, 2015). In MARS, *de novo* QTL mapping is done for each breeding population. Once major QTLs of interest are identified, selected individuals are subjected to controlled pollination to develop lines with optimum complement of QTL's from both parents (Figure 2.3b). With the use of continuous nursery programs during off-season, pre-flowering genotyping and controlled pollinations, multiple cycles of MARS can be completed within one year (Gazal *et al.*, 2015).

2.20.1 Procedure of MARS

1. Fingerprint progenies from a given breeding population derived from biparental cross with specific molecular markers. This means that QTLs of



(2.3a)



(2.3b)

Figure 2.3. Marker-assisted recurrent selection scheme (a) and its mechanism (b) (Adapted from Gokidi *et al*., 2016)

200-300 progenies from F_2 or more than 300 progenies from F_3 population are identified to enable the calculation of genotypic value for each progeny.

2. About 200-300 progenies from the F_3 derived population i.e., $F_{3:4}$ or $F_{3:5}$ are evaluated at multi-location trials for phenotypic data.
3. Based on the genotypic and phenotypic data, few plants are selected and intermated for two or three cycle.
4. Recombined lines are self-pollinated for two to three years and then subjected to a final phenotypic screening at multi-location trials to select the best lines to release as varieties (Figure 2.3b) (Eathington *et al*., 2007; Gazal *et al.,* 2015).

2.20.2 Response to selection and genetic gains

Response to selection is one of the ways to track progress made in selection. Improvement of grain yield, resistance to *Striga hermonthica* and drought stress in maize have been achieved through MARS (Beyene *et al*., 2016; Bankole *et al*., 2017). Beyene *et al*. (2016) reported an increase of 0.5 to 46.3% in GY of maize hybrid enhanced through MARS over hybrid developed using pedigree selection under drought stress and 3.4 to 13.3% increase under well-watered condition. Similarly, Bankole *et al*. (2017) stated a 7% gain/cycle of MARS for GY under drought stress, 3% under rainfed and 1% under irrigation condition. Other studies showed MARS' superiority over phenotypic recurrent selection in accumulating favourable alleles from different parents into one individual (Moreau *et al*., 2004; Eathington, 2005; Crosbie *et al*., 2006). They found that gain made in some maize genotypes as a result of MARS was almost two-folds of those selected phenotypically. Eathington *et al*. (2007) demonstrated that the improvement achieved in grain moisture at harvest, grain yield and percent oil content in MARS lines was comparable to conventionally selected lines in European sunflower (*Helianthus annuus* L.) breeding population. Bernardo (2008) reported that MARS increased the frequency of favourable marker allele from 0.50 to 0.80 in F_2 population of sweet corn.

The merit of the use of MARS over phenotypic selection was found to be more when the population under selection was exceedingly diverse, as is often the case with tropical and subtropical materials (Gokidi *et al*., 2016). Simulation studies showed that the comparative advantage of MARS in relation to phenotypic selection decreases rapidly when the fraction of the total genotypic variance explained by the QTLs included in the selection index decreased (Van Berloo and Stam, 2001). The results

from several simulation and experimental studies indicate that gains per selection cycle for grain yield in maize are 25 -50% lower for MARS than for phenotypic selection based on testcross performance. Howevr, phenotypic selection requires 2 years per cycle, while up to three cycles of MARS can be completed each year by using off-season nursery/greenhouse facilities. Therefore, gains per year are much higher for MARS than those for phenotypic selection (Bernardo 2008). MARS has become highly competitive with phenotypic selection in large scale breeding programmes of private sector because of return per unit cost per unit time (Bernardo 2008).

There is a dearth of reports on the effects of MARS on PVA carotenoids, probably due to huge cost of large-scale phenotyping with HPLC quantification (Prasanna *et al*., 2020). Dhliwayo *et al*. (2014) studied the effect of S_1 recurrent selection on PVA carotenoids of three open-pollinated varieties (OPVs) of maize. They reported linear increase of 25 - 67% per cycle for PVA, 28 - 60% for βC, 18 - 70% for βCX and 11 - 46% for ZXT. A two cycle recurrent selection increased ZXT, LUT, βC and total carotenoid content by a range of 18.5 to 196.6%, and grain yield by 23.2% in orange waxy corn (Khamkoh *et al*., 2019). In a similar study on two IITA maize synthetics (HGA and HGB) and their improved cycles (C_1 and C_2) developed through MARS, according to Astatke (2018), the improvement of PVA, βC and TC in HGA was 30%, 40%, and 36%, respectively, while the improvement of αC and βCX in HGB was 20% and 5%, respectively. There was an increase in the rate of occurrence of four favourable alleles of *crtRB1*-KASP markers. This study showed the potential benefits of using MARS to improve PVA carotenoids in maize. However, more studies still have to be done to ascertain its effects on different maize germplasm.

Response to selection per generation can be determined from genetic gain formula, i.e. h^2S (Falconer and Mackay, 1996); where h^2 is narrow sense heritability for random mating population and S is selection differential. Selection differential (S) is the difference between the mean of selected genotypes (μ_s) and the base population mean itself (μ) (Singh, 2012). The difference between the mean of the offspring of the selected individuals (μ') and the mean of the total population (μ) is known as response (R) of the selection, ($R = \mu' - \mu$) (Ponta, 2001). The ratio of R/S is called realized heritability. A trait's heritability determines how much genotypic value is passed on to offspring from superior parents (Hallauer *et al*., 2010). If the selection unit is normally distributed, it can therefore be represented in a frequency distribution curve (Figure

2.4a). Selection differential can be standardized as $Z/B = (\mu_s - \mu)/ \sigma^2$; where Z is height at which selection is truncated, B is the area of selected individuals, μ_s is the mean of selected individuals, μ is the mean of total population and σ^2 is the variance.

2.20.3 Measuring response to selection

To make accurate measurement of response to selection, the breeder must adhere to the following: maintain seeds capable of germinating for each selection cycle, possess a large sample size of each cycle; make use of active population in recombination and develop the seed a season prior to evaluation (Hallauer *et al*., 2010). The goal of every selection experiment is to acquire improve varieties or hybrids. The feat depends on the efficiency of selection. Response to selection (R) could be measured using either of the following methods:

1. Total gain method

   The formula of percentage total gain is given as:

$$R = \frac{Cn - C0}{C0} \times 100$$

Where:

R: is percentage total gain (%)

Cn: is mean performance of the final cycle under consideration.

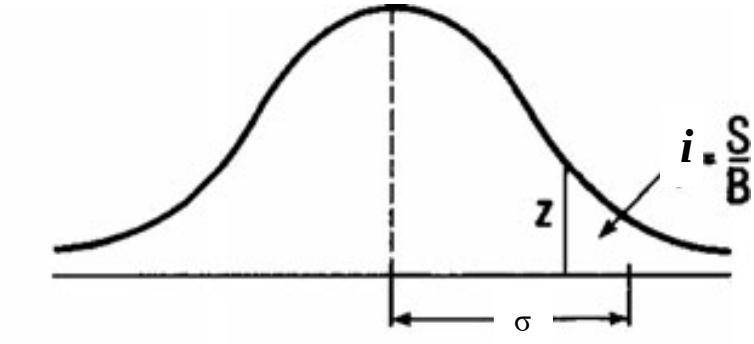
C0: is the mean performanceof the original cycle

To obtain gain per cycle or year, percentage total gain will be divided by the number of selection cycle. Response to selection/year is considered as the most effective means of comparison amongst the selection methods (Hallauer *et al*., 2010).

2. Method of regression

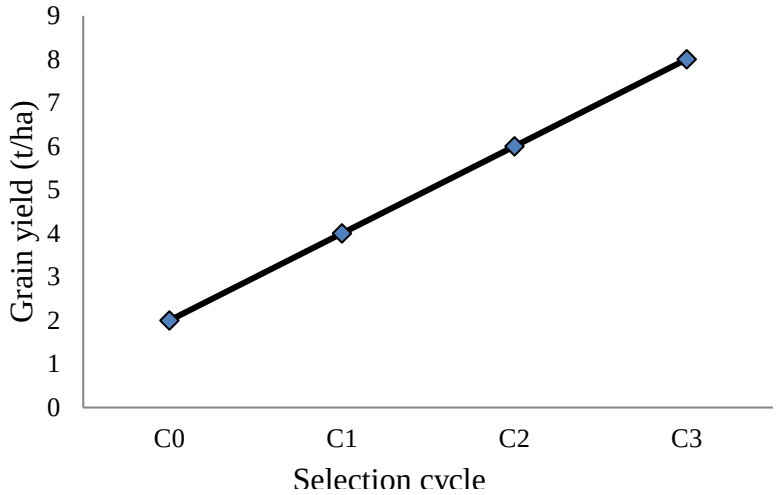
It is the mean response to selection per cycle i.e. regression of trait of interest on selection cycle. The gain/cycle is a based on the estimation of the regression coefficient (*b*) (Figure 2.4b).

To obtain percentage gain/cycle, the linear regression coefficient (*b*) is divided by the mean performance of the initial or base population (C_0). A gain from selection can be calculated as a percentage of the observed original mean, or as a percentage of the predicted original mean using linear regression. Another way to determine selection gain is to measure the mean changes of selection units which are the families evaluated in replicated trials across locations and years and adjusted to a check performance (usually a commercial hybrid). The Gains are expressed as a percentage of check performance.



(2.4a)

$$Yi = \mu_0 + \beta_{1Xi} + \delta_i$$



(2.4b)

Figure 2.4: Frequency distribution of normally distributed population (2.4a) with subpopulation (B), selection intensity (*i*) and selection differential (S) and response to selection of grain yield in maize over three cycles through regression method (2.4b) (Adapted from Hallauer *et al*., 2010)

Eberhart (1964) suggested simple or multiple regression models for estimating the rate of response in continuous selection program. The simple linear regression model for a one population undergoing selection is

$$Yi = \mu 0 + \beta 1Xi + \delta i$$

Where:

Yi: is the observed means over cycles of selection (i= 0, 1….c)

μ0: is the mean of the original population,

β1: is the coefficient of linear regression,

Xi: is the cycle of selection and

δi: is the deviation from the linear model.

For the improvement of two different populations where information on the effects of selection on the parental populations, crossed population and heterosis of the crosses is desired, more complex regression models can be used. Using modified Reciprocal Recurrent Selection (RRS), Patterniani and Vencovsky (1977) analyzed the effects of reciprocal crosses on the mean of the original populations, heterosis in the original cross, changes in populations, and changes in heterosis between advanced cycles.

3. Smith's Model

The two methods above do not provide information on how changes in mean relate to variations in allele frequencies and inbreeding depression owing to genetic drift and selection. Based on allelic frequencies and effects, Smith (1979) explained changes in population mean under selection. The model takes into account how inbreeding depression limits genetic gains. The model can estimate for the contribution of the effects of additive and dominance genes to population means as well quantify the magnitude of heterosis as well as response to selection (Hallauer *et al*., 2010). Data on selection cycles, selfed and cross-cycles is used Smith model. It is based on the following assumptions: Hardy-Weinberg equilibrium of random mating population, trait of diploid inheritance and no epistasis. The model was first applied in RRS of BSSS and BSCB1 populations. Non-significant regression coefficients for the per se performance of the two populations was reported by Eberhart *et al*. (1973). However, based on the model, significant variations in allele frequencies in the populations were reported, showing that RRS was effectual in increasing the frequencies of favourable

alleles. Smith, (1979) reported substantial inbreeding depression in the populations which was linked to non-significant mean changes in selection.

2.21 Basic principle of combining ability

The estimate of the potential of parent lines on the basis of their offspring performance in a hybridization process based on a definite mating design is known as combining ability (Allard, 1960). It is simply the ability for cultivar to combine for optimum yield in hybrid combination. It was proposed by Griffing in 1956 and thus also known as Griffing's approach or principle of combining ability. It is a diallel analysis used in plant improvement programs to identify superior parents for crosses, estimates general, specific and reciprocal effects (Stattuck *et al*., 1993). The development of superior offsprings in crosses and the determination of the magnitude of gene action of the trait of interests are dependent on the combining ability of parents. The GCA effect of the the parent is the average performance of the progeny of a parent when mated with a series of genotypes, while the SCA effect is the performance of the progeny of a pair of parental cross. A parent is considered an average combiner if the GCA effect is zero and considered to have positive or negative GCA effect its performance is above or below average; SCA effects are either positive or negative (Stattuck *et al*., 1993). General combining ability represents additive, additive × additive (where epistasis is significant) gene actions while SCA indicates nonadditive (dominance) gene actions. The presence of epistasis associated with additive effects in segregating populations can cause bias in predicted heterosis and repeatability (Stattuck *et al*., 1993).

Environment can significantly influence GCA and SCA and changes the parental combining ability. Therefore, evaluation of potential parental materials in multi-locational trial is necessary for precise and accurate estimation of combining ability.

2.21.1 Griffing's models of combining ability

Griffing proposed four models which considered genotype and block effects. In model one also known as the fixed effect model; the genotypes and blocks effects are constant. Model two (random model), the genotype and block effects are random. Model three (mixed model), the genotype effects are constant while the block effects are random. Model four (mixed model), the genotype effects are random while the block effects are constant. Models one and two are most commonly used in many researches. In addition to the models, Griffing presented four methods of diallel

crossing scheme to produce 16 different model/method combinations. The method depends on the material included in experimentation. Method one involves the inclusion of parents, F_1 and reciprocal crosses, method two includes parents and F_1 progenies, method three includes F_1 progenies and reciprocal crosses and method 4 includes F_1 progenies in experimentations.

2.21.2 Combining ability for PVA carotenoids

To efficiently explore the genetic potential of ILs or genotypes of different genetic backgrounds for increased PVA content in maize endosperm, the influence of the gene for carotenoids must be assessed. The contributions of ILs in formation of different hybrids depend on the gene action, heritability and existiting variability among the ILs for the trait. Since early 20^{th} century, carotenoid content of maize endosperm is been a trait of interest and research amongst scientists (Hauge and Trost, 1928). However, there had not been report on combining ability of PVA-enriched maize synthetics and their advanced cycles belonging to different heterotic groups. Grogan *et al*. (1963) reported significant male and female effects as well as additive gene action in the control of carotenes and xanthophylls among 10 maize inbred lines (ILs), while substantial GCA and SCA with GCA being more important was reported by Egesel *et al*. (2003) among 10 maize ILs for the generation of 45 maize hybrids. In a similar study, Senete *et al*. (2011) reported that GCA effects were significant for all the carotenoids measured, except LUT for which SCA was significant. Other authors (Suwarno *et al*., 2015; Azmach *et al*., 2021) also reported preponderance of additive effect over dominance effects for the gene action of βC and other PVA carotenoids in different maize germplasm. Halilu *et al*. (2016) however reported slightly different results of preponderance non-additive gene effect over additive gene effect for all carotenoids assessed in some tropical maize ILs.

The above studies underscored the significance of additive gene effect more than the nonadditive effects in the genetic control of maize carotenoids. In addition, molecular studies have also revealed that the major genes (*PSY1*, *LCYE* and *crtRB1*) regulating the synthesis of carotenoids in the biosynthetic pathway have additive effects (Yan *et al*., 2010; Azmach *et al*., 2013). This is probably the reason for lack of heterosis for PVA in most maize genetic background as heterosis leverages on SCA effects in crosses. Due to this fact, there is the need for more studies that involve broad base

genotypes such as synthetic populations to explore gene actions and heterosis for PVA carotenoids.

Preponderance of additive gene action for βC and total carotene content has been established in cassava, orange fleshed sweet potato and carrot (Jagosz, 2012; Njenga *et al*., 2014; Baafi *et al*., 2017). However, nonadditive gene effect has been reported to be responsible in the inheritance of carotenoids in vegetables including pumpkin, cauliflower; chilli peper, cucumber, broccoli, (Pandey *et al*., 2010; Bhutia *et al*., 2015 and Kaur *et al*., 2016).

2.22 Heterosis for PVA carotenoids

The phenomenon of heterosis is poorly understood, but it has been exploited extensively in breeding and commercial production of hybrids. The term heterosis refers to the superior performance of hybrids compared with their parents (Hallauer *et al*., 2010). In maize, heterosis is demonstrated by offsprings of inbred lines that manifest significant SCA. In order for hybrid vigour to be expressed, parental crosses must exhibit genetic diversity. It is possible to infer heterosis from heterotic patterns (Hallauer and Carena, 2009). According to Carena and Hallauer (2001), crosses between genotypes with a high level of heterosis are termed heterotic patterns. For instance, Iowa Stiff Stalk Synthetic crosses well with Lancaster Sure Crop derived ILs; therefore, they are heterotic to each other (Hallauer *et al*., 2010). When heterotic pattern is known, it reduces the cost of producing high performing hybrids since the performance of crosses from the heterotic groups is predetermined. Analysis of genetic distance is used to categorize genotypes into heterotic groups. Genetic distance of maize ILs for catenoid contents have been estimated through molecular approaches (Menkir *et al*., 2014; Suwarno *et al*., 2014). Suwarno *et al*. (2014) used 402 SNPs information on GD to classify 127 CIMMYT's PVA maize ILs into three heterotic groups. Furthermore, the pedigree information was applied to categorize the three groups into sub-groups to avoid crossing closely related ILs. The GD effect had significant heterosis for GY of the maize hybrids but not on PVA content. However, some of the hybrids had elevated levels of PVA. Assessing the effect of hybridization of parental lines of AFLP-based heterotic groups on carotenoid content, Menkir *et al*. (2014) reported that hybrids of elevated PVA content could result from parents in marker-based groups and with high PVA contents. Althought hybrids developed from

ILs exhibit higher level of heterosis for GY and disease resistance, but not much study has been reported on the expression of heterosis for PVA carotenoids of varietal-cross hybrids derived from maize synthetic. Open-pollinated varieties, composites, synthetic varieties and improved varieties are being used in many diallel crosses to obtain heterosis in hybrids. To measure hybrid performance relative to its parents, two methods are proposed:

(1) **Mid-parent heterosis (MPH)**: It's a measure of a hybrid's performance relative to its parents' average performance.

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

(2) **Better-parent heterosis (BPH)**: It is a measure of the performance of a hybrid in comparison to its superior parent.

$$BPH = \frac{F_1 - BP}{BP} \times 100$$

Where:

MP: is the value of mid-parent

BP: is the value of superior parent.

Another type of heterosis that is of practical and economic use is the **standard heterosis.** It estimates the performance of the hybrid (F_1) in relation to the **standard check**. Standard heterosis is calculated as

$$STH = \frac{F_1 - SCV}{SCV} \times 100$$

Where:

STH: is standard heterosis (%) and

SCV: is the mean of standard check variety included in the trial.

Estimation of heterosis has been largely reported in quality protein, GY and yield associated traits of different maize lines (Souza *et al*., 2009; Kolawole *et al*., 2017 and Tulu *et al*., 2018), but limited information is available on heterosis of PVA (Suwarno, 2012; Alfieri *et al.*, 2014; Azmach *et al*., 2021). To enhance carotenoid accumulation in maize kernels, Pfeiffer and McClafferty (2007) proposed exploiting heterosis. Heterosis for carotenoids in the crosses of yellow dent maize kernels was reported to be uncommon and irregular (Burt *et al*., 2011). They stated that heterosis is partly due

to QTLs that influence carotenoid biosynthesis flux and QTLs affecting one branch over the other. However, with increase in breeding efforts and development of improved ILs, hybrids are now known to express mid and better-parent heterosis (Alfieri *et al.*, 2014; Azmach *et al.*, 2021).

Alfieri *et al.* (2014) reported significant MPH and BPH range of (17.95% to 94.17%) and (0.31% to 17.88%), respectively for βC in maize hybrids developed from 19 Italian parental lines. The highest percent significant heterotic effect was observed for LUT, and the lowest was for ZXT. This confirms the study of Burt *et al*. (2011) that heterosis is influenced by the total influx through the carotenoid pathway which favours one branch than the other. Suwarno (2014) reported average BPH of -0.4% to 1.0% for PVA concentration within a mating set of maize parents. Azmach *et al*. (2021) estimated the heterosis of 80 maize hybrids obtained from crossing 24 maize ILs using North Carolina Design II (NCDII). Forty two hybrids displayed significant positive MPH, while 15 hybrids demonstrated BPH for at least one of the carotenoids. Twenty two hybrids demonstrated MPH in βC while 21 hybrids in βCX, 17 in ZXT and 14 in LUT. The MPH heterosis in βC, PVA and TC ranged between 15% and 56%. One hybrid demonstrated MPH for all the studied carotenoids and its PVA content was 5.5μg g^{-1} but was poor in GY and most of the agronomic traits. Hybrids 22 and 23 registered significant positive BPH for both βCX (30%) and βC (56%), each with mean PVA concentration of 5.27 μg/g and 6.0 μg/g. One of the hybrids (Entry 12) which exhibited significant SCA effects for relevant growth and yield-related traits also had desireable BPH (32%) for PVA and 37% for TC. In his study, he found that majority of the hybrids which manifested remarkable heterosis for beta branch carotenoids had no desirable heterosis for alpha branch carotenoids, such as LUT. Also, most hybrids which had desirable heterosis for any of the PVA carotenoid (αC**,** βCX and βC) did not express significant heterosis for the xanthophylls (ZXT and LUT). The report agrees with the results of Burt *et al*. (2011).

2.23 Inbreeding depression

Inbreeding is a mating system which occurs between relatives. It leads to increase level of homozygosity which results in depressive effect in traits (Filho, 1999). Inbreeding depression arises from the inevitable consequence of inbreeding and it is quantified by reduced mean performance in trait under nonadditive genetic effects. Inbreeding depression is caused by recessive deleterious alleles in the homozygous state. A

population's inbreeding depression is an intrapopulation effect, whereas a population's heterosis is an interpopulation effect (Filho, 1999). Selfing is the most extreme form of inbreeding; it increases homozygosity and gives rapid decrease in fitness. Selfing leads to reduced population size with Ne=1; where Ne is the effective population size. Random drift occasioned by a small size of population could also cause inbreeding depression due to high frequency of homozygotes at the rate of F = 1/2Ne per generation. After reduction of large population over generations, some genes become fixed (p = 1) in the subpopulation while others get lost (p = 0). The gene frequency of a quantitative trait in a population ranges between 0 and 1 depending on the type of population. Completely inbred line has genes at frequencies 0 or 1 while partially inbred populations have a proportion of fixed alleles at frequencies 0 or 1 and in the range $0 < P < 1$. Synthetics developed from many inbred lines and broad based open pollinated populations have distributions with intermediate gene frequencies with heterozygosity of 50% for locus with two alleles. Synthetics have less inbreeding depression and recurrent selection has been recommended for eliminating deleterious recessive genes causing inbreeding depression (Hallauer and Filho, 1995).

2.24 Genetic bases of heterosis and inbreeding depression

Inbreeding depression is direct opposite of heterosis, hence, are governed by same genetic hypothesis. The genetic bases of these phenomena have been debated for year without consensus (Kaeppler, 2012). However, the general bases commonly alluded to are:

1. Dominance hypothesis

This hypothesis states that the dominant allele has favourable effect at each locus while the recessive allele has unfavourable effect (Singh, 2012). The lethal effects of recessive allele are masked by the favourable effect of the dominant allele in a heterozygous locus. Heterosis occurs when the lethal effects of recessive alleles are masked by dominant alleles while inbreeding depression occurs when the lethal recessive allele is homozygous at several loci due to inbreeding. Inbred line carrying harmful recessive alleles will have weak vigour because inbred lines are homozygous at many loci. It is however possible to isolate superior inbred with high vigour through selections and hybrid vigour is obtained from the crosses of unrelated inbred lines. In

open-pollinated populations, plants are highly heterozygous and do not express the harmful effects of recessive alleles present in the population.

2. Overdominance hypothesis

It states that heterozygote loci are superior to both relevant homozygote loci. In this, heterozygosity causes heterosis while homozygosity of either dominant or recessive alleles causes inbreeding depression. The hypothesis is also called single gene heterosis. East (1936) stated that there are several alleles for each locus showing overdominance with increasingly different functions. The more divergent each allele in heterozygote state, the more heterotic they are. In this hypothesis, it is impossible to isolate inbred lines as homozygosity of either dominant or recessive alleles result in depression. There have not been strong evidences of overdominance in polygenic traits but in oligonenic traits such as days to anthesis and maturity in maize. Interpretation of overdominance is however confounded with pseudo-overdominance in most studies (Kaeppler, 2012).

3. Epistasis hypothesis

Epistasis is the influence of one locus on the expression of another. It is also referred to as non-allelic interaction. The role of epistasis in heterosis has become clearer with accumulation of data over the years, utilization of recent molecular markers and modern statistical approaches. These have led to the detection of many heterotic crosses that have shown significant epistasis (Singh, 2012; Kaeppler, 2012). For epistasis to have significant contribution to heterosis, it should be of complementary type. This means that dominance effects and dominance × dominance interaction effects of genes should have same sign such that they do not cancel out. In addition, the interacting pairs of genes should be dispersed in both parents of the hybrids. It is suggested that in the absence of overdominance, dispersion of genes showing complementary epistasis seems to be the main cause of heterosis; while multiplicative interacting gene effects have been linked to the cause of heterosis in polygenic traits such as yield (Singh, 2012).

Large QTL mapping studies find little evidence for epistatic interactions for specific developmental, architectural, and biochemical traits (Tian *et al*., 2011). Heterosis caused by epistasis is greater in quantitative traits such as grain yield. Genes/QTLs of

small effects must interact in the same way to have sufficient magnitude to cause detectable heterosis. Epistatic variance contributes little to the total genetic variance compared to additive and dominance variance. Understanding of the role of epistasis to heterosis will continue to improve with advances in molecular and statistical tools as current evidence suggests that there is much more to learn (Kaeppler, 2012).

2.25 The role of heterotic grouping in hybrid development

Heterotic groups are useful in hybrid development in that it helps breeders to efficiently utilize their germplasm by avoiding unnecessary crosses. Mere increase in genetic diversity of germplasm is not sufficient to maximize heterosis; therefore, development of heterotic population or assigning genotypes into heterotic groups and routine assessment of their combining abilities is an integral part of hybrid breeding (Gopi and Hampannavar, 2018). Heterotic group was explained by Melchinger and Gumber (1998) as a set of genetically distinct germplasm that combine well with genotypes from other genetically distinct group and exhibit a similar response when crossed with them. On the other hand, heterotic pattern refers to a pair of heterotic groups that result in high hybrid vigour when crossed. Populations with good heterotic patterns are often improved through reciprocal recurrent selections and are useful in long term hybrid breeding programme, inbred recycling and population improvement. Heterotic grouping reduces SCA variance to GCA variance ratio. This makes early testing more effective in that superior hybrids are identified based on the prediction of the GCA effects.

Different approaches are suggested in establishing heterotic groups, depending on the germplasm. They include the use of good average performance and high genetic variance of hybrid population in the target region(s), good adaptability and excellent *per se* performance of the parent populations, high ratio of GCA variance to SCA variance, source materials for inbred development that have low inbreeding depression (Melchinger and Gumber, 1998).

2.26 Correlated effects of grain yield and provitamin-A carotenoids

A major challenge associated with selection for trait of interest using recurrent selection is the likelihood of having traits-correlated effects. For example, Dudley and Lambert (2004) reported that increasing the oil content in Illinois maize populations through recurrent selection, gave rise to a significant decline in GY and starch content,

but increased the embryo size of the maize population. Likewise, Below *et al.* (2004) reported that a recurrent selection focused on increasing protein content also enhanced GY, some physiological traits such as high rate of N absorption and translocation, increased asparagine status and enzymatic rate in nitrogen metabolism in maize, but with decreased the sugar content in the grains. Therefore, in as much as recurrent selection has been proven effective in enhancing qualitative traits in maize breeding, the correlated effects amongst traits is unpredictable (Below *et al*., 2004).

In the study of assessing the effects of S_1 recurrent selection on GY and PVA carotenoids in three OPVs maize, Dhliwayo *et al*. (2014) reported that selection enhanced ZXT, βCX, βC, PVA and TC but only increased GY in one of the OPVs but decreased it ($P < 0.01$) in two others. They assumed that the reason for this negative effect could be as a result of linkage drag accompanying PVA-enhancing genes of exotic donor maize ILs. In a contrary result, Azmach *et al*. (2021) reported a strong negative relationship between GY and PVA concentration in most tropical maize ILs and hybrids studied. Similarly, Ortis-Covarrubias *et al*., (2019) described significant negative association between GY and PVA carotenoids in 55 hybrids derived from 11-line diallel crosses. Assessment of correlated effect between GY and PVA carotenoids in maize synthetics, selection cycles and their crosses will guide silmutaneous selection of these traits for optimizing heterosis.

2.27 Effects of drought stress on grain yield and agronomic traits of maize

Drought stress has been defined as a period when there is soil moisture deficit occasioned by insufficient rainfall and incessant loss of soil water due to evaporation, transpiration and evapotranspiration (Iseghohi *et al*., 2021). Most times in practice, drought stress and water deficit are used interchangeably. The terms are meteorological and relate to availability of soil moisture to plants. It is often known as ‘soil moisture stresses’. Soil moisture stress varies depending on the soil properties such as soil moisture holding and releasing capacity, the textural class ratio of clay, loam, sand and silt (Ismail, 1991). In a study of regional and global patterns in drought for a period of 1950 – 2000, Sheffied and Wood (2008) reported apparent regional variation in drought and a significant drying, particularly in West Africa occasioned by a continuous decline in Sahel precipitation.

Drought stress induces many physiological, biochemical and molecular processes in plants such as stomata closure and reduced transpiration rates, a decline in water potential and Relative Water Content, decrease in photosynthesis, decrease in photosynthetic assimilate formation and translocation, and ultimately, poor plant growth and reduced yield (Hasanuzzaman et *al*., 2019). There is no other stress that affects agricultural crops more than drought, which is becoming increasingly severe in different regions (Aslam *et al*., 2015). Statistical data showed that areas subjected to drought stress globally have doubled from 1970 to 2000 and its occurrence is predicted to increase (The Intergovernmental Panel on Climate Change IPCC, 2013). Drought often afflicts whole regions, creating regional food shortages. Its effects are particularly severe in southern and eastern Africa, the Sahel and Sudan savannas of West Africa where most maize production is rainfed. Rainfed maize covers 160 million hectares worldwide. Therefore, maize production for major regions of the world is subject to moisture stress arising from erratic climate conditions. Approximately 120 million tonnes of grain yield of maize are lost annually to drought in the world, representing 15% of well-watered yield potential (Edmeades, 2013).

The first plant organ to be exposed to soil moisture stress is the root and may lodge in severe water stress. Water stress can inhibit the growth of leaves because they are highly sensitive to it. It results in leaf rolling and wilting. Plants respond to drought stress mainly in three ways: (i) stress escape, (ii) stress avoidance and (iii) stress tolerance. The development of maize varieties that are tolerant and resistant to drought stress is one strategy to mitigate the adverse effect of drought. On a global scale, maize is grown across versed agro-climatic zones, therefore, drought tolerant maize is important in ensuring food security. The crop requires different amounts of water at different stages of growth, from germination to maturity (Ihsan *et al*., 2016). At early and terminal stages of growth, maize has a low water requirement, but at reproductive stages, it needs a lot of water. One week before and two weeks after flowering, the crop is particularly susceptible to drought stress; hence, drought stress at this stage will result in significant yield loss (Araus *et al*., 2012). At this stage, maize plants can continue to wilt and decrease yield by three to eight percent per day, while insufficient water can delay silking and causes emerged silks to be non-receptive to pollens (Hasanuzzaman et *al*., 2019). Grain filling drought stress can reduce GY by 2-6% per day and kernel abortion 2 weeks after pollination is common. Drought stress impacts

severely on maize during grain filling and dough formation while the stage before tasseling and physiological maturity are not as sensitive to drought stress as the two previous stages (Ahmed-Amal and Mekki, 2005).

Many reports have shown that the GY of maize is significantly reduced by drought stress (Meseka *et al*., 2015; Abdulmalik *et al*., 2017); however, there is limited report of the effects of drought stress on PVA-enriched maize (Ortiz-Covurrubias *et al*., 2019; Kondwakwenda *et al*., 2019). Manjeru, (2017) reported that 30 PVA-enriched hybrids responded differently in grain yield to drought stress in different environments. Therefore, development of varieties with appreciable yield and PVA under drought stress would be important for areas with recurrent drought stress.

2.27.1 Drought stress management

Technical management of drought stress under irrigation controlled practices in dry season field trials or simulated screenhouse experimental condition is the standard procedure for breeding for droughts tolerance in maize (Bänziger *et al*., 2000). Timing, intensity, and uniformity of the stress are factors to consider in stress management. Timing should be such that the growth stages targeted are susceptible to drought stress, for example, the anthesis-silking and the grain-filling phases (Bänziger *et al*., 2000). The intensity of drought stress should be severe enough so that traits become important for yield distinct from those which affect yield under non-stress conditions. Managed drought stress under irrigation, imposed at anthesis stage is designed to slow down silking and causes ear abortion. In general, a drought stress is considered intermediate when mean yield of the drought trial ranges between 40-50 percent of yield under optimal moisture, and severe when it goes down below 30 percent (Zaidi, 2019).

2.27.2 Managing irrigation schedule in drought stress trial

Efficient management of irrigation schedule is paramount for successful evaluations of genotypes under moisture stress and optimal soil water conditions. It is important to monitor the soil moisture level during drought stress. Tensiometer is usually used to monitor soil moisture content in drought stress trials (Hensley and Deputy, 1999). It measures soil water potential or tension equivalent to its moisture content, and readings are in centibars (cb) or *kilopascals (Kpa)*. The vacuum gauge dial measures from 0 to 100 cb, and the operational range of the instrument is between 0 and 85 cb. A reading of zero indicates a saturated soil while a reading of 10 to 25 centibars reflects a

soil at field capacity. The decision to irrigate is made when the average tensiometer reading exceeds a given critical value. The specific critical value depends on soil and crop type (Hensley and Deputy, 1999). For sandy loam, the field capacity for maize is 2.5 inches/foot of soil (i.e.10 to 20 cb) and the recommended soil moisture tension for maize is 45-60 centibar (Hanson *et al*., 2000). Another method to ascertain the threshold for irrigation is when the available soil moisture is depleted to an allowable value, called the allowable depletion (Doorenbos and Pruitt, 1977). Recommended allowable depletions are expressed as a percentage of the available water. For most crops, an allowable depletion of 50% is used.

Various methods are available to determine the day of last irrigation for imposing drought stress at targeted crop growth stage, for example, in case of flowering stage drought stress in maize, depends upon soil type. Irrigation should be stopped about two weeks before anthesis in a medium texture soils (Bänziger *et al*., 2000). Uniformity of last irrigation before imposition of drought stress is critical for uniform moisture regime across field, and therefore development of uniform drought stress. The best option for achieving this uniformity is to use drip irrigation system until full saturation. The second best option is sprinkler irrigation system, applied in two installments: first round of 3-4 hours and after a gap of few hours second round until full saturation (Zaidi, 2019).

2.27.3 Drought-related traits and selection indices

In considering drought-related traits for breeding drought tolerant maize genotypes, Araus *et al*. (2012) outlined the following criteria that a secondary trait should meet: (i) There should be a genetic correlation between the trait and GY under the test condition (ii) it should be less influenced by environment than grain yield is affected (iii) genetic variability must exist within the genotype for the trait (iv) the trait should be correlated with high yield under optimum condition and (v) it must be easy to measure.

When maize is exposed to stress at flowering there is an increase in the interval between pollen shedding and silk emergence. This has commonly been referred to as silk delay, loss of synchrony, or anthesis-silking interval (ASI) (Bänziger *et al*., 2000). Days to anthesis is little affected by drought stress but its effect on days to silking mostly results in slow extrusion of silk thereby resulting in long ASI (Araus *et al* 2012). Plants with a long ASI under moisture stress condition are usually infertile, having scanty kernels per ear. Anthesis-silking interval is one of the few examples of

secondary traits widely used for maize selection under drought. The use of ASI directly in selection has resulted in increased yield and reduced infertility under stress. Anthesis-silking interval is an excellent secondary trait since it exhibits a significant negative correlation with grain yield and relatively high heritability (Araus *et al*., 2012).

Delayed senescence commonly known as stay-green characteristics is often scored on a scale of 1-9 in drought experiments. After maize ears have been formed under terminal moisture stress, stay green has been linked with plant ability to redirect reservered carbohydrates in stems and husks for increased yield (Araus *et al*., 2012). There is usually a feeble relationship between leafy stay-green and yield (Bolaños and Edmeades, 1996), reason being that nitrogen uptake and use efficiency are low under drought stress (Chapman and Edmeades, 1999). Therefore, Stay-green may be a consequence of a plant being able to keep better water or nitrogen status rather than a primary factor in itself (Araus *et al*., 2012).

The number of grains per plant in maize under moisture stress is dependent largely on the rate of flow of photosynthates within the two weeks of anthesis and silking (Schussler and Westgate, 1995). It seems that stored photosynthates produced prio to anthesis are not translocated to the ears, thereby impairing sink strength (Westgate, 1997). Nevertheless, kernels improve the sink strength required to redirect carbon flow once they enter the stage of building up biomass. This process, alongside continuous photoassimilate translocation, defines the final weight and yield of kernel (Arau *et al*., 2012).

Drought indices which provide a measure of drought based on loss of yield under drought conditions in comparison to normal conditions have been used for screening drought tolerant genotypes (Mitra, 2001). These indices are either based on drought resistance or susceptibility of genotypes (Fernandez, 1992). Drought resistance is defined by Hall (1993) as the relative yield of a genotype compared to other genotypes subjected to the same drought stress. Drought susceptibility of a genotype is often measured as a function of the reduction in yield under drought stress (Blum, 1985). Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the differences in yield between the stress (Ys) and non-stress (Yn) environments. Fernandez (1992) defined a new advanced index, the stress tolerance index (STI), which can be used to identify

genotypes that produce high yield under both moisture-stress and optimum conditions. The yield stability index (YSI) suggested by Bouslama and Schapaugh (1984) is a yield-based estimate which evaluates the stability of genotypes in both stress and non-stress conditions. Different Scientists have made diverse comparisons among the drought indices (Fernandez, 1992; Richard, 1996) and assessed their genetic parameters (Golabadi *et al.*, 2006). For drought resistant coefficient (DRC), STI and YSI, higher values indicate genotypes tolerant to drought or stable in the case of YSI in diverse environments, and these indices generally show greater efficiency in identifying superior genotypes in both environments (Santos *et al*., 2020). However, higher values of stress susceptibility indices (SSI) signify susceptibility while low values indicate resistance.

CHAPTER 3

MATERIALS AND METHODS

3.1 Description of Locations of experimental sites

The experiments were conducted in four experimental stations of the International Institute of Tropical Agriculture (IITA), namely: Ikenne (6°54' N, 3°42' E), Ogun State; Mokwa (9°18' N, 5°4' E), Niger State; Saminaka (10°34' N, 8°39' E), Kaduna State, and Zaria (11°8' N, 7°45' E), Kaduna State (Figure 3.1).

Ikenne lies 60 m above the sea level (asl) and it is located in the tropical rainforest region of Nigeria. It has an average annual temperature range of 17 to 36°C and average rainfall of 1636 mm per annum (Table 3.1). The soil types in Ikenne according to FAO classification are Luvisols, Acrisols, Ferrasols and Lithosols (Mbagwu, 1985; Ogunkunle, 1998). Mokwa is located in the southern Guinea savanna of Nigeria. It is 457 m asl with annual temperature range of 14 to 45°C and average rainfall of 1002 mm per annum. The soil type is Luvisol. Saminaka is a major agricultural town in Kaduna state known for growing different crops especially maize. It lies 760 m asl and located in the northern Guinea ecology of Nigeria. It has an annual temperature range of 10 to 44°C and average annual rainfall of 838 mm. The soil type is Luvisols. Zaria lies 622 m asl and it is located in Sudan savanna agro-ecological zone of Nigeria. It has an average annual temperature range of 9 to 44°C and average rainfall of 782 mm per annum. The soil type in Zaria is Luvisols.

The fields were previously sown to maize in the last three cropping seasons. Soil samples were randomly augered to 30 cm depth at each site for the analyses of the physical and chemical properties of the soil prior to land preparations. The fields were cleared, ploughed and harrowed.

3.2 Genetic materials

Genetic materials (Table 3.2) were obtained from the Maize Improvement Programme (MIP) of the International Institute of Tropical Agriculture (IITA). The materials comprised three selection cycles (SC) of two maize synthetics [PVASYNHGA (HGA)

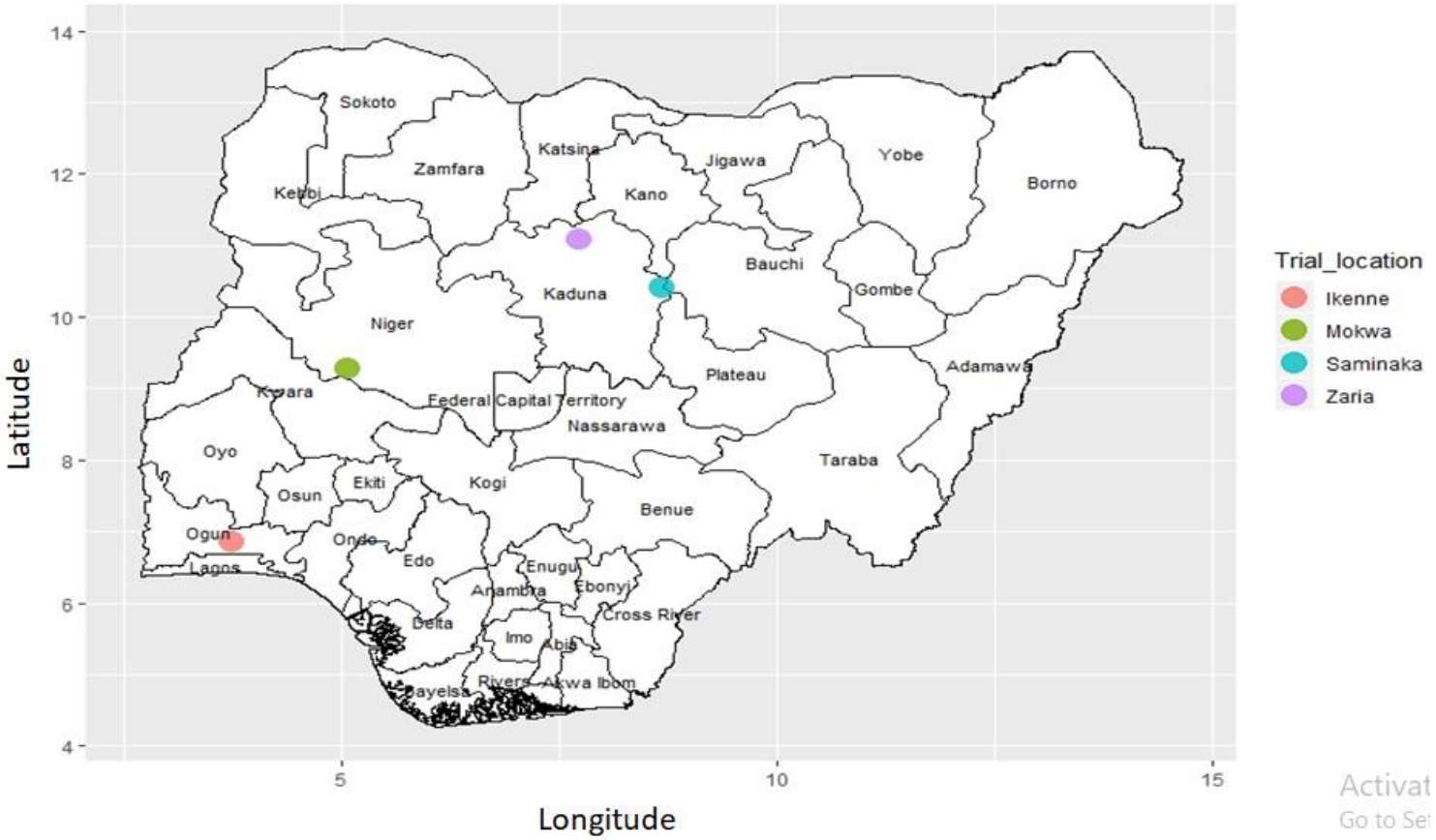


Figure 3.1. Locations of sites for the evaluation of sixteen provitamin-A maize genotypes in Nigeria

Table 3.1. Weather data and soil type of the study sites

Location	Agroecology	Altitude (M' asl)	Average Rainfall/annum (mm)	Average Temperature/annum (°C)		Soil Type (FAO Classification)
				Minimum	Maximum	
Ikenne	Rainforest	60	1636	17	36	Luvisols, Acrisols, Ferrasols and Lithosols
Mokwa	Southern Guinea savanna	457	1002	14	45	Luvisols
Saminaka	Northern Guinea savanna	760	838	10	44	Luvisols
Zaria	Sudan savanna	622	782	9	44	Luvisols

M′ asl = Meters above sea level

Table 3.2 Sixteen provitamin-A maize genotypes used in the study

S/N	Pedigree
	Selection cycles of maize synthetics
1	PVASYNHGAC$_0$
2	PVASYNHGAC$_1$
3	PVASYNHGAC$_2$
4	PVASYNHGBC$_0$
5	PVASYNHGBC$_1$
6	PVASYNHGBC$_2$
	Varietal-cross hybrids
7	PVASYN HGBC$_0$/PVASYN HGAC$_0$
8	PVASYN HGBC$_1$/PVASYN HGAC$_0$
9	PVASYN HGBC$_2$/PVASYN HGAC$_0$
10	PVASYN HGBC$_0$/PVASYN HGAC$_1$
11	PVASYN HGBC$_1$/PVASYN HGAC$_1$
12	PVASYN HGBC$_2$/PVASYN HGAC$_1$
13	PVASYN HGBC$_0$/PVASYN HGAC$_2$
14	PVASYN HGBC$_1$/PVASYN HGAC$_2$
15	PVASYN HGBC$_2$/PVASYN HGAC$_2$
16	PVASYN13 (Check)

and PVASYNHGB (HGB)] and a released check (PVASYN13). The two maize synthetics (MS) were each developed from eight elite maize inbred lines (ILs) with intermediate to high provitamin-A content and were then independently subjected to two cycles of MARS at IITA. Breifly, three gene specific markers (*crtRB1-5'TE*, *LycE-3'Indel*, *LycE-SNP-216*) were initially used for cycle selection based on PCR and gel electrophoresis (Gebremeskel *et al*., 2018). Subsequently, seven KASP-SNP assays (appendix 1) linked to *crtRB1* gene were used for high throughput genotyping at Intertek for verification. A total of 167 KASP-SNPs (14–23 per chromosome) were then used to assess genetic diversity and changes in allele frequencies caused by MARS after two cycles of selection (Astatke, 2018). In the present study, cycle 0, 1 and 2 of PVASYNHGA were crossed with cycle 0, 1 and 2 of PVASYHGB using 3 × 3 diallel without recirocal (Hallauer *et al*., 2010) to generate nine varietal-cross hybrids (VH). The check variety (PVASYN13) was developed by IITA in collaboration with IAR and released by National Agricultural Seeds Council of Nigeria (NASC) in 2017 as SAMMAZ 52 with national code NGZM-17-133 (NASC, 2017). It has an Intermediate level of PVA content of 9.8 µg/g and grain yield of 6.0 t/ha.

3.3 Experimental design and procedures for the evaluation of genetic materials

The experimental materials (Table 3.2) also known as sixteen genotypes of PVA-enriched maize in this study were evaluated for agronomic performance in the 2018 and 2019 rainy seasons at four locations, namely: Ikenne, Ogun State; Mokwa, Niger State; Saminaka and Zaria, Kaduna State. EXCEL Fieldbook software (CIMMYT, 1999) developed by CIMMYT was used to randomise the entries. The trials were arranged in a 4 × 4 lattice design with four replicates (Figure 3.2). Plots consisted of four rows of 5 m long with inter- and intra- row spacing of 0.75 m and 0.5 m, respectively. Plot size was 5 × 3 m (15 m^2). Each field trial comprised 64 plots, giving a total field area of 960 m^2. Three seeds were sown per hill and later thinned to two stands per hill to give 88 stands/plot and a plant population of 53,333 plants/ha. Fertilizer application was based on recommendation following soil test (Chude *et al*., 2012). Fertilizer in the form of NPK 15:15:15 was applied at the time of sowing at the rate of 400 kg/ha to supply 60 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, and 60 kg K_2O ha^{-1}. This was top-dressed with 60 kg N ha^{-1} using urea four weeks after planting. Weeds were managed with the application of 500 g/L of atrazine and 200 g/L of paraquat as pre- and post-emergence herbicides, respectively which was complemented with hand

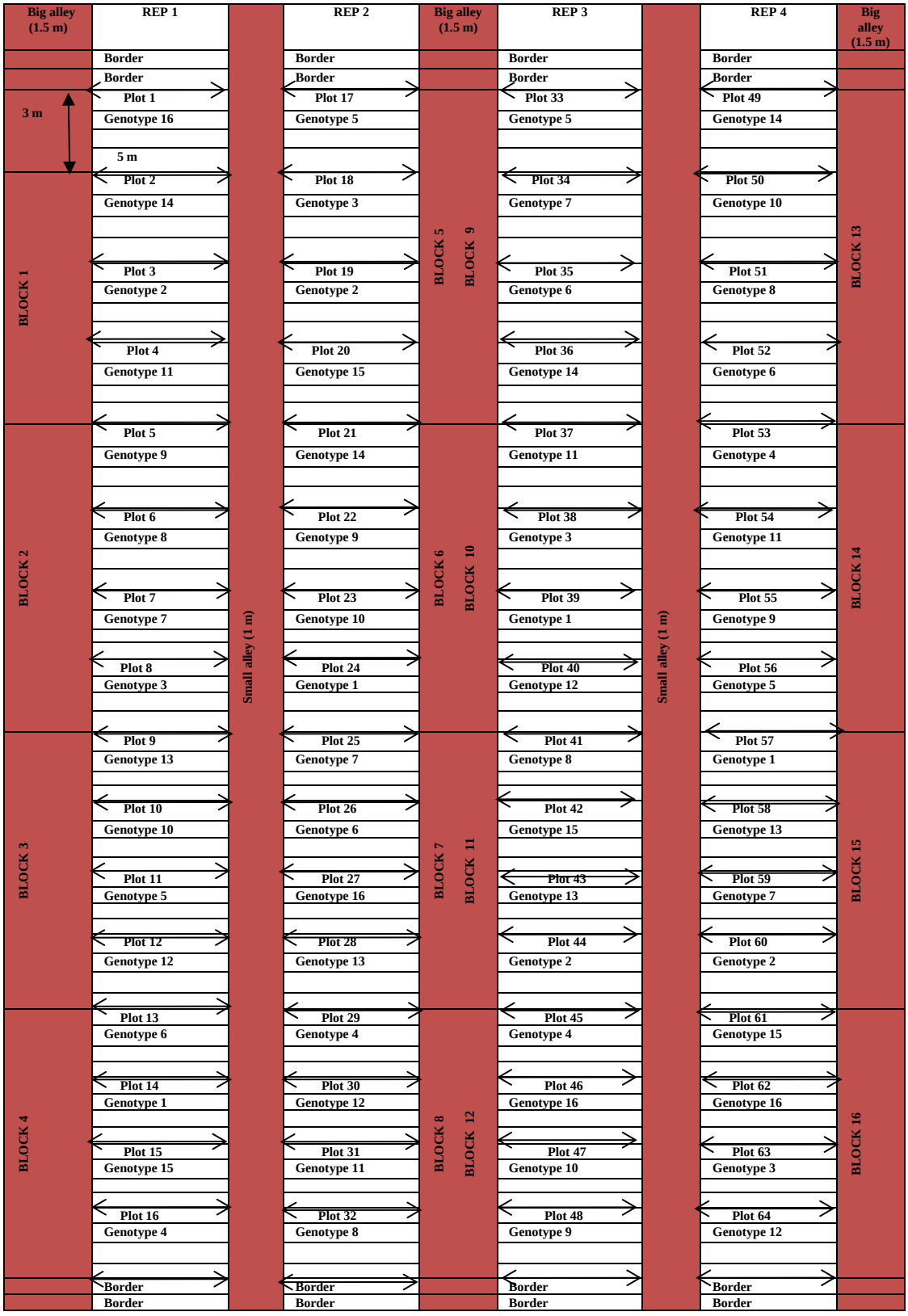


Figure 3.2. Field layout showing a 4 × 4 lattice design with four replicates used in evaluating sixteen provitamin-A maize genotypes across four locations in Nigeria

weeding two weeks after (2 WAP) and (4 WAP) to keep the weeds below economic level. Agronomic and yield data were recorded from the two middle rows while the two outer rows were self-pollinated for carotenoid analysis.

3.4 Agronomic and yield data collection

Days to anthesis (DA) and days to silking (DS) were recorded as number of days from planting to when 50% of the plants in a plot shed pollen and had emerged silks, respectively. Anthesis-silking interval (ASI) was calculated as the difference between DS and DA. Plant height (PH) and ear height (EH) were measured in cm as the distance from the base of the plant to first tassel branch and the node bearing the upper ear, respectively. Plant aspect (PA) was scored on a 1 to 5 scale (Table 3.3) as described by Badu-Apraku *et al*. (2012); where 1 represented uniform, clean, vigorous and good overall phenotypic appeal, while 5 represented weak, diseased and poor overall phenotypic appeal. All ears in the two rows were harvested to determine grain yield per plot. Ear aspect (EA) was scored on a 1 to 5 scale (Table 3.3), where 1 represented clean, well filled, uniform and large ears, while 5 represented diseased, poorly filled, variable and small ears. Ears harvested were shelled and grain moisture content of shelled grains was determined using a portable Dickey-John moisture tester. The grain weight and moisture content were used to compute grain yield adjusted to 15% moisture as follows:

$$\text{Grain yield (t ha}^{-1}) = \frac{\text{GWT x (100 – moisture content \%)}}{85} \times \frac{1000}{\text{plot size}}$$

Where:

GWT is the grain weight (kg/plot).

Weather data comprising rainfall, temperature, relative humidity and sunshine were recorded throughout the growing seasons (Appendices 2 and 3).

3.5 Evaluation of genetic materials under managed-drought stress conditions

The genetic materials were evaluated at Ikenne, Ogun State, under managed-drought stress (MDS) and well-watered condition (WWC) in two dry seasons (December to March of 2018/2019 and 2019/2020). Experimental design and procedures followed the pattern described in section 3.3, except that each plot was a two-row plot.

Table 3.3: Rating scale for plant and ear aspects of maize

Score	Plant aspect	Ear aspect
1	Clean, even, vigorous and excellent overall appeal of the whole plants per plot	Excellent overall phenotypic appeal of ears per plot: Large and uniform ears, fully filled grains, disease and insect damage free with uniformity in grain colour
2	Uniform, clean, vigorous and very good overall appeal of the whole plants per plot	Large ears with 1 to 2 variabilities in cob size, insect and disease damage free and fully filled grains
3	Slightly uniform with mild disease symptoms and good overall phenotypic appeal of some plants per plot	Ears with mild insect damage, 1 to 2 variabilities in cob size, disease free and fully filled grains
4	Variable, mild disease symptoms and poor overall phenotypic appeal plants per plot	Serious ears damage caused by insects and diseases, few cobs and grains/cob, and non-uniform cobs
5	Variable, weak, diseased and very poor overall phenotypic plants per plot	Diseased, poorly filled, variable and small ears

Adapted from Badu-Apraku *et al*. (2012)

In each year, the genotypes were planted in two blocks, with one block well-watered, while the other was subjected to MDS. The two blocks were separated by a distance of 20 m to avoid underground seepage and lateral movement of water from the WW block to the MDS block. The WW block received full irrigation every week using sprinkler irrigation system from planting till physiological maturity. The irrigation system dispensed 17 mm water week^{-1}. In the manage drought plots, irrigation was withdrawn five weeks after planting to impose drought stress two weeks before flowering until harvesting. Soil moisture status was monitored by installing two tensiometers per replicate at 30 cm depth in the drought trial, while two were installed in well-watered trial as control.

3.6 Data collection under drought trial

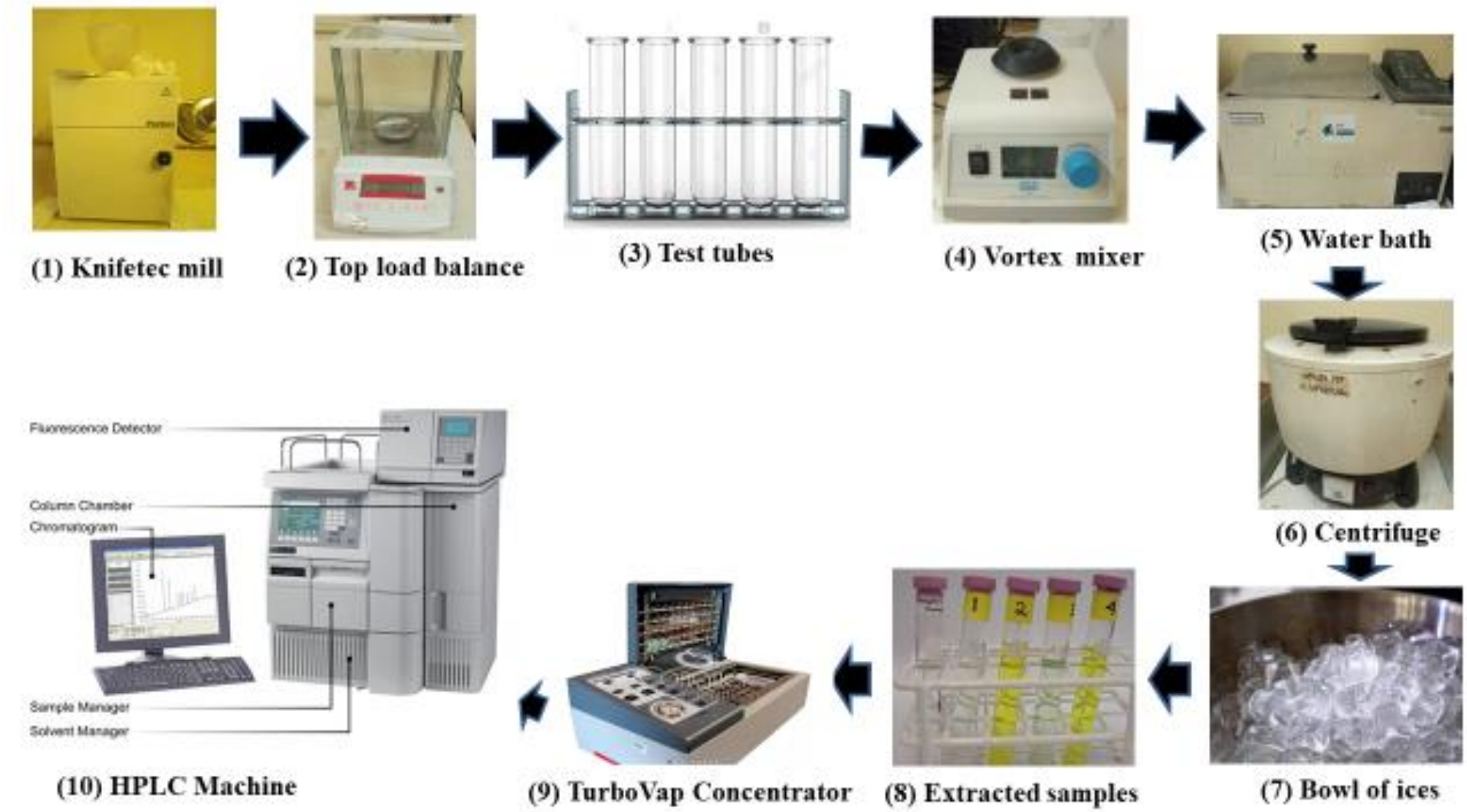
In the drought trials, the procedures for the measurement of agronomic and yield traits as explained in section 3.4 were used. In addition, tensiometer readings were recorded twice a week until drought stress was terminated.

3.7 Carotenoid extraction and analysis

At each location and year, self-pollinated plants from each plot were harvested separately and a minimum of ten clean ears were selected, air-dried under ambient temperatures, hand shelled and the grains bulked. Samples drawn from the bulk grain were analysed for carotenoid content at the Food and Nutrition Laboratory of IITA using high-performance liquid chromatography (HPLC) machine (Water Corporation, Milford, Massachuset, USA). The extraction followed a modified procedure described by Howe and Tanumihardjo (2006) and Menkir *et al*. (2015) as described below.

The following materials were used: 50 mL glass centrifuge tubes, screw caps, weighing balance, vortex mixer, water bath, centrifuge, concentrator tubes, TurboVap nitrogen gas concentrator and High Performance Liquid Chromatography (HPLC) (Plate 3.1)

The solvents and reagents included 0.01% butylated hydroxyl toluene (BHT) ethanol, 80% potassium hydroxide (KOH), Hexane, cold water, β-Apo 8'-carotenal, 50:50 dichloromethane: Methanol, 100% Methyl Tertiary Butyl Ether (MTBE) and HPLC grade Methanol: water (92%: 8%).



HPLC: High Performance Liquid Chromatography

Plate 3.1. Schematic representation of the extraction and quantification processes of maize carotenoids at the Food and Nutrition laboratory of the International Institute of Tropical Agriculture (IITA)

Ten grams of grain sample was drawn for each entry from the first two replicates and ground using a knifetec 1095 small mill (FOSS, Eden Prairie, MN, USA). A 0.6 g of each ground sample was weighed and transferred into a 50 mL glass centrifuge tube. A 6 mL of ethanol containing 0.01% butyl hydroxyl toluene was added and vortex at 1000 revolution per minute (rpm) for 15 seconds. Samples were incubated in water-bath at 85ºC for 5 minutes. Samples were taken out of water bath and 0.5 mL of 80% KOH was added to each. Samples were vortex at 1000 rpm for 15 seconds. Samples were placed in water bath at 85ºC for 5 minutes**.** Vortexing and incubation in water bath at 85ºC for 5 minutes was repeated thrice. Samples were taken out of water bath, put on ice and 3 mL of cold water was added. To each sample, 200 µL of internal standard β-Apo 8'-carotenal and 3 mL of hexane were added. Samples were vortex for 10 seconds and centrifuged at 1000 rpm for 10 seconds. The upper phase of the solvent was pipetted into 50 mL concentrator tube for each sample. Vortexing and pipetting were repeated three times to ensure adequate quantity of carotenoids is extracted. Extracts were dried under nitrogen gas at 40°C for 25 minutes using a TurboVap concentrator (Caliper LifeSciences, Hopkinton, Massachusetts, USA). Samples were reconstituted in 1 mL of 50:50 dichloromethane: Methanol and vortex for 10 seconds. Samples were transferred to HPLC vials, placed in auto-sampler tray and slid into the HPLC machine.

For each sample, 50 µL aliquots were injected into the HPLC system and run for major carotenoids based on the calibration of the standard of each carotenoid. Carotenoids were separated by C_{30} Column (4.6 × 250 mm; 3 µm) eluted by a mobile phase using methanol/water (92: 8 v/v) as solvent A and 100% MTBE as solvent B. The flow rate of solvent was 1 mL/min. and absorbance was measured at 450 nm for carotenoid detection.

Chromatograms (Appendices 4 and 5) were extracted after the runs. Major carotenoids were identified and each carotenoid was calculated following the procedure of Galicia *et al*. (2012) as:

$$C_X\ (\mathrm{ug/g}) = \frac{\mathrm{A_X} \times \mathrm{C_s\ (ug/mL)} \times \text{total volume of extact (mL)}}{\mathrm{A_s} \times \text{sample weight (g)}}$$

Where:

C_X and $\mathrm{A_X}$ were concentration and peak area of carotenoid X,

C_s and A_s were the concentration and peak area of the standard.

Total carotenoid (TC) was computed as the sum of concentrations of LUT, ZXT, βCX, αC and βC (13-*cis*, *trans* and 9-*cis* isomers) while PVA content was calculated as the sum of βC and half of βCX and αC.

3.8 Data analyses

Combined analysis of variance (ANOVA) was done using PROC MIXED procedure in SAS version 9.4 (SAS Institute, 2012). Entry was considered as fixed effect, whereas year, location, year × location and their interactions with entries were regarded as random effects. The significances of entry and interaction effects were tested using the appropriate mean squares. Spearman's rank correlation coefficients between pairs of environment means were calculated for traits with significant location × entry interaction to determine the consistency of ranking of the entries across locations (Menkir *et al*., 2008). Mean separations were done using Least Significant Difference (LSD) at 0.05 level of probability. For each trait, cycle means of parental synthetics were regressed on cycles of selection (C_0 – C_2) using PROC REG in SAS 9.4 (SAS Institute, 2012). Genetic gain per cycle was calculated as the regression coefficient (*b*) divided by the corresponding cycle zero (C_0) mean (Menkir and Kling, 2007). Total gain (TG) at final cycle of selection was estimated as:

$$\mathrm{TG} = \frac{C_2 - C_0}{C_0} \times 100$$

Where:

C_0 and C_2 are the mean performances of the initial and final cycles under consideration.

Proportions of each carotenoid in the parents and varietal-cross hybrids were calculated as:

$$\frac{\text{Mean of a carotenoid}}{\text{mean of total carotenoid}} \times 100$$

After the removal of the check variety and the selection cycles (the parental genotypes) from the entry list, each location-year combination was considered an environment and

combined ANOVA based on North Carolina design II was carried out using a modification of DIALLEL-SAS program developed by Zhang *et al*., (2005). The analysis was based on the model:

$$Y_{ijk} = \mu + E_d + REP_k(E_d) + \text{BLK}\,(REP_k) + g_i + g_l + s_{ij} + E_d \times g_i + E_d \times g_j + E_d \times g_{ij} + e_{ijk}$$

Where:

Y_{ijk} is the observed mean performance of the cross between i^{th} and j^{th} parents in the k^{th} replication

μ is the overall mean,

E_d is the environmental effect

REP_k (E_d) and $\text{BLK}(\text{REP}_k)$ are the replicate and block effects nested in environment

g_i is the HGA cycle GCA effect

g_j is the HGB cycle GCA effect

s_{ij} is the SCA i.e HGA × HGB effect

$E_d \times g_i$, $E_d \times g_j$, and $E_d \times g_{ij}$ are the environmental interaction with HGA and HGB cycle GCA and SCA effects, respectively

e_{ijk} is the random error.

Entries were considered as fixed effects, while environment and effects nested within it were regarded as random effects. Significant mean square of each main effect was tested using its respective interaction with the environment; whereas HGA × HGB × environment mean square was tested using the pooled error mean square. The GCA and SCA effects of the parental synthetics as well as the variance components for each trait were calculated with Analysis of Genetic Design (AGD-R, V.5.0). Variance components were based on Restricted Maximum Likelihood Method (REML) in AGD-R (Rodriguez *et al*., 2018). The relative importance of GCA and SCA also known as predictability ratio was calculated following the procedure of Baker (1978) as:

$$\frac{2\sigma^2\text{GCA}}{2\sigma^2\text{GCA} + \sigma^2\text{SCA}}$$

Where:

σ^2GCA and σ^2SCA are variances due to GCA and SCA, respectively.

Repeatability values and narrow sense heritability for each trait were calculated in AGD-R (Rodriguez *et al*., 2018). Mid-parent, better-parent and standard heterosis were calculated according to the formulae of Falconer and Mackay (1996) and Hallauer *et al*. (2010) as:

$$\text{MPH} = \frac{\text{F1}-\text{MP}}{\text{MP}} \times 100, \qquad \text{BPH} = \frac{\text{F1}-\text{BP}}{\text{BP}} \times 100, \qquad \text{STH} = \frac{\text{F1}-\text{SC}}{\text{SC}} \times 100$$

Significances of heterosis were tested with t-statistic as:

$$\text{Tmp} = \frac{\text{F1}-\text{MP}}{\sqrt{\frac{3\ \text{EMS}}{2\text{r}}}}, \qquad \text{Tbp} = \frac{\text{F1}-\text{BP}}{\sqrt{\frac{2\ \text{EMS}}{\text{r}}}}, \qquad \text{Tsc} = \frac{\text{F1}-\text{SC}}{\sqrt{\frac{2\ \text{EMS}}{\text{r}}}}$$

Where:

MPH, BPH and STH are mid-parent, better-parent and standard heterosis, respectively. F_1, MP, BP and SC are the means of hybrids, mid-parents, better parents and standard check variety, respectively.

Tmp, Tbp and Tsc are the calculated t of MP and BP and SC, respectively

EMS is the error mean square and

r is the number of replicates.

Pearson correlation coefficicient (r) was calculated amongst F_1, MP, MPH and SCA and also among agronomic traits and carotenoids using PROC CORR in SAS.

For drought stress trial, Drought Tolerance Index (DTI) and Yield Stability Index (YSI) were calculated based on the formulae of Fernandez (1992) and Bouslama and Schapaugh (1984) as:

$$\text{DTI} = \frac{\text{GYi(n)} \times \text{GYi(s)}}{\text{GY}^2}, \quad \text{YSI} = \frac{GYi(s)}{GYi(n)}$$

Where:

GY*i*(n) and GY*i*(s) are grain yields of genotype *i* under well-watered and drought stress conditions, respectively. GY is the average of grain yield of all genotypes under well-watered condition.

Drought Tolerance Index (DTI) of 0 – 0.49 = low, 0.50 – 0.69 = moderate and 0.70 – 1.0 = high (Fernandez 1992).

Percentage (%) yield reduction under MDS was estimated as:

$$\frac{Grain\ yield\ under\ well\ watered\ condition - Grain\ yield\ under\ managed\ drought\ stress}{Grain\ yield\ under\ well\ watered\ condition} \times 100$$

Analysis of variance was performed for drought stress, well-watered conditions, as well as combined water regimes following the procedure described above. Each year-water condition was considered an environment, and another ANOVA was performed for the combined environments. Genotype (G) main effect and genotype × environment (E) interaction (GGE) biplot was used to determine the performance and stability of genotypes across the combined drought and well-watered conditions (Yan, 2001, Badu-Apraku *et al*., 2019).

CHAPTER 4
RESULTS

4.1 The physical and chemical properties of soil of the study sites

The soil in the four study sites was slightly acidic with low organic carbon and total nitrogen contents (Table 4.1). Available phosphorus was high in Ikenne, Mokwa and Saminaka, but moderate in Zaria. Exchangeable cations (potassium, magnesium, calcium and sodium) were highest in Ikenne, but moderate in Saminaka and Zaria, except sodium which was below critical level in those two sites. Exchangeable cations were generally low in Mokwa. Based on the United States Department of Agriculture (USDA) Classification, the textural class of soil in Ikenne and Zaria were loamy sand and loam, respectively; whereas, Mokwa and Saminaka were both sandy loam (Table 4.1).

4.2 Variability for agronomic traits and carotenoids among PVA maize genotypes

In the combined analysis, genotype, year, location and year × location had significant effects on most or all measured agronomic traits and carotenoids (Tables 4.2 and 4.3). Genotype × location effect was significant on days to Flowering (DA), Days to Silking (DS) Plant Aspect (PA) and Ear Aspect (EA). Similarly, genotype × year, genotype × location had significant effects on PVA and βC, while genotype × year × location effect was significant on PVA (Table 4.3). Making each year-location as environment, Spearman's rank correlation coefficient (r) for a pair of environment ranged from 0.33 to 0.84 ($p \leq 0.22$ to $p \leq 0.001$) for βC and 0.32 to 0.76 ($p \leq 0.23$ to $p \leq 0.001$) for PVA (Table 4.4).

Partitioning genotypic source of variation into GCA and SCA components, the GCA of PVASYNHGA (GCA-HGA) cycles was significant for GY, DA and DS, while the GCA of PVASYNHGB (GCA-HGB) cycles and the hybrid effects were significant for GY and most agronomic traits, except PH (Table 4.5). The SCA effect was significant for GY and most agronomic traits, except ASI and PH (Table 4.5). On the contrary, the interactions of the environment effect with GCA-HGA cycles, GCA-HGB cycles, SCA and hybrid effects were not significant, except for DA and PH. Although the effects of GCA-HGA cycles, GCA-HGB cycles and hybrids were significant for almost or all the carotenoids, the SCA had no significant effects (Table 4.6).

Table 4.1. The Physical and chemical properties of soils (0 – 30 cm) of the study sites

Soil Properties	Ikenne	Mokwa	Saminaka	Zaria	Critical level
		Value			Chude *et al*. (2012)
pH (1:1, H_2O)	4.8	5.7	5.2	5.1	Neutral 6.6-7.2
Organic carbon (g/kg)	9.4	7.2	7.5	9.1	10-14
Total nitrogen (g/kg)	0.9	0.8	1.0	1.0	1.6-2.0
Available P (mg/kg)	21.5	31.5	89.5	16.4	7.2
Exchangeable acidity	1.0	0.9	0.9	2.0	
Exchangeable cations (cmol/kg)					
K	1.2	0.1	0.3	0.3	
Mg	1.2	0.1	0.3	0.9	0.3-0.6
Ca	2.5	0.1	0.3	0.4	
Na	0.6	0.1	0.2	0.2	
Extractable micronutrients (mg/kg)					
Fe	19.3	172.0	180.0	126.0	
Mn	55.6	121.0	17.2	29.1	
Cu	2.9	1.0	1.4	1.4	
Zn	2.2	1.8	7.3	2.2	
Particle size distribution (g/kg)					
Sand	811.0	740.0	740.0	460.0	
Silt	87.0	174.0	194.0	414.0	
Clay	102.0	86.0	66.0	126.0	
Textural class (USDA)	Loamy Sand	Sandy Loam	Sandy Loam	Loam	

Table 4.2. Mean squares for grain yield and agronomic traits of sixteen provitamin-A maize genotypes evaluated across four locations in 2018 and 2019 seasons in Nigeria

Source	DF	Grain yield	Days to anthesis	Days to silking	Anthesis-silking interval	Plant height	Ear height	Plant aspect	Ear aspect
Year (Y)	1	14.85***	217.88***	371.28***	20.32***	23476.74***	2346.13***	4.31***	0.26
Location (L)	3	177.83***	1838.40***	1919.91***	12.05***	6991.87***	8118.06***	2.54***	2.71***
Y×L	3	13.60***	154.59***	134.91***	6.64***	9209.32***	8937.61***	0.55*	3.37***
Rep (Y×L)	24	1.84***	3.68***	4.18***	0.39	1222.50***	666.37***	0.31**	0.34***
Block(Y×L×Rep)	96	0.71**	2.21*	2.46*	0.29	266.68*	296.99***	0.19	0.17
Genotype	15	10.45***	16.21***	19.72***	0.76*	559.70***	207.23	0.99***	1.52***
Genotype × Y	15	0.51	0.91	1.32	0.26	306.49	114.65	0.25	0.18
Genotype × L	45	0.67	2.62*	2.64*	0.31	239.91	124.40	0.23*	0.22*
Genotype ×Y×L	45	0.43	2.33*	2.21	0.33	224.70	145.05	0.17	0.15
Error	264	0.48	1.64	1.71	0.44	193.71	178.45	0.15	0.14

*, **, ***: $p \leq 0.05$, 0.01 and 0.001

Table 4.3. Mean squares for provitamin-A and other carotenoids of sixteen PVA-enriched maize genotypes evaluated across four locations in 2018 and 2019 seasons in Nigeria

Source	DF	Lutein	Zeaxanthin	βcryptoxanthin	αcarotene	βcarotene	Provitamin-A	Total carotenoid
Year (Y)	1	963.33***	78.74***	80.99***	16.13***	698.61***	1084.76***	6297.71***
Location (L)	3	147.64***	51.93***	13.31***	0.75***	29.69***	57.95***	412.94***
Y×L	3	24.50***	16.35***	1.02**	0.09	7.23***	9.30***	101.98***
Rep (Y×L)	8	18.20***	6.82**	0.61**	0.15**	0.45	0.45	35.39***
Genotype	15	64.31***	29.90***	3.49***	0.20***	12.57***	9.99***	60.19***
Genotype × Y	15	2.93	2.29	0.44	0.07	3.06***	2.59***	9.15
Genotype × L	45	3.43	3.47	0.25	0.05	1.07*	1.12*	7.89
Genotype × Y× L	45	3.55	3.08	0.21	0.05	1.02	1.20*	11.10
Error	120	3.49	2.52	0.18	0.04	0.71	0.72	8.04

*, **, ***: $p \leq 0.05$, 0.01 and 0.001

Table 4.4. Spearman's rank correlation coefficient for βcarotene and provitamin-A content of sixteen maize genotypes evaluated across eight environments (E1-E8) in Nigeria

Env	E1	E2	E3	E4	E5	E6	E7	E8
				B-carotene				
E1	-							
E2	0.45	-						
E3	0.78***	0.52*	-					
E4	0.82***	0.55*	0.57*	-				
E5	0.60*	0.55*	0.67**	0.65**	-			
E6	0.47	0.67**	0.65**	0.33	0.55*	-		
E7	0.70**	0.58*	0.77***	0.74***	0.83***	0.69**	-	
E8	0.68**	0.58*	0.84***	0.67**	0.56*	0.57*	0.81***	-
				Provitamin A				
E1	-							
E2	0.53*	-						
E3	0.73**	0.50*	-					
E4	0.76***	0.43	0.49	-				
E5	0.50*	0.55*	0.39	0.63**	-			
E6	0.52*	0.66**	0.65**	0.32	0.31	-		
E7	0.61*	0.58*	0.74***	0.60*	0.55*	0.71**	-	
E8	0.66**	0.58*	0.64**	0.68**	0.40	0.56*	0.70**	-

*, **, ***: $p \leq 0.05$, 0.01 and 0.001

E1, E2, E3 and E4: Ikenne, Mokwa, Saminaka and Zaria 2018 rainy season
E5, E6, E7 and E8: Ikenne, Mokwa, Saminaka and Zaria 2019 rainy season

Table 4.5. Mean squares for grain yield and agronomic traits of three selection cycles of two maize synthetics and their hybrids evaluated across eight environments in Nigeria

Source	DF	Grain yield	Days to anthesis	Days to silking	Anthesis-silking interval	Plant height	Plant aspect	Ear aspect
Environment (Env)	7	47.65***	369.35***	374.30***	3.27***	4355.21***	1.18***	1.46***
Rep(Env)	24	1.38***	3.67***	4.15***	0.49	798.95***	0.27*	0.32**
Block(Env×Rep)	96	0.55	2.04ns	2.13**	0.31	279.67**	0.17	0.14
Hybrid	8	10.71***	12.51***	15.00***	0.92**	341.78	1.05***	1.45***
Env×Hybrid	56	0.31	1.96	1.93*	0.28	208.81	0.15	0.12
GCA-HGA cycles	2	9.88***	13.66**	8.94**	0.66	502.83	0.04	0.10
GCA-HGB cycles	2	0.80*	11.01**	23.48**	2.33**	249.21	0.86*	1.55***
SCA (HGB×HGA)	4	14.62***	14.50***	16.37***	0.30	269.45	1.67***	2.19***
Env × GCA-HGA cycles	14	0.35	1.95	1.33	0.19	358.58*	0.17	0.11
Env × GCA-HGB cycles	14	0.21	1.59	2.19*	0.22	105.03	0.18	0.04
Env×SCA (HGB×HGA)	28	0.38	2.15	2.00*	0.37	182.24	0.11	0.16
Error	96	0.49	1.47	1.21	0.46	167.35	0.16	0.14
Repeatability (H^2)		0.99	0.99	0.99	0.82	0.83	0.98	0.99
Narrow-sense heritability (h^2)		0.50	0.49	0.53	0.82	0.45	0.49	0.52

*, **, ***: $p \leq 0.05$, 0.01 and 0.001, GCA: General combining ability, SCA: Specific combining ability

Table 4.6. Mean squares for provitamin-A and other carotenoids of three selection cycles of two maize synthetics and their hybrids evaluated across eight environments in Nigeria

Source	DF	Lutein	Zeaxanthin	βcryptoxanthin	αcarotene	βcarotene	Provitamin-A	Total carotenoid
Environment (Env)	7	111.65***	28.01***	9.77***	1.50***	59.71***	96.06***	633.73***
Rep(Env)	8	13.26***	4.99*	0.33	0.09	0.53	0.45	27.88**
Hybrid	8	44.28***	9.67***	0.80**	0.04	11.54***	8.75***	43.82***
Env×hybrid	56	3.81	2.48	0.24	0.06	0.74	0.80	8.39
GCA-HGA cycles	2	57.15***	25.34**	1.60*	0.02	22.71***	16.50***	35.32*
GCA-HGB cycles	2	99.19***	6.95	1.04*	0.03	22.67***	17.74***	108.77**
SCA (HGB×HGA)	4	10.39	3.20	0.29	0.05	0.39	0.39	15.59
Env×GCA-HGA cycles	14	1.66	3.40	0.30	0.08	1.25	1.19	8.82
Env×GCA-HGB cycles	14	4.20	2.04	0.18	0.07	0.85	1.10	11.16
Env×SCA (HGB×HGA)	28	4.70	2.24	0.24	0.04	0.43	0.46	6.80
Error	64	2.87	2.22	0.17	0.06	0.70	0.81	8.68
Repeatability (H^2)		0.98	0.93	0.91	0.00	0.98	0.98	0.95
Narrow-sense heritability (h^2)		0.84	0.82	0.82	0.00	0.98	0.98	0.79

*, **, ***: $p \leq 0.05$, 0.01 and 0.001, GCA: General combining ability, SCA: Specific combining ability

The broad-sense heritability estimates also known as repeatability estimates were high for agronomic and carotenoid traits of the maize genotypes (Tables 4.5 and 4.6); whereas, narrow-sense heritability estimates (h^2) ranged from moderate to high (0.45 - 0.82) for most agronomic traits and high (0.79 - 0.98) for all carotenoids, except for αC which had $h^2 = 0$.

4.3 Mean performance for agronomic traits and carotenoids of PVA maize genotypes

The mean performance of the maize genotypes for GY and most agronomic traits were consistent in the two years of evaluation at almost all test locations except in Zaria where GY declined by 20% in 2019 (Table 4.7). There was also decrease in plant height in 2019 compared to 2018 in Mokwa and Zaria. Provitamin-A and all carotenoids measured decreased in 2019 in all test locations (Table 4.8). The highest average GY and PVA content in the two years of evaluation were recorded in Mokwa (6.35 t/ha, 8.7 µg/g), followed by Zaria (5.9 t/ha, 8.47 µg/g), Saminaka (5.6 t/ha, 7.2 µg/g) and Ikenne (3.7 t/ha, 6.8 µg/g), respectively (Tables 4.9 and 4.10). The DA and DS were shortest in Mokwa and longest in Zaria, whereas, PH was shortest at Ikenne (206.4 cm) and highest in Saminaka (222.5 cm). Average lowest PA and EA scores were recorded in Mokwa while the highest PA and EA scores were recorded in Zaria and Ikenne, respectively (Table 4.9).

All PVA carotenoids were lowest in Ikenne and highest in Mokwa (Table 4.10). In each location and across locations, αC was the lowest carotenoid in the grain of the PVA-enriched maize genotypes while LUT was the most abundant carotenoids (Table 4.10). Lutein accounted for 28% of the total carotenoid in the maize genotypes, followed by PVA (22%) and ZXT (22%). The proportion of βC, βCX and αC was 17%, 8% and 3%, respectively (Appendix 6).

4.4 Marker-assisted recurrent selection effect on agronomic traits and carotenoids of PVA maize synthetics

Marker-assisted recurrent selection (MARS) did not have significant effect on the GY of HGA cycles but significantly increased GY in HGB cycles (Table 4.11). The MARS increased GY by 13.3% from C_0 to C_1 and by 8.9% from C_0 to C_2 in HGB. However, the three selection cycles (SCs) of each maize synthetics (MS) produced significantly lesser GYs than the check (PVASYN13). There was no genetic gain per

Table 4.7. Effect of year on of grain yield and agronomic traits of sixteen PVA-enriched maize genotypes evaluated in four locations in 2018 and 2019 in Nigeria

TRAIT	IKENNE		MOKWA		SAMINAKA		ZARIA		Mean±SE
	2018	2019	2018	2019	2018	2019	2018	2019	
Grain yield (t/ha)	3.6	3.7	6.5	6.2	5.5	5.6	6.5	5.2	5.4±0.13
Days to anthesis	54.2	53.9	54.5	50.2	58.7	57.1	60.3	61.2	56.3±0.25
Days to silking	55.9	55.7	56.5	52.2	60.6	58.3	63.0	63.0	58.2±0.26
Anthesis-silking interval (days)	1.7	1.8	2.1	2.0	1.9	1.2	2.7	1.8	1.9±0.13
Plant height (cm)	220.2	192.6	224.3	203.1	217.0	228.0	215.6	199.3	212.5±2.77
Plant aspect (1-5)	2.9	2.9	3.0	2.8	2.7	2.5	3.1	2.8	2.8±0.08
Ear aspect (1-5)	3.0	2.5	2.6	2.7	2.3	2.5	2.6	2.5	2.6±0.07

SE: Standard error

Table 4.8. Effect of year on provitamin-A and other carotenoids of sixteen maize genotypes evaluated in four locations in 2018 and 2019 in Nigeria

TRAIT	IKENNE		MOKWA		SAMINAKA		ZARIA		Mean±SE
	2018	2019	2018	2019	2018	2019	2018	2019	
Lutein (µg/g)	10.9	7.8	9.7	7.0	13.8	9.4	13.8	8.5	10.1±0.43
Zeaxanthin (µg/g)	7.0	6.2	7.3	6.6	8.5	8.3	9.5	7.0	7.6±0.42
βcryptoxanthin (µg/g)	2.8	2.0	4.0	2.6	3.1	2.0	3.8	2.6	2.9±0.12
αcarotene (µg/g)	1.0	0.6	1.3	0.7	1.0	0.5	1.1	0.6	0.86±0.06
βcarotene (µg/g)	6.6	3.6	8.3	4.7	6.8	4.3	8.5	4.4	5.9±0.28
Provitamin-A (µg/g)	8.6	4.9	11.0	6.4	8.8	5.5	10.9	6.0	7.8±0.28
Total carotenoid (µg/g)	28.4	20.2	30.6	21.6	33.1	24.4	36.7	23.1	27.3±0.70

SE: Standard error

Table 4.9. Means and standard error of grain yield and agronomic traits of sixteen PVA-enriched maize genotypes evaluated across four locations in two years in Nigeria

Trait	Ikenne	Mokwa	Saminaka	Zaria	Mean±SE
Grain yield (t/ha)	3.65±0.3	6.35±0.4	5.54±0.2	5.84±0.3	5.35±0.13
Days to anthesis	54.05±04	52.34±0.6	57.91±0.4	60.74±0.6	56.26±0.25
Days to silking	55.80±0.4	54.35+0.8	59.45±0.4	62.98±0.8	58.15±0.26
Anthesis-silking interval	1.75±0.2	2.02±0.1	1.54±0.2	2.24±0.4	1.89±0.13
Plant height (cm)	206.39±5.9	213.68±5.4	222.50±8.0	207.48±5.8	212.51±2.77
Ear height (cm)	106.16±4.1	124.01±4.7	108.28±4.1	112.46±7.6	112.73±2.28
Plant aspect (1-5)	2.87±0.2	2.87±0.2	2.61±0.1	2.93±0.2	2.82±0.08
Ear aspect (1-5)	2.72±0.1	2.63±0.2	2.38±0.1	2.54±0.2	2.57±0.07

SE: Standard error

Table 4.10. Means and standard error of provitamin-A and other carotenoids of sixteen PVA-enriched maize genotypes evaluated across four locations in two years in Nigeria

Trait	Ikenne	Mokwa	Saminaka	Zaria	Mean±SE
Lutein (µg/g)	9.23±1.0	8.35±1.3	11.56±0.8	11.16±1.1	10.08±0.43
Zeaxanthin (µg/g)	6.62±0.8	6.94±1.0	8.41±0.8	8.24±1.0	7.55±0.42
βcryptoxanthin (µg/g)	2.41±0.3	3.30±0.4	2.51±0.2	3.19±0.3	2.85±0.12
αcarotene (µg/g)	0.80±0.1	1.00±0.1	0.76±0.1	0.87±0.2	0.86±0.06
βcarotene (µg/g)	5.14±0.3	6.52±0.6	5.54±0.4	6.44±0.4	5.91±0.28
Provitamin-A (µg/g)	6.75±0.3	8.67±0.8	7.17±0.4	8.47±0.5	7.77±0.28
Total carotenoid (µg/g)	24.29±1.6	26.11±1.5	28.78±1.5	29.89±1.8	27.27±0.70

SE: Standard error

Table 4.11. Effect of MARS on grain yield and agronomic traits of two maize synthetics evaluated across multi-environments in Nigeria

ENTRY	Grain yield (t/ha)	Days to anthesis (d)	Days to silking (d)	Anthesis-silking interval (d)	Plant height (cm)	Plant aspect (1-5)	Ear aspect (1-5)
$PVASYNHGAC_0$	4.9	57.3	59.5	2.1	200.0	3.0	3.0
$PVASYNHGAC_1$	4.8	57.3	59.3	2.0	213.6	3.1	2.9
$PVASYNHGAC_2$	4.8	56.7	58.7	2.0	215.4	2.7	2.6
$PVASYNHGBC_0$	4.5	56.6	58.5	1.9	214.4	2.9	2.6
$PVASYNHGBC_1$	5.1	57.1	59.0	1.8	211.5	3.1	2.9
$PVASYNHGBC_2$	4.9	56.0	57.7	1.8	216.8	2.7	2.6
PVASYN13(Check)	5.5	56.6	58.7	2.1	217.0	2.8	2.4
Mean	4.9	56.8	58.8	2.0	212.7	2.9	2.7
SED (0.05)	0.2	0.3	0.4	0.1	4.0	0.1	0.1
Repeatability	0.8	0.6	0.7	0.3	0.7	0.7	0.8
CV (%)	15.0	2.0	3.0	34.0	7.2	14.0	14.0
% gain/cycle (HGA)	-1.6	-0.5	-0.7	-4.0	4.0	-5.0	-6.3
% total gain (HGA)	-2.0	-1.0	-1.3	-4.8	7.7	-10	-13.3
% gain/cycle (HGB)	4.0	-0.6	-0.6	-3.0	0.6	-2.2	-1.0
% total gain (HGB)	8.8	-1.1	-1.4	-5.3	1.1	-6.9	0.0

SED: Standard error of difference, CV: Coefficient of variation

SC in HGA for GY but the genetic gain/cycle in HGB for GY was 4%. Furthermore, while there was no marked effect of MARS on the two MS for DA, there was a significant reduction in DS by 1.4% and 2.3% from C_0 to C_2 and C_1 to C_2, respectively in HGB (Table 4.11). There was marked improvement for PH, PA and EA in HGA by 7.7%, 10% and 13.3%, repectively but not for PH and EA in HGB, except PA which also improved by 6.9% in HGB (Table 4.11). The MARS improved LUT, βC, PVA and TC per SC by 26%, 25%, 15% and 8%, respectively in HGA but not in HGB. The total genetic gain from $C_0 – C_2$ for LUT, βC, PVA and TC in HGA was 50.6%, 50%, 30.4% and 15%, respectively (Table 4.12). However, the improvements in LUT, βC and PVA contents in HGA were associated with decreases in ZXT, βCX and αC. Although MARS had no significant effect on carotenoids in HGB but ZXT and αC increased by 3% and 5%, respectively per SC and each improved by 6.1% and 9.1% from $C_0 – C_2$ (Table 4.12).

The broad-sense heritability (H^2) estimate among the SC of the MS was high for most agronomic traits, except for ASI (H^2= 0.3) which was low (Table 4.11). The broad-sense heritability estimate among the SC was generally high for PVA and all carotenoids measured (Table 4.12).

4.5 Varietal-cross hybrids' agronomic traits and carotenoid content

The top five varietal-cross hybrids (VH) were not significantly different for GY but had GY that was significantly higher than the yield of the PVA-enriched check (PVASYN13). Also, there were no marked differences among the VH in other agronomic traits but all had significantly lower DA, DS and ASI than the check. Nevertheless, the VH had similar performance with the check in terms of PH, PA and EA (Table 4.13). One VH ($PVASYNHGBC_1/PVASYNHGAC_1$) had comparable GY, DA and ASI as the check (PVASYN13), while three other VH ($PVASYNHGBC_1/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_2$ and $PVASYNHGBC_2/PVASYNHGAC_2$) had significantly lower GY than the check (Table 4.13).

The average PVA, βC and TC in the VH was 7.8 µg/g, 5.9 µg/g and 27.3 µg/g, respectively (Table 4.14). Four VH ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$, $PVASYNHGBC_0/PVASYNHGAC_2$ and

Table 4.12. Effect of MARS on PVA content and other carotenoids of two maize synthetics evaluated across multi-environments in Nigeria

ENTRY	Lutein (µg/g)	Zeaxanthin (µg/g)	βcryptoxanthin (µg/g)	αcarotene (µg/g)	βcarotene (µg/g)	Provitamin-A (µg/g)	Total carotenoid (µg/g)
$PVASYNHGAC_0$	8.1	7.7	3.0	0.88	5.0	6.9	24.6
$PVASYNHGAC_1$	8.4	8.3	3.0	0.82	5.1	7.0	25.6
$PVASYNHGAC_2$	12.2	5.6	2.2	0.77	7.5	9.0	28.3
$PVASYNHGBC_0$	12.4	6.6	2.5	0.77	6.8	8.4	29.1
$PVASYNHGBC_1$	7.8	7.9	3.0	0.76	5.0	6.9	24.5
$PVASYNHGBC_2$	12.5	7.0	2.5	0.84	6.5	8.2	29.4
PVASYN13(Check)	7.0	11.7	4.3	1.23	5.8	8.6	30.1
Mean	9.8	7.8	2.9	0.87	6.0	7.9	27.3
SED (0.05)	0.7	0.6	0.1	0.1	0.3	0.3	1.0
Repeatability	1.0	0.9	1.0	0.91	0.9	0.8	0.9
CV (%)	20.0	22.0	16.0	21.0	15.0	10.0	9.0
% gain/cycle (HGA)	26.0	-13.5	-13.0	-6.3	25.0	15.0	8.0
% total gain (HGA)	50.6	-27.3	-26.7	-11.1	50.0	30.4	15.0
% gain/cycle (HGB)	1.0	3.0	-0.2	5.0	-2.0	-1.4	1.0
% total gain (HGB)	0.8	6.1	0.0	9.1	-4.4	-2.4	1.0

SED: Standard error of difference, CV: Coefficient of variation

Table 4.13. Means of grain yield and agronomic traits of varietal-cross and a check variety evaluated across multi-environments in Nigeria

ENTRY	Grain yield (t/ha)	Days to anthesis (d)	Days to silking (d)	Anthesi-silking interval (d)	Plant height (cm)	Plant aspect (1-5)	Ear aspect (1-5)
$PVASYNHGBC_0/PVASYNHGAC_0$	6.2	55.0	56.7	1.8	213.2	2.6	2.3
$PVASYNHGBC_1/PVASYNHGAC_0$	5.1	57.3	59.3	2.1	208.8	3.1	2.9
$PVASYNHGBC_2/PVASYNHGAC_0$	6.4	55.5	57.3	1.8	215.9	2.6	2.2
$PVASYNHGBC_0/PVASYNHGAC_1$	6.2	55.3	57.1	1.8	211.0	2.7	2.5
$PVASYNHGBC_1/PVASYNHGAC_1$	5.3	56.2	58.2	2.1	204.5	3.0	2.7
$PVASYNHGBC_2/PVASYNHGAC_1$	6.2	54.9	56.8	1.9	214.6	2.7	2.3
$PVASYNHGBC_0/PVASYNHGAC_2$	4.6	56.7	58.3	1.6	211.9	2.9	2.6
$PVASYNHGBC_1/PVASYNHGAC_2$	6.2	55.6	57.4	1.8	216.9	2.6	2.3
$PVASYNHGBC_2/PVASYNHGAC_2$	5.0	56.2	57.9	1.8	214.8	2.8	2.5
PVASYN13	5.5	56.6	58.7	2.1	217.0	2.8	2.4
Mean	5.3	56.3	58.2	1.9	212.5	2.8	2.6
SED (0.05)	0.2	0.4	0.4	0.1	4.0	0.1	0.1
CV (%)	13.0	2.3	2.3	35.3	6.6	13.8	14.5

SED: Standard error of difference, CV: Coefficient of variation

Table 4.14. Means for provitamin-A and other carotenoids of nine varietal-cross hybrids and a check variety evaluated across multi-environments in Nigeria

ENTRY	Lutein (µg/g)	Zeaxanthin (µg/g)	βcryptoxanthin (µg/g)	αcarotene (µg/g)	βcarotene (µg/g)	Provitamin-A (µg/g)	Total carotenoid (µg/g)
$PVASYNHGBC_0/PVASYNHGAC_0$	10.3	7.1	2.8	0.8	6.2	8.0	27.3
$PVASYNHGBC_1/PVASYNHGAC_0$	7.9	8.4	3.0	0.8	4.6	6.6	24.8
$PVASYNHGBC_2/PVASYNHGAC_0$	10.6	8.1	3.0	0.9	5.5	7.4	28.1
$PVASYNHGBC_0/PVASYNHGAC_1$	10.1	7.8	2.9	0.9	6.0	7.9	27.7
$PVASYNHGBC_1/PVASYNHGAC_1$	9.2	7.9	3.0	0.9	5.0	6.9	26.0
$PVASYNHGBC_2/PVASYNHGAC_1$	10.4	7.1	2.7	0.8	5.5	7.2	26.4
$PVASYNHGBC_0/PVASYNHGAC_2$	12.6	6.1	2.4	0.8	7.4	9.0	29.3
$PVASYNHGBC_1/PVASYNHGAC_2$	9.1	7.0	2.9	0.9	5.8	7.7	25.6
$PVASYNHGBC_2/PVASYNHGAC_2$	13.2	6.5	2.5	0.8	6.8	8.4	29.7
PVASYN13	7.0	11.7	4.3	1.2	5.8	8.6	30.1
Mean	10.1	7.6	2.9	0.9	5.9	7.8	27.3
SED (0.05)	0.6	0.6	0.1	0.1	0.4	0.4	1.0
CV (%)	15.3	20.7	13.8	21.4	13.0	10.0	9.7

SED: Standard error of difference, CV: Coefficient of variation

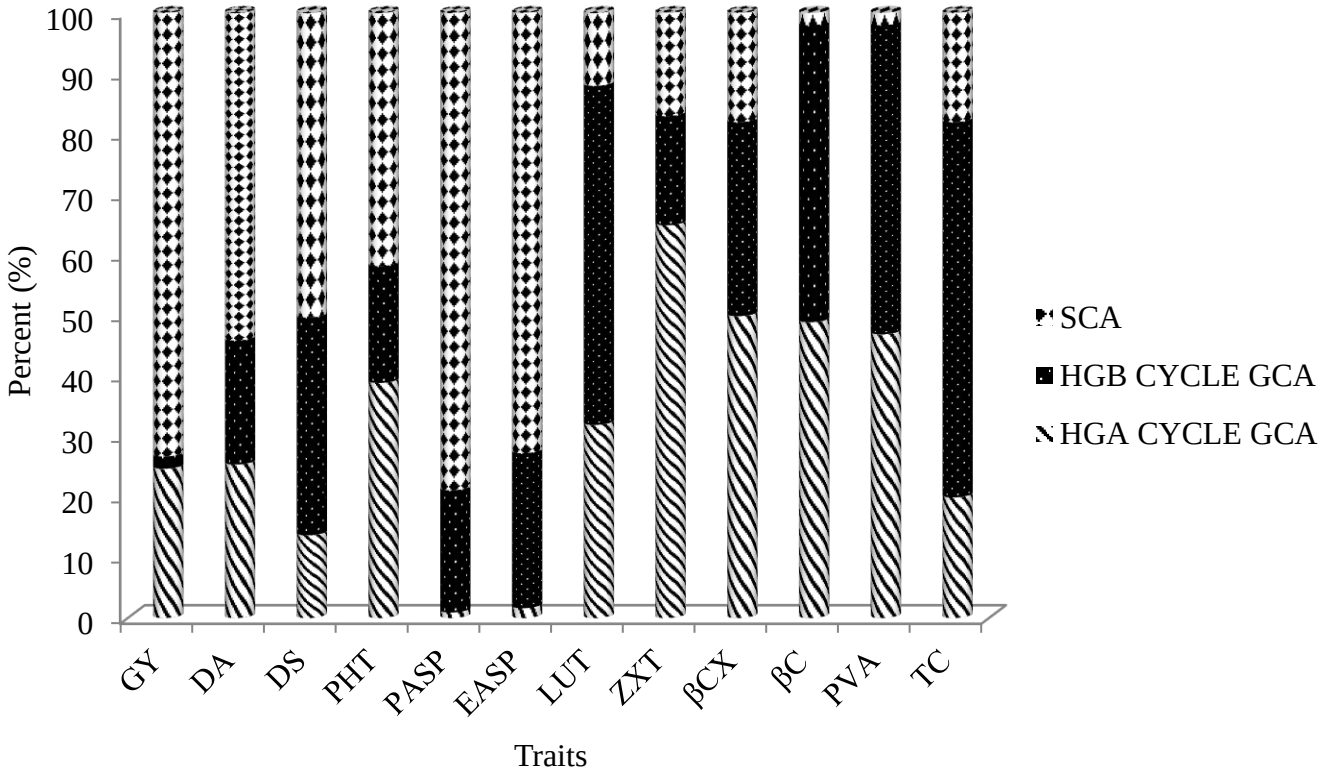
PVASYNHGBC$_2$/PVASYNHGAC$_2$) had comparable PVA contents as the released check (PVASYN13); but the best VH (PVASYNHGBC$_0$/PVASYNHGAC$_2$) had PVA content of 4.7% more than PVASYN13 (Table 4.14). Five VH had comparable βC content as the check, whereas the βC contents of two VH (PVASYNHGBC$_0$/PVASYNHGAC$_2$ and PVASYNHGBC$_2$/PVASYNHGAC$_2$) were significantly higer than that of PVASYN13 (Table 4.14). The lutein contents of all the VH were also significantly higher than the check. However, the check had considerably more ZXT, βCX and αC contents than all the VH included in the trial. In addition, the Total Carotenoid Content (TCC) of the check variety was markedly higher than the TCC of most VH, except PVASYNHGBC$_2$/PVASYNHGAC$_2$ and PVASYNHGBC$_0$/PVASYNHGAC$_2$ which had similar TCC as the check variety (Table 4.14).

4.6 The GCA and SCA proportional contributions to traits inheritance

The proportion of HGA cycle GCA was more than HGB cycle GCA for the inheritance of GY, DA and PH, while HGB cycle GCA effect was more in the inheritance of DS, PA, EA and most carotenoids (Figure 4.2). Furthermore, the GCA effects of the maize synthetics and their selection cycles were more than the SCA effects for PVA and other carotenoids but not for GY and most agronomic traits (Figure 4.2). In the inheritance of GY, SCA effects accounted for 73% while the combined GCA effects of HGA and HGB accounted for 27%. For DA, DS, PA and EA, SCA effects accounted for 54%, 50%, 79% and 73%, respectively (Figure 4.2). On the other hand, the inheritances of the carotenoids were largely due to the GCA effects. The contribution due to GCA effects for the inheritance of LUT, ZXT, βCX, βC, PVA and TC in the VH was 88%, 83%, 82%, 98%, 98% and 82%, respectively (Figure 4.1).

4.7 The GCA effect for agronomic traits and carotenoid content

Three SCs (PVASYNHGAC$_0$, PVASYNHGAC$_1$ and PVASYNHGBC$_2$) had significant positive GCA effects for GY, while PVASYNHGAC$_2$ and PVASYNHGBC$_1$ had significant negative GCA effects for the trait (Table 4.15). Although PVASYNHGBC$_0$ had positive GCA effects for GY, it was however not significant. Associated with the positive GCA effects of PVASYNHGAC$_1$, PVASYNHGBC$_0$ and PVASYNHGBC$_2$ for GY were negative GCA effects for DA,



GY: Grain Yield, DA: Days to Anthesis, DS: Days to Silking, PH: Plant Height, PA: Plant Aspect, EA: Ear Espect, LUT: Lutein, ZXT: Zeaxanthin, βCX: βcryptoxanthin, βC: βcarotene, PVA: Provitamin-A, TC: Total Carotenoid

Figure 4.1. The relative contribution of selection cycles of two maize synthetics to the inheritance of grain yield, agronomic traits and carotenoids in hybrids evaluated in multi-environments in Nigeria

Table 4.15. General combining ability for grain yield and agronomic traits of selection cycles of two maize synthetics evaluated across multi-environments in Nigeria

Parent	Grain yield	Days to anthesis	Days to silking	Anthesis-silking interval	Plant height	Plant aspect	Ear aspect
$PVASYNHGAC_0$	0.17**	0.13	0.16	0.03	2.19	-0.02	0.04*
$PVASYNHGAC_1$	0.30***	-0.55***	-0.46**	0.09	-3.55**	-0.01	0.00
$PVASYNHGAC_2$	-0.47***	0.42**	0.30*	-0.12*	1.36	0.03	-0.04*
$PVASYNHGBC_0$	0.04	-0.34*	-0.50**	-0.16**	1.18	-0.09*	-0.03
$PVASYNHGBC_1$	-0.14**	0.51***	0.75***	0.24***	-2.51	0.14***	0.19***
$PVASYNHGBC_2$	0.10*	-0.17	-0.25	-0.08	1.33	-0.05	-0.16***
SED (0.05)	0.05	0.13	0.15	0.05	1.05	0.04	0.02
σ^2 GCA	0.45	0.57	0.65	0.01	5.5	0.04	0.05

*, **, ***: $p \leq 0.05$, 0.01 and 0.001, SED: Standard error of difference, σ^2GCA: General Combining Ability variance

DS and PA which is desirable. Nevertheless, $PVASYNHGAC_2$ and $PVASYNHGBC_1$ had significant and positive GCA effects for these traits and EA score, which is adverse. Two SCs: $PVASYNHGAC_2$ and $PVASYNHGBC_0$ consistently had significant positive GCA effects for PVA, βC, LUT and TC (Table 4.16), while $PVASYNHGBC_2$ had positive GCA effects for these carotenoids, except for PVA (Table 4.16). Futhermore, these three SCs had significant and negative GCA effects for ZXT and βCX, while others had positive and significant GCA effects for the two carotenoids, but had negative and significant GCA effects for PVA, βC LUT and TC. The GCA effect for GY and the GCA effect for PVA were inversely related in HGA, but not in HGB (Tables 4.15 and 4.16). Two SCs, $PVASYNHGBC_0$ and $PVASYNHGBC_2$ combined positive and significant GCA effects for both GY and βC, while $PVASYNHGBC_0$ combined for both GY and PVA.

4.8 The SCA effect for agronomic traits and carotenoid content

Four VH ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_2/PVASYNHGAC_0$, $PVASYNHGBC_2/PVASYNHGAC_1$ and $PVASYNHGBC_1/PVASYNHGAC_2$) had significant positive SCA effects for GY, while four others ($PVASYNHGBC_1/PVASYNHGAC_0$, $PVASYNHGBC_1/PVASYNHGAC_1$, $PVASYNHGBC_0/PVASYNHGAC_2$ and $PVASYNHGBC_2/PVASYNHGAC_2$) had significant negative SCA effects for the trait (Table 4.17). Although $PVASYNHGBC_0/PVASYNHGAC_1$ had positive SCA effect for GY, it was however not significant. All five VH which had either significant positive/positive SCA effects for GY also had desirable negative SCA effects for DA, DS, PA and EA scores. Associated with the VH which had significant negative SCA effects for GY were also undersirable SCA effects for other agronomic traits (Table 4.17).

No VH had significant SCA effect for PVA or any PVA carotenoid. However, five VH were promising as all five had positive SCA effects for βC, four of which were also positive for PVA and LUT (Table 4.18). All four VH had similar pattern of SCA effects for PVA, βC and LUT with corresponding negative SCA effects for ZXT and βCX, except $PVASYNHGBC_2/PVASYNHGAC_2$ which had positive SCA effect for ZXT (Table 4.18). The predictability ratio (i.e. a measure of the proportional relevance of GCA and SCA in trait inheritance) ranged from moderate to high (0.67 – 1.00) for GY and most agronomic traits, but was high (0.92 – 1.00) for PVA and other carotenoids (Tables 4.17 and 4.18).

Table 4.16. General combining ability for provitamin-A content and other carotenoids of selection cycles of two maize synthetics evaluated across multi-environments in Nigeria

Parent	Lutein	Zeaxanthin	βcryptoxanthin	βcarotene	Provitamin-A	Total carotenoid
$PVASYNHGAC_0$	-0.99***	0.36	0.09	-0.26*	-0.23*	-0.82*
$PVASYNHGAC_1$	-0.10	0.43*	0.09	-0.44***	-0.40***	-0.03
$PVASYNHGAC_2$	1.09***	-0.79***	-0.18***	0.70***	0.62***	0.85*
$PVASYNHGBC_0$	1.27***	-0.12	-0.05	0.62***	0.60***	1.73***
$PVASYNHGBC_1$	-1.97***	0.32	0.17**	-0.63***	-0.54***	-2.11***
$PVASYNHGBC_2$	0.70**	-0.20	-0.12*	0.01	-0.06	0.38
SED (0.05)	0.25	0.19	0.05	0.10	0.11	0.36
σ^2 GCA	2.53	0.45	0.04	0.45	0.34	0.22

*, **, ***: $p \leq 0.05$, 0.01 and 0.001, SED: Standard error of difference, σ^2GCA: General Combining Ability variance

Table 4.17. Specific combining ability for grain yield and agronomic traits of selection cycles of two maize synthetics evaluated across multi-environments in Nigeria

Hybrid	Grain yield	Days to anthesis	Days to silking	Anthesis-silking interval	Plant height	Plant aspect	Ear aspect
$PVASYNHGBC_0/PVASYNHGAC_0$	0.32**	-0.71**	-0.68**	0.03	2.76	-0.07	-0.12
$PVASYNHGBC_1/PVASYNHGAC_0$	-0.58***	0.68*	0.73**	0.06	-1.63	0.24***	0.29***
$PVASYNHGBC_2/PVASYNHGAC_0$	0.26*	0.03	-0.06	-0.09	-1.13	-0.17**	-0.17*
$PVASYNHGBC_0/PVASYNHGAC_1$	0.20	-0.10	-0.08	0.03	-1.17	-0.05	-0.03
$PVASYNHGBC_1/PVASYNHGAC_1$	-0.51***	0.27	0.33	0.06	-2.20	0.12*	0.12
$PVASYNHGBC_2/PVASYNHGAC_1$	0.31**	-0.17	-0.25	-0.08	3.37	-0.07	-0.09
$PVASYNHGBC_0/PVASYNHGAC_2$	-0.52***	0.81**	0.75**	-0.05	-1.59	0.12*	0.15*
$PVASYNHGBC_1/PVASYNHGAC_2$	1.09***	-0.95***	-1.06***	-0.11	3.82	-0.36***	-0.41***
$PVASYNHGBC_2/PVASYNHGAC_2$	-0.57***	0.14	0.31	0.17	-2.23	0.24***	0.26***
SED (0.05)	0.11	0.26	0.25	0.11	2.39	0.06	0.07
σ^2SCA	0.45	0.57	0.56	0.00	4.56	0.04	0.05
Predictability ratio	0.67	0.67	0.70	1.00	0.71	0.67	0.67

*, **, ***: $p \leq 0.05$, 0.01 and 0.001, SED: Standard error of difference, σ^2SCA: Specific Combining Ability variance

Table 4.18. Specific combining ability for provitamin-A and other carotenoids of selection cycles of two maize synthetics evaluated across multi-environments in Nigeria

Hybrid	Lutein	Zeaxanthin	βcryptoxanthin	βcarotene	Provitamin-A	Total carotenoid
$PVASYNHGBC_0/PVASYNHGAC_0$	0.86	-0.13	-0.07	0.03	0.00	0.70
$PVASYNHGBC_1/PVASYNHGAC_0$	-0.37	-0.05	-0.10	-0.03	-0.10	-0.61
$PVASYNHGBC_2/PVASYNHGAC_0$	-0.48	0.18	0.17	0.01	0.11	-0.09
$PVASYNHGBC_0/PVASYNHGAC_1$	-1.06*	0.28	0.19	-0.16	-0.04	-0.69
$PVASYNHGBC_1/PVASYNHGAC_1$	1.04*	-0.09	-0.08	0.27	0.21	1.08
$PVASYNHGBC_2/PVASYNHGAC_1$	0.02	-0.19	-0.10	-0.11	-0.17	-0.39
$PVASYNHGBC_0/PVASYNHGAC_2$	0.20	-0.15	-0.12	0.14	0.04	-0.01
$PVASYNHGBC_1/PVASYNHGAC_2$	-0.66	0.15	0.18	-0.24	-0.10	-0.47
$PVASYNHGBC_2/PVASYNHGAC_2$	0.46	0.01	-0.06	0.10	0.06	0.48
SED (0.05)	0.43	0.33	0.10	0.20	0.20	0.60
σ^2SCA	0.42	0.06	0.01	0.00	0.00	0.47
Predictability ratio	0.92	0.94	0.94	1.00	1.00	0.90

*: $p \leq 0.05$ levels, SED: Standard error of difference, σ^2SCA: Specific Combining Ability variance

4.9 Heterosis estimate for agronomic traits and carotenoid content

The variations in the magnitude of Mid-parent Heterosis (MPH) among the VH were significant for GY, DA, DS and EA but not for ASI, PH and PA score (Table 4.19). Five VH ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_2/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$ $PVASYNHGBC_2/PVASYNHGAC_1$ and $PVASYNHGBC_1/PVASYNHGAC_2$) had significant MPH for GY, whereas the MPH of three other VH was not significant for GY (Table 4.19). The five VH also exhibited consideration level of MPH for DA, DS and EA score; four of which were significant for DS and EA score while two had significant MPH for DA (Table 4.19).

There was no significant MPH for PVA and any other carotenoid measured in this study. However, three VH ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$ and $PVASYNHGBC_0/PVASYNHGAC_2$) manifested appreciable levels of MPH for key PVA carotenoids (αC, βCX, βC) and PVA content, while the other six VH exhibited negative MPH (depression) for PVA and βC (Table 4.20). Two ($PVASYNHGBC_2/PVASYNHGAC_0$ and $PVASYNHGBC_1/PVASYNHGAC_2$) of the six VH had outstanding MPH for ZXT, αC and βCX, while $PVASYNHGBC_0/PVASYNHGAC_0$ and $PVASYNHGBC_0/PVASYNHGAC_1$ combined significant positive MPH for GY with positive MPH for PVA (Tables 4.19 and 4.20).

4.10 Relationships between mid-parent, VH, SCA and MPH

The *per se* performance of each selection cycle had no significant correlation with the mean performance of VH for GY and all agronomic traits assessed (Table 4.21) but significantly correlated for PVA and all measured carotenoids, except αC (Table 4.22). Also, the SCA effects of the selection cycles had significant positive correlation with the mean performance and MPH of VH for GY and most agronomic traits but not for PVA. However, SCA effect had significant positive correlation with MPH for LUT, ZXT and βCX (Table 4.22).

4.11 Correlation among agronomic traits and carotenoids

There was significant negative correlation of GY with DA, DS, PA and EA, whereas the relation of GY and other agronomic traits with carotenoids was not significant (Table 4.23). Conversely, DA and DS were positively correlated with each other and with PA and EA, while PH had a significant negative correlation coefficient with EA

Table 4.19. Mid-parent heterosis (%) for grain yield and agronomic traits of maize varietal-cross hybrids evaluated across multi environments in Nigeria

Varietal-cross hybrid	Grain yield	Days to anthesis	Days to silking	Anthesis-silking interval	Plant height	Plant aspect	Ear aspect
$PVASYNHGBC_0/PVASYNHGAC_0$	30.2***	-3.5**	-3.8**	-12.7	2.9	-13.3	-18.3*
$PVASYNHGBC_1/PVASYNHGAC_0$	1.3	0.0	0.2	3.8	1.4	2.5	-1.9
$PVASYNHGBC_2/PVASYNHGAC_0$	31.0***	-2.0	-2.2	-9.8	3.6	-11.3	-21.9**
$PVASYNHGBC_0/PVASYNHGAC_1$	32.6***	-2.9	-3.0*	-5.9	-1.4	-10.4	-11.1
$PVASYNHGBC_1/PVASYNHGAC_1$	6.9	-1.8	-1.6	6.5	-3.8	-4.4	-5.6
$PVASYNHGBC_2/PVASYNHGAC_1$	27.9***	-3.0*	-3.0*	-0.5	-0.3	-7.4	-15.8*
$PVASYNHGBC_0/PVASYNHGAC_2$	-1.8	0.1	-0.5	-19.0	-1.4	3.6	0.2
$PVASYNHGBC_1/PVASYNHGAC_2$	25.6**	-2.4	-2.4*	-3.4	1.6	-11.2	-16.5*
$PVASYNHGBC_2/PVASYNHGAC_2$	3.0	-0.3	-0.5	-4.3	-0.6	4.2	-3.1
SED (0.05)	0.4	0.7	0.7	ns	ns	ns	0.2

*, **, ***: $p \leq 0.05$, 0.01 and 0.001, ns: nonsigficance, SED: Standard error of difference

Table 4.20. Mid-parent heterosis (%) for provitami-A and other carotenoids of varietal-cross hybrids evaluated across multi-environments in Nigeria

Varietal-cross hybrid	Lutein	Zeaxanthin	βcrypthoxanthin	αcarotene	βcarotene	Provitamin-A	Total carotenoid
$PVASYNHGBC_0/PVASYNHGAC_0$	1.0	0.0	0.9	0.6	4.8	4.0	1.6
$PVASYNHGBC_1/PVASYNHGAC_0$	-0.7	8.2	0.8	1.2	-7.4	-5.1	1.0
$PVASYNHGBC_2/PVASYNHGAC_0$	3.1	10.8	7.7	8.1	-4.9	-1.9	4.1
$PVASYNHGBC_0/PVASYNHGAC_1$	-2.5	5.0	6.2	6.9	1.1	2.3	1.5
$PVASYNHGBC_1/PVASYNHGAC_1$	13.7	-2.2	0.7	13.9	-1.6	-0.1	3.9
$PVASYNHGBC_2/PVASYNHGAC_1$	-0.7	-7.3	-0.5	-2.4	-6.2	-5.0	-3.8
$PVASYNHGBC_0/PVASYNHGAC_2$	2.6	0.5	2.1	2.6	2.7	2.6	2.2
$PVASYNHGBC_1/PVASYNHGAC_2$	-9.5	2.9	11.2	16.3	-6.7	-2.8	-2.9
$PVASYNHGBC_2/PVASYNHGAC_2$	6.4	2.9	4.5	-0.6	-3.6	-2.3	2.9
SED (0.05)	1.47	1.29	0.35	0.20	0.72	0.78	2.55

SED: Standard error of difference

Table 4.21. Pearson's correlation coeficients among mid-parent, mean performance of hybrids, specific combining ability and mid-parent heterosis for agronomic traits of selection cycles of two maize synthetics and varietal-cross hybrids evaluated across multi-environments in Nigeria

	Grain yield	Days to anthesis	Days to silking	Anthesis-silking interval	Plant height	Plant aspect	Ear aspect
MP vs F_1	0.00	0.33	0.32	-0.23	0.40	0.18	0.28
MP vs SCA	0.00	0.04	0.00	-0.01	-0.11	-0.27	-0.10
MP Vs MPH	-0.20	-0.03	-0.05	-0.58	-0.3	-0.46	-0.22
SCA Vs F_1	0.84**	0.77*	0.77*	0.32	0.55	0.81**	0.8**
SCA Vs MPH	0.82**	0.80**	0.80**	0.29	0.43	0.84**	0.85**
F_1 Vs MPH	0.98***	0.92***	0.93***	0.92***	0.65	0.78*	0.86**

*, **, ***: $p \leq 0.05$, 0.01 and 0.001, MP: Mid-parent, F1: Varietal-cross hybrid, SCA: Specific combining ability, MPH: Mid-parent heterosis

Table 4.22. Pearson's correlation coeficients among mid-parent, mean performance of hybrids, specific combining ability and mid-parent heterosis for provitamin-A and carotenoid contents of selection cycles of two maize synthetics and varietal-cross hybrids evaluated across multi-environments in Nigeria

	Lutein	Zeaxanthin	βcrypthoxanthin	αcarotene	βcarotene	Provitamin-A	Total carotenoid
MP vs F_1	0.93***	0.85**	0.87**	-0.13	0.95***	0.94***	0.88**
MP vs SCA	0.00	0.00	0.00	0.00	0.00	-0.01	0.05
MP Vs MPH	-0.08	-0.05	-0.3	-0.43	0.32	0.29	0.00
SCA Vs F_1	0.25	0.36	0.46	-0.19	0.14	0.11	0.25
SCA Vs MPH	0.73*	0.68*	0.89**	-0.08	0.37	0.41	0.54
F_1 Vs MPH	0.27	0.47	0.20	0.88**	0.58	0.60	0.46

*, **, ***: $p \leq 0.05$, 0.01 and 0.001, MP: Mid-parent, F1: Varietal-cross hybrid, SCA: Specific combining ability, MPH: Mid parent heterosis

Table 4.23. Pearson's correlation coefficient among agronomic traits and carotenoids of sixteen provitamin-A maize genotypes evaluated across eight environments in Nigeria

Trait	GY	DA	DS	PH	PA	EA	LUT	ZXT	βCX	αC	βC	PVA
DA	-0.72***	-										
DS	-0.70***	0.95***	-									
PH	0.01	-0.25	-0.17	-								
PA	-0.68***	0.76***	0.67***	-0.19	-							
EA	-0.64**	0.72***	0.70***	-0.53*	0.76***	-						
LUT	-0.20	-0.24	-0.28	0.15	-0.20	-0.30	-					
ZXT	-0.02	0.27	0.33	0.12	0.23	0.13	-0.76***	-				
βCX	0.04	0.22	0.30	0.11	0.17	0.10	-0.79***	0.98***	-			
αC	0.03	0.16	0.27	0.14	-0.01	-0.09	-0.51*	0.86***	0.99***	-		
βC	-0.27	-0.16	-0.17	0.32	-0.22	-0.31	0.83***	-0.56*	-0.53*	-0.23	-	
PVA	-0.28	-0.10	-0.09	0.40	-0.20	-0.33	0.66**	-0.27	-0.24	0.08	0.95***	-
TC	-0.33	-0.07	-0.05	0.42	-0.11	-0.34	0.66**	-0.05	-0.08	0.25	0.78***	0.87***

*, **, ***: $p \leq 0.05$, 0.01 and 0.001, GY: Grain yield, DA: Days to anthesis, DS: Days to silking, PH: Plant height, PA: Plant aspect, EA: Ear aspect, LUT: Lutein, ZXT: Zeaxanthin, βCX: βcryptoxanthin, αC: αcarotene, βC: βcarotene, PVA: Provitamin-A, TC: Total carotenoid

score. Provitamin-A had significant positive correlation with βC, LUT and TC but non-significant negative correlation with ZXT and βCX. Meanwhile, βC had significant negative correlation with ZXT and βCX (Table 4.23).

4.12 Performance of PVA-enriched maize genotypes under drought stress

4.12.1 Genetic variation among the genotypes

In the ANOVA of the PVA maize genotypes under Managed Drought Stress (MDS), Well-watered Condition (WWC) and combined water regime, the effect of year was significant for GY and all or most agronomic traits (Table 4.24). The genotypes differed significantly for GY, DS, ASI, PA, Drought-stress Tolerance Index (DTI) and Yield Stability Index (YSI) under MDS; whereas under WWC, significant differences were observed for GY, DA, DS, PH and EA. Genotype × year interaction effect was significant for GY under MDS condition but not under WWC. The effect of water regime differed significantly for GY and most agronomic traits measured, except PH (Table 4.24). Also, each environment (i.e. year-water regime) differed for all traits assessed, whereas, GEI effect was significant for GY and DS under the combined test condition (Table 4.25).

4.12.2 Weather conditions and soil moisture content during drought-stress trials

Monthly weather data (rainfall, relative humidity, solar radiation, minimum and maximum temperature) recorded during drought stress trials showed that rainfall and relative humidity were higher between December and February of 2018/2019 than in 2019/2020 (Figures 4.2 and 4.3). At anthesis, solar radiation was higher in 2020 than in 2019 with corresponding lower night temperature. Tensiometer reading shows that moisture tension was high (45 to 73 centibars) under drought stress during anthesis and silking of the maize genotypes, while the moisture tension of well-watered trial was below the field capacity throughout the trial (Figure 4.4).

4.12.3 Agronomic performance of PVA maize genotypes under MDS and WWC

Grain yield was higher in 2018 than in 2019 both under MDS and WWC (Table 4.26). Under MDS, ASI was longer in 2018 than in 2019, while DTI and YSI were also higher in 2018 than in 2019 (Table 4.26). The mean performance of most agronomic traits, except EA score was better in 2018 than in 2019 under WWC (Table 4.26).

Table 4.24. Mean squares for grain yield and agronomic traits of sixteen PVA maize genotypes evaluated under managed drought stress, well-watered condition and the combined water regime in 2018 and 2019 at Ikenne, Nigeria

Source	DF	Grain yield (t/ha)	Days to anthesis (days)	Days to silking (days)	Anthesis-silking interval (days)	Plant height (cm)	Plant aspect (1-5)	Ear aspect (1-5)	Drought tolerance index	Yield stability index
Managed drought stress										
Year (Y)	1	51.40***	16.26***	16.59**	65.71***	1412.48	0.00	2.41***	9.87***	0.02
REP (Year)	6	1.91***	4.33***	14.25***	4.90**	562.20	0.39*	0.41**	0.24***	0.21***
BLK (REP×Y)	24	0.69***	1.30	4.10*	1.59	1396.06	0.16	0.18	0.08***	0.04
Genotype	15	0.88***	1.69	6.73***	2.12*	1337.60	0.31*	0.15	0.09***	0.08*
Y×Genotype	15	0.55*	1.02	2.25	1.34	1396.20	0.12	0.08	0.07**	0.04
Error	66	0.26	0.95	2.05	1.25	1161.76	0.13	0.11	0.03	0.04
Well-watered condition										
Year (Y)	1	162.18***	72.34***	85.64***	0.56*	34081.58***	6.32***	4.46***		
REP (Year)	6	2.25**	0.75	0.56	0.10	237.26	0.38*	1.42***		
BLK (REP×Y)	24	0.51	0.91	0.85*	0.10	94.01	0.15	0.17		
Genotype	15	2.21***	2.14***	2.85***	0.12	256.67*	0.14	0.25*		
Y×Genotype	15	0.52	0.99	1.28**	0.17	113.02	0.30*	0.29*		
Error	66	0.65	0.56	0.51	0.14	118.42	0.16	0.13		
Combined water regimes										
Water regime (WR)	1	256.82***	36.08***	195.86***	63.81***	27791.82***	0.15	5.29***		
REP (WR)	6	2.66	2.56	6.55**	2.30	492.94	0.29	0.45*		
BLK (REP×WR)	24	0.75	1.27	2.89	0.70	597.27	0.12	0.21		
Genotype	15	2.78*	3.11*	7.46*	1.39	863.00	0.40*	0.39*		
Genotype × WR	15	1.01	1.25	2.62	0.91	789.28	0.17	0.17		
Error	194	1.70	1.35	2.20	1.12	867.34	0.21	0.21		

*, **, ***: $p \leq 0.05$, 0.01 and 0.001, respectively

Table 4.25. Mean squares of sixteen PVA maize genotypes evaluated under combined environments of drought stress and well-watered conditions in Ikenne, Nigeria

Source	DF	Grain yield (t/ha)	Days to anthesis (days)	Days to silking (days)	Anthesis-silking interval (days)	Plant height (cm)	Plant aspect (1-5)	Ear aspect (1-5)
Environment (ENV)	3	171.10***	42.59***	100.32***	42.57***	22343.84***	2.22***	4.34***
REP (ENV)	12	2.08***	2.54***	7.40***	2.50***	399.73	0.38**	0.92***
BLK (REP×ENV)	48	0.60	1.11*	2.48**	0.85	745.04	0.15	0.18*
Genotype	15	2.25***	3.00***	7.62***	1.44*	882.14	0.31*	0.27**
Genotype × ENV	45	0.67*	0.99	2.00*	0.83	764.48	0.19	0.18
Error	132	0.46	0.75	1.30	0.69	640.09	0.15	0.12

*, **, ***: $p \leq 0.05$, 0.01 and 0.001, respectively

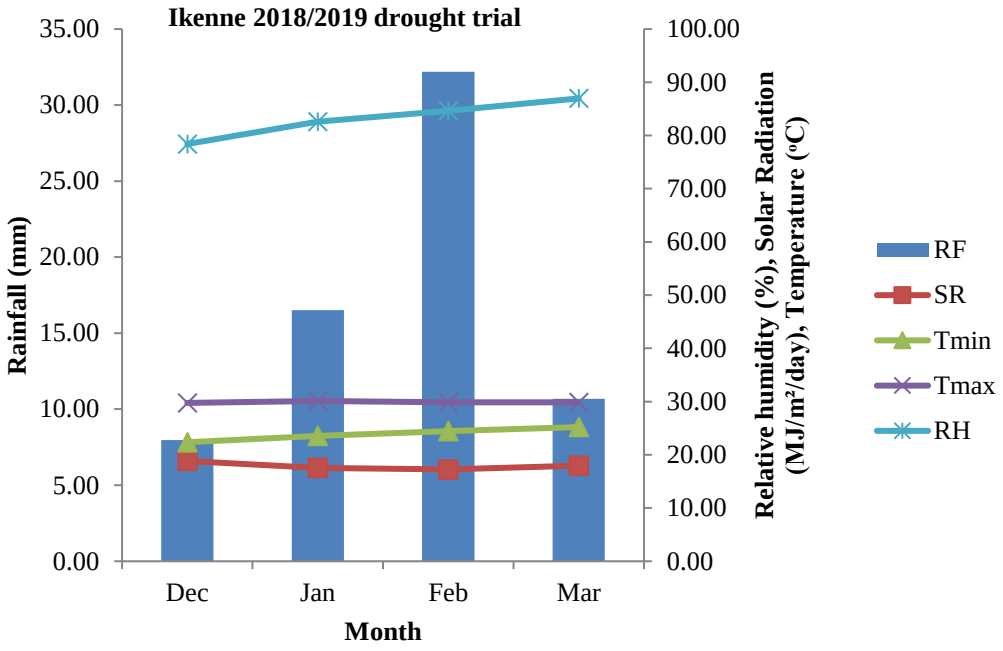


Figure 4.2. Monthly rainfall (RF), relative humidity (RH), solar radiation (SR), Minimum and maximum temperature (Tmin and Tmax) measured during the evaluation of sixteen provitamin-A maize genotypes at Ikenne in 2018/2019 dry season

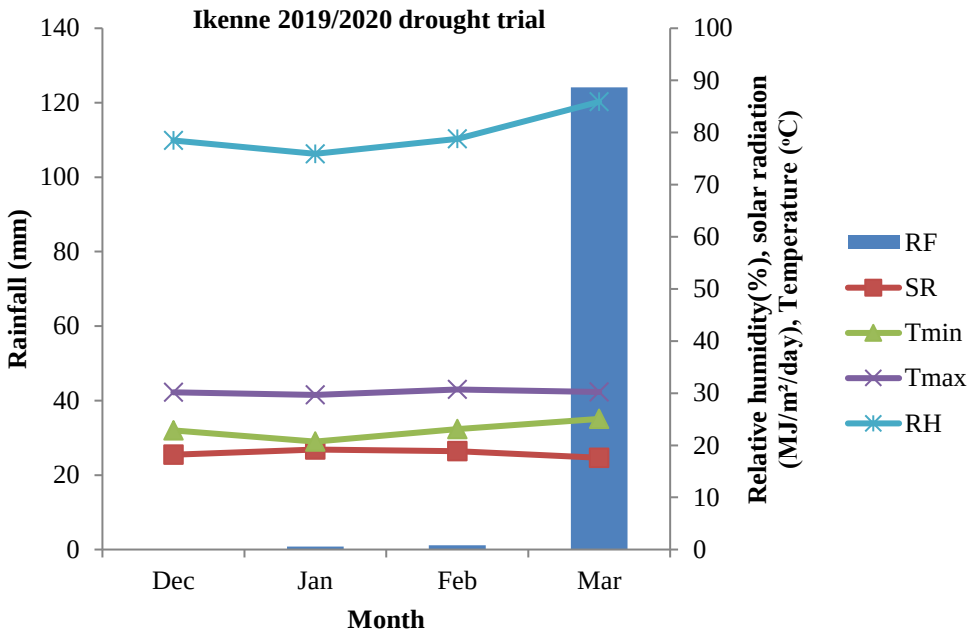
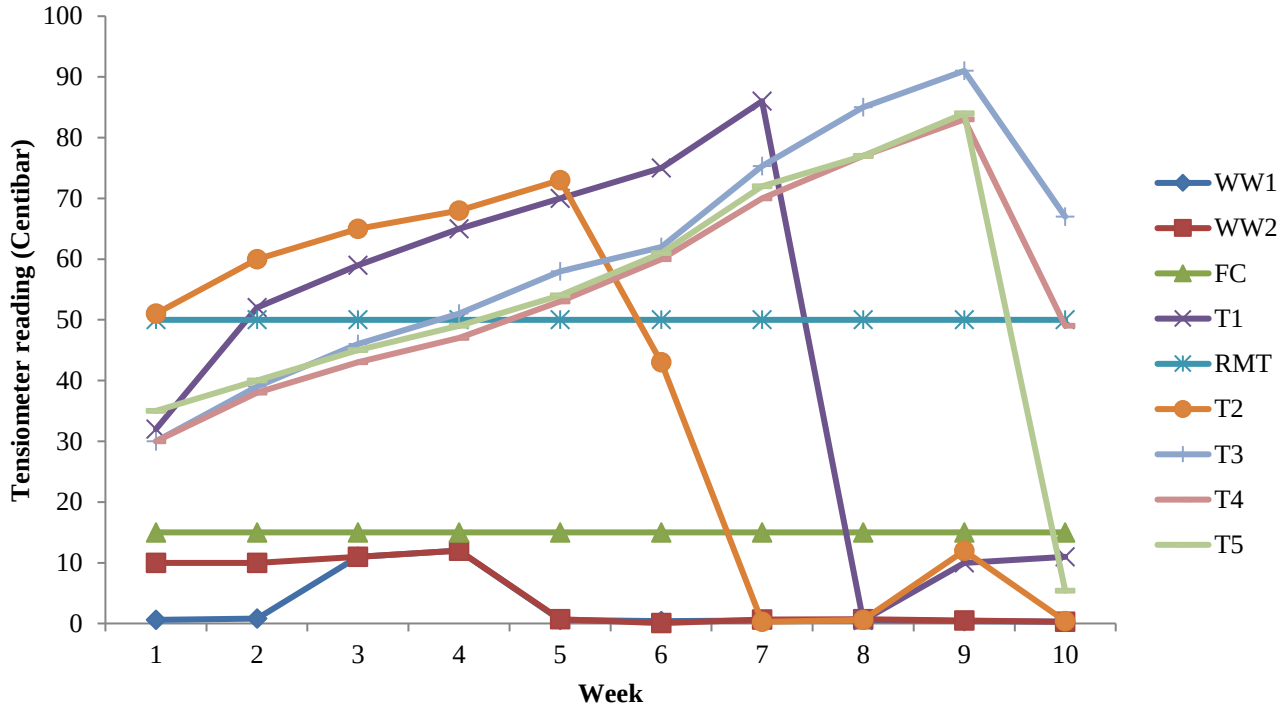


Figure 4.3. Monthly rainfall (RF), relative humidity (RH), solar radiation (SR), Minimum and maximum temperature (Tmin and Tmax) measured during the evaluation of sixteen provitamin-A maize genotypes at Ikenne in 2019/2020 dry season



WW1, WW2: Tensiometers under well-watered condition, T1-T5: Tensiometers under drought stress, FC: Tensiometer reading at field capacity, RMT: Recommended moisture tension

Figure 4.4: Tesionmeter readings under drought stress and well-watered conditions during the evaluation of sixteen maize genotypes at Ikenne in 2018/2019 and 2019/2020 dry seasons

Table 4.26. Agronomic performance of sixteen PVA maize genotypes evaluated under drought stress and well-watered conditions in 2018 and 2019 at Ikenne, Nigeria

Trait	**Drought stress trial**		**Well-watered trial**	
	2018	2019	2018	2019
Grain yield (t/ha)	2.5	1.2	5.2	2.9
Days to anthesis	55.1	55.8	53.9	55.4
Days to silking	58.8	58.1	55.8	57.4
Anthesis-silking interval (days)	3.7	2.3	1.88	2.03
Plant height (cm)	146.9	139.2	183.7	149.4
Plant aspect (1-5)	2.7	2.7	2.6	3.0
Ear aspect (1-5)	3.0	2.7	2.8	2.3
Stress tolerance index	0.77	0.20	-	-
Yield stability index	0.49	0.45	-	-

In the combined year-water regime analysis, drought stress reduced GY and agronomic performance of the PVA maize genotypes (Table 4.27, Plates 4.1). The average GY under drought stress was 1.8 t/ha, while the average GY under WWC was 4.1 t/ha (Table 4.27). The average GY of the maize genotypes under MDS was 44% of the average yield under WWC, resulting in an average yield reduction of 56%. Percentage yield reduction ranged from 31.4% [PVASYNHGBC$_1$/PVASYNHGAC$_2$ (Plate 4.2a$_1$)] to 69.8% [PVASYNHGBC$_1$/PVASYNHGAC$_0$ (Plate 4.2b$_1$)]. Under MDS, there was no significant difference in the mean GY performance among all the SCs of the MS. However, all six SCs had GY which were significantly lower than that of the drought tolerant check (PVASYN13), but the GY and other agronomic traits of three (PVASYNHGBC$_0$/PVASYNHGAC$_0$, PVASYNHGBC$_2$/PVASYNHGAC$_0$, and PVASYNHGBC$_0$/PVASYNHGAC$_1$) of the VH were comparable with those of the check variety (Table 4.27). One hybrid (PVASYNHGBC$_1$/PVASYNHGAC$_2$) produced 12.5% more yield than the check under MDS condition. These four hybrids had moderate to high DTI and YSI (Table 4.27). Under MDS, the SCs had similar mean performance for DA but not for DS, ASI and EA score. Three SCs (PVASYNHGAC$_0$, PVASYNHGAC$_1$ and PVASYNHGBC$_1$) had relatively longer DS and ASI above the trial average and the check variety and also had relatively poorer EA scores under MDS. Thus, the crosses (PVASYNHGBC$_1$/PVASYNHGAC$_0$ and PVASYNHGBC$_1$/PVASYNHGAC$_1$) of the SCs resulted in relatively low GY performance and DTI when compared to other VH (Table 4.27; Plates 4.2 to 4.4).

Under WWC, most of the SCs had similar GY and agronomic performance with the check variety. Five of the VH had significantly higher GY, ranging from 37 to 46% than the check variety, and also had shorter DA and DS (Table 4.27). Estimates of broad-sense heritability for GY and most agronomic traits were moderate to high under MDS, but were relatively high for GY, DA and DS under WWC.

4.13 Yield stability of PVA maize genotypes under MDS and WWC

The results of the Genotype main effect and Genotype by Environment (GGE) biplots (Figures 4.5 and 4.6) for GY of the sixteen PVA-enriched maize genotypes evaluated under MDS and WWC conditions revealed that the first and second principal components (PC 1 and 2) axis described 90.3% of the total variability in GY. The

Table 4.27. Mean performance, stress tolerance and yield stability indices of sixteen PVA maize genotype evaluated under managed drought stress and well-watered condition in 2018/2019 and 2019/2020 at Ikenne, Nigeria

Entry	Grain yield (t/ha)	Days to anthesis (days)	Days to silking (days)	Anthesis-silking interval (days)	Plant height (cm)	Ear aspect (1-5)	Drought tolerance index	Yield stability index	% yield reduction	Grain yield (t/ha)	Days to anthesis (days)	Days to silking (days)	Anthesis-silking interval (days)	Plant height (cm)	Ear aspect (1-5)
	Managed drought stress									Well-watered condition					
$PVASYNHGAC_0$	1.6	56.0	60.1	4.1	145.1	3.1	0.40	0.44	54.3	3.5	55.4	57.5	2.1	154.9	2.9
$PVASYNHGAC_1$	1.6	55.9	59.3	3.4	139.6	2.9	0.43	0.38	57.9	3.8	54.4	56.5	2.1	165.4	2.7
$PVASYNHGAC_2$	1.6	55.6	58.4	2.8	150.8	2.8	0.41	0.47	52.9	3.4	54.8	56.8	2.0	172.3	2.6
$PVASYNHGBC_0$	1.6	55.0	57.9	2.9	148.9	2.8	0.50	0.36	50.0	3.2	55.0	56.9	1.9	167.1	2.9
$PVASYNHGBC_1$	1.4	56.0	59.5	3.5	129.4	3.0	0.38	0.36	65.9	4.1	55.9	58.1	2.3	157.6	2.6
$PVASYNHGBC_2$	1.6	55.4	58.0	2.6	136.1	2.9	0.41	0.44	55.6	3.6	54.0	55.6	1.6	162.9	2.4
$PVASYNHGBC_0/PVASYNHGAC_0$	2.2	55.1	57.6	2.5	113.6	2.8	0.62	0.51	54.2	4.8	53.9	56.0	2.1	174.0	2.3
$PVASYNHGBC_1/PVASYNHGAC_0$	1.3	56.5	60.6	4.1	140.5	3.0	0.32	0.32	69.8	4.3	54.4	56.3	1.9	157.0	2.5
$PVASYNHGBC_2/PVASYNHGAC_0$	2.1	55.3	57.9	2.6	157.6	2.9	0.55	0.55	57.1	4.9	54.4	56.1	1.8	171.0	2.3
$PVASYNHGBC_0/PVASYNHGAC_1$	2.3	55.0	57.6	2.6	139.8	2.6	0.70	0.53	52.1	4.8	54.1	56.1	2.0	170.4	2.4
$PVASYNHGBC_1/PVASYNHGAC_1$	1.7	56.0	59.1	3.1	151.3	2.9	0.43	0.46	55.3	3.8	55.5	57.5	2.0	158.0	2.8
$PVASYNHGBC_2/PVASYNHGAC_1$	1.8	54.5	57.1	2.6	150.5	2.9	0.46	0.49	63.3	4.9	54.0	55.9	1.9	175.5	2.3
$PVASYNHGBC_0/PVASYNHGAC_2$	1.4	55.5	58.6	3.1	130.9	3.0	0.35	0.34	60.0	3.5	54.9	56.8	1.9	167.6	2.7
$PVASYNHGBC_1/PVASYNHGAC_2$	2.7	55.0	57.8	2.8	140.9	2.5	0.72	0.81	31.4	5.1	54.6	56.5	1.9	173.9	2.4
$PVASYNHGBC_2/PVASYNHGAC_2$	1.8	55.1	57.8	2.6	152.0	2.8	0.48	0.47	52.6	3.8	54.0	56.0	2.0	172.9	2.3
PVASYN13	2.4	55.0	57.8	2.8	161.8	2.6	0.63	0.61	47.1	3.5	55.0	56.9	1.9	164.8	2.6
Mean	1.8	55.4	58.4	3.0	143.0	2.9	0.49	0.47	56.1	4.1	54.6	56.6	2.0	166.6	2.6
SED (0.05)	0.3	0.5	0.7	0.6	17.0	0.2	0.1	0.1	-	0.4	0.4	0.4	0.2	5.5	0.2
CV (%)	28.2	1.8	2.5	37.2	23.8	11.7	34.2	41.6	-	19.9	1.4	1.3	19.1	6.5	14.4
Heritability	0.4	0.5	0.7	0.4	0.0	0.4	0.20	0.64	-	0.8	0.8	0.7	0.0	0.6	0.1

SED: Standard error of difference, CV: Coefficient of variation

(a) **Drought stress condition** (b) **Well-watered condition**

Plate 4.1. Field trial of provitamin-A maize genotypes evaluated under (a) managed drought stress and (b) well-watered conditions in 2019 dry seasons at IITA station, Ikenne, Nigeria

(a_1) **Drought stress condition**

(a_2) **Well-watered condition**

PVASYNHGBC$_1$/PVASYNHGAC$_0$

(b_1) **Drought stress condition**

(b_2) **Well-watered condition**

PVASYNHGBC$_1$/PVASYNHGAC$_2$

Plate 4.2. The worse performing varietal-cross hybrid (PVASYNHGBC$_1$/PVASYNHGAC$_0$) and the best performing varietal-cross hybrid (PVASYNHGBC$_1$/PVASYNHGAC$_2$) evaluated under (1) managed drought stress and (2) well-watered conditions in 2018 and 2019 dry seasons at IITA station, Ikenne, Nigeria

(a$_1$) **Drought stress condition**

(a$_2$) **Well-watered condition**

PVASYNHGBC$_0$/PVASYNHGAC$_2$

(b$_1$) Drought stress condition

(b$_2$) Well-watered condition

PVASYNHGBC$_2$/PVASYNHGAC$_2$

Plate 4.3. Maize ears of PVASYNHGBC$_0$/PVASYNHGAC$_2$ and PVASYNHGBC$_2$/PVASYNHGAC$_2$ evaluated under (1) managed drought stress and (2) well-watered conditions in 2018 and 2019 dry seasons at IITA station, Ikenne, Nigeria

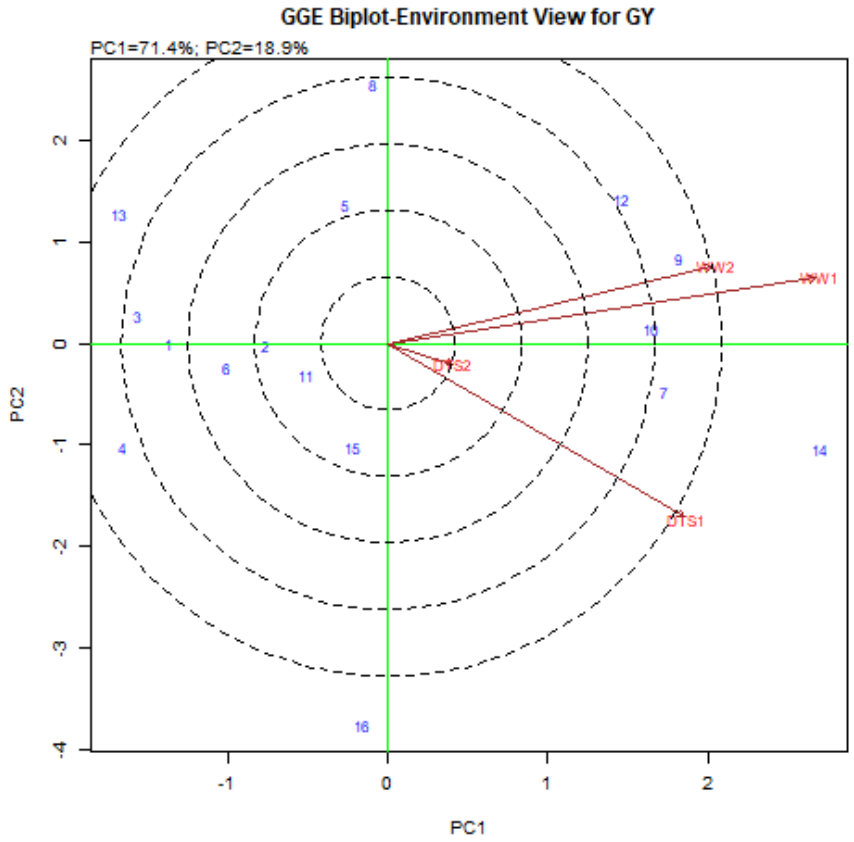
(a1) Drought stress condition PVASYNHGBC$_2$

(a$_2$) Well-watered condition

(b$_1$) Drought stress condition Check veriety (PVASYN13)

(b$_2$) Well-watered condition

Plate 4.4. Maize ears of PVASYNHGBC$_2$ and a check variety (PVASYN13) evaluated (1) under managed drought stress and (2) well-watered conditions in 2018 and 2019 dry seasons at IITA station, Ikenne, Nigeria

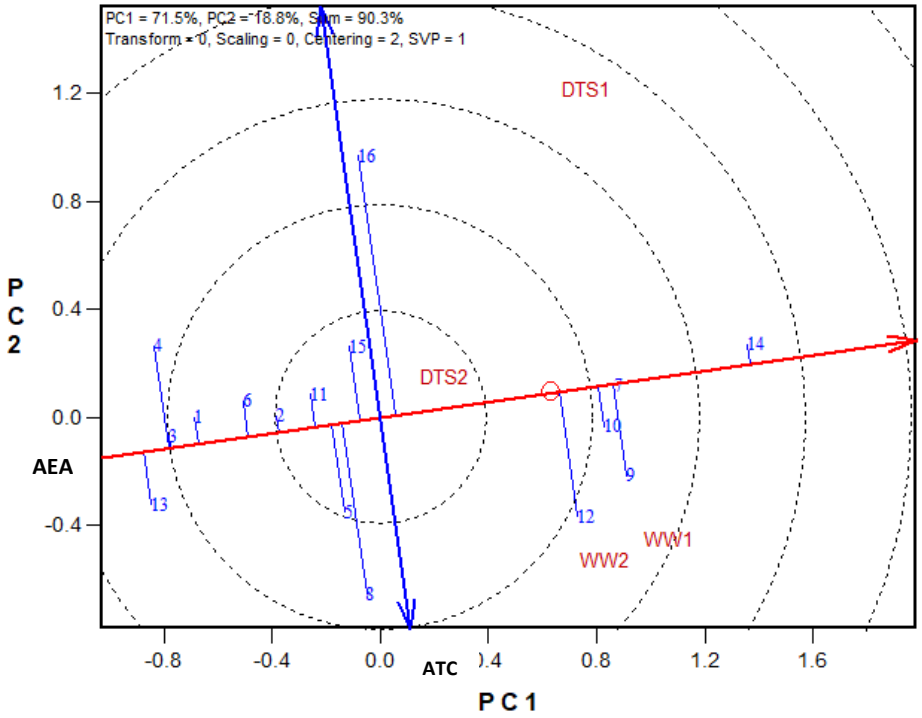


Key

Entry	Pedigree
1	$PVASYNHGAC_0$
2	$PVASYNHGAC_1$
3	$PVASYNHGAC_2$
4	$PVASYNHGBC_0$
5	$PVASYNHGBC_1$
6	$PVASYNHGBC_2$
7	$PVASYNHGBC_0/PVASYNHGAC_0$
8	$PVASYNHGBC_1/PVASYNHGAC_0$
9	$PVASYNHGBC_2/PVASYNHGAC_0$
10	$PVASYNHGBC_0/PVASYNHGAC_1$
11	$PVASYNHGBC_1/PVASYNHGAC_1$
12	$PVASYNHGBC_2/PVASYNHGAC_1$
13	$PVASYNHGBC_0/PVASYNHGAC_2$
14	$PVASYNHGBC_1/PVASYNHGAC_2$
15	$PVASYNHGBC_2/PVASYNHGAC_2$
16	PVASYN13

PC: Principal component, DTS: Drought stress, WW: Well-watered environment

Figure 4.5. The environment-vector view of GGE biplot for grain yield of sixteen provitamin-A maize genotypes evaluated under drought stress (DTS) and well-watered conditions (WW) in 2018 and 2019 at Ikenne, Nigeria



Key

Entry	Pedigree
1	$PVASYNHGAC_0$
2	$PVASYNHGAC_1$
3	$PVASYNHGAC_2$
4	$PVASYNHGBC_0$
5	$PVASYNHGBC_1$
6	$PVASYNHGBC_2$
7	$PVASYNHGBC_0/PVASYNHGAC_0$
8	$PVASYNHGBC_1/PVASYNHGAC_0$
9	$PVASYNHGBC_2/PVASYNHGAC_0$
10	$PVASYNHGBC_0/PVASYNHGAC_1$
11	$PVASYNHGBC_1/PVASYNHGAC_1$
12	$PVASYNHGBC_2/PVASYNHGAC_1$
13	$PVASYNHGBC_0/PVASYNHGAC_2$
14	$PVASYNHGBC_1/PVASYNHGAC_2$
15	$PVASYNHGBC_2/PVASYNHGAC_2$
16	PVASYN13

PC: Principal component, SVP: Singular value partitioning, AEA: Average environment axis, ATC: Average tester coordinate

Figure 4.6. The Genotype-vector view of GGE biplot for grain yield of sixteen provitamin-A maize genotypes evaluated under drought stress (DTS) and well-watered conditions (WW) in 2018 and 2019 at Ikenne, Nigeria

GGE biplot environment view (Figure 4.5) indicated that two VH ($PVASYNHGBC_0/PVASYNHGAC_0$ and $PVASYNHGBC_1/PVASYNHGAC_2$) were well adapted to drought stress condition, while $PVASYNHGBC_2/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$ and $PVASYNHGBC_2/PVASYNHGAC_1$ were well adapted to well-watered environments. The well-watered condition of 2018 (WW1) was the most discriminating, informative and representative of all the environments, followed by 2018 drought stress (DTS1) and 2019 well-watered environments (WW2), whereas 2019 drought stress (DTS2) was the least discriminating environment (Figure 4.5).

The red single-arrowed line (average environment axis, AEA) of the GGE biplot points to the highest mean yield across environments, and the concentric circle on the axis is the average environment, while the blue double-arrowed line (average tester coordinate, ATC) points to greater instability of genotypes in either direction (Figure 4.6). Based on the aforementioned background, in ranking of the genotypes for grain GY across the test environments, $PVASYNHGBC_1/PVASYNHGAC_2$ was the best, followed by $PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_2/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$ and $PVASYNHGBC_2/PVASYNHGAC_1$ having mean yields higher than the check and the trial average (Figure 4.6). All the SCs and four VH had means lower than the check and trial average with $PVASYNHGBC_0/PVASYNHGAC_2$ being the worse performing genotype across the test environments. The genotype with a relatively high yield and most stable across all environments was PVASYNHGBC0/PVASYNHGAC0, and thus the 'idea genotype' (Figure 4.6). This is followed by $PVASYNHGBC_1/PVASYNHGAC_2$. Although $PVASYNHGAC_0$, $PVASYNHGAC_1$, $PVASYNHGAC_2$ and $PVASYNHGBC_2$ had relatively lower yield than the check, they were however more stable across the test environments (Figure 4.6).

4.14 Heterosis for grain yield under drought stress and well-watered condition

The VH differed in heterosis for GY both in magnitude and direction under MDS and WWC. Three VH ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$ and $PVASYNHGBC_1/PVASYNHGAC_2$) expressed significant MPH under MDS, while two of the hybrids ($PVASYNHGBC_0/PVASYNHGAC_1$ and $PVASYNHGBC_1/PVASYNHGAC_2$) exhibited significant positive better-parent heterosis (BPH) under the water deficit

condition (Table 4.28). Conversely, two VH (PVASYNHGBC$_1$/PVASYNHGAC$_0$ and PVASYNHGBC$_0$/PVASYNHGAC$_2$) expressed negative MPH, BPH and significant negative standard heterosis (STH) for GY under MDS. On the other hand, five VH expressed significant positive MPH and STH for GY under WWC. Two VH (PVASYNHGBC$_0$/PVASYNHGAC$_0$ and PVASYNHGBC$_2$/PVASYNHGAC$_0$) also had significant positive BPH (Table 4.28).

4.15 Relationship between GY and agronomic traits under MDS and WWC

Pearson's correlation coefficients show that the relationship between GY and the flowering traits under the two water regimes were negative but significant only under MDS (Table 4.29). Nonetheless, under both test conditions, the relationships of GY with PA and EA were significant and similar. In addition, the associations of GY with DTI and YSI were significant and positive. Under drought stress, all the agronomic traits, except PH, were significantly correlated with each other. On the other hand, ASI had no significant relationship with any trait under WWC, except DS.

Table 4.28. Percentage heterosis for grain yield of nine maize varietal-cross hybrids derived from selction cycles of two maize synthetics evaluated under drought stress and well-watered conditions in 2018 and 2019 dry season at Ikenne, Nigeria

Hybrids	Managed drought stress			Well-watered condition		
	MPH	BPH	STH	MPH	BPH	STH
$PVASYNHGBC_0/PVASYNHGAC_0$	33.33*	32.52	-8.33	42.94**	37.97*	37.14*
$PVASYNHGBC_1/PVASYNHGAC_0$	-16.45	-21.12	-45.83**	14.13	4.85	22.86
$PVASYNHGBC_2/PVASYNHGAC_0$	30.86	30.06	-12.50	39.94**	36.84*	40.00*
$PVASYNHGBC_0/PVASYNHGAC_1$	43.13*	40.49*	-4.17	37.07*	27.20	37.14*
$PVASYNHGBC_1/PVASYNHGAC_1$	12.67	7.64	-29.17*	-2.67	-7.04	8.57
$PVASYNHGBC_2/PVASYNHGAC_1$	11.25	9.20	-25.00	32.88*	30.40	40.00*
$PVASYNHGBC_0/PVASYNHGAC_2$	-13.93	-14.72	-41.67**	4.96	1.45	0.00
$PVASYNHGBC_1/PVASYNHGAC_2$	74.92***	65.63**	12.50	34.92*	23.79	45.71*
$PVASYNHGBC_2/PVASYNHGAC_2$	11.46	10.43	-25.00	8.94	6.37	8.57
SED (0.05)	0.30	0.34	0.34	0.50	0.58	0.58

*, **, ***: $p \leq 0.05$, 0.01 and 0.001, MPH: Mid-parent heterosis, BPH: Better-parent heterosis, STH: Standard heterosis, SED: Standard error of difference

Table 4.29. **Pearson's Correlation coefficients of grain yield and agronomic traits of sixteen provitamin-A maize genotypes evaluated under drought stress (above the diagonal) and well-watered condition (below the diagonal) in 2018 and 2019 dry seasons at Ikenne, Nigeria**

	Grain yield	Days to anthesis	Days to silking	Anthesis-silking interval	Plant height	Plant aspect	Ear aspect	Drought tolerance index	Yield stability index
Grain yield	-	-0.64**	-0.65**	-0.59*	0.17	-0.79***	-0.84***	0.96***	0.93***
Days to anthesis	-0.44	-	0.96***	0.83***	-0.18	0.71**	0.62*	-0.67***	-0.53*
Days to silking	-0.40	0.98***	-	0.96***	-0.14	0.71**	0.67***	-0.67***	-0.55*
Anthesis-silking interval	-0.05	0.40	0.59*	-	-0.10	0.66**	0.67***	-0.60**	-0.51*
Plant height	0.46	-0.65**	-0.63**	-0.21	-	-0.29	-0.18	0.06	0.23
Plant aspect	-0.79**	0.59*	0.57*	0.21	-0.83***	-	0.89***	-0.86***	-0.68***
Ear aspect	-0.71***	0.76***	0.75***	0.31	-0.68***	0.83***	-	-0.86***	-0.78***
Drought tolerance index	-	-	-	-	-	-	-	-	0.82***
Yield stability index	-	-	-	-	-	-	-	-	-

*, **, ***: $p \leq 0.05$, 0.01 and 0.001, respectively

CHAPTER 5
DISCUSSION

This research was carried out to obtain genetic information such as gene action, heritability estimates, combining abilities and predictability ratio for agronomic traits and carotenoid content of two maize synthetics (MS) improved for Provitamin-A (PVA) using high throughput markers in Marker-assisted Recurrent Selection (MARS). The effect of MARS on the agronomic performance, carotenoid content and combining ability of the selection cycles (SCs) of the two MS as well as heterosis of their crosses [Varietal-cross Hybrid (VH)] were assessed. The effects of drought stress on the agronomic performance and yield stability of the PVA-enriched maize genotypes included in this study was also assessed.

The significant difference among the genoypes for grain yield (GY), agronomic traits and PVA accumulation in the two years of study indicated that variation exists among the genotypes and thus selection can be made. The significant year effect for the agronomic and carotenoid traits could be due to differences in climatic and edaphic factors in the different locations in 2018 and 2019. The non-significant genotype × year, genotype × location and genotype × year × location effects on GY indicated that genotype was stable across test years and locations, suggesting that genotypes had broad adaptation to diverse field environments (Kutka, 2011). The significant genotype × location effect for days to anthesis (DA), days to silking (DS), βcarotene (βC) and PVA could be due to the variations in the climate conditions and soil nutrient status at the different locations (Menkir *et al*., 2015). It may have affected enzymatic activity in the pathway that synthesizes carotenoids (Kopsell and Kopsell, 2008; Efeoglua *et al*., 2009; Ali *et al*., 2010).

The significant genotype × year effects on βC and PVA signified that growing season affected accumulation of these carotenoids, in line with the findings of Egesel *et al*. (2003) and Menkir *et al*. (2015). The reduction in carotenoid content in 2019 compared to 2018 may probably be due to carotenoid degradation in storage before the next planting season. Consequently, efforts must be intensified to reduce variation due to season by enhancing efficiency of sample handling and storage prior to carotenoid

analysis to reduce carotenoid degredation arising from environmental effects (Pixley *et al*., 2013). Nevertheless, the significant Spearman's correlation coefficients among environments suggested that the genotype × environment interaction for βC and PVA were not of serious crossover types in most environments. This supports earlier findings which indicated that expression of βC and PVA are more affected by genotype and environment than by GEI effects (Menkir and Maziya-Dixon, 2004; Menkir *et al*., 2008; Suwarno *et al*., 2014). In addition, the stability of βCX in the present study is noteworthy as increased breeding effort for βCX as a means of enhancing PVA has been advocated, primarily because of its relative stability. Prasanna *et al*. (2020) recommended greater exploitation of βCX for increased PVA content due to its higher stability and bioavailability than βC. There is also the need to prioritise research to minimize carotenoid degradation, especially βC in maize.

Upon partitioning of genotypic effect, significant HGA and HGB cycle (GCA) effects for GY, some agronomic traits and most measured carotenoids including PVA indicated that additive effects were important in the maize synthetics and selection cycles improved through MARS. Also, the significant specific combining ability (SCA) for GY and most agronomic traits signified the presence of non-additive gene effects in the inheritance of these traits (Hallauer *et al*., 2010; Badu *et al*., 2015). The significant hybrid effect for GY and PVA as well as most agronomic traits and carotenoids indicated that there was marked variation among the VH, suggesting that progress from selection and genetic gains can be made (Falconer and Mackay, 1996).

Variable environmental conditions have been reported to affect GY, agronomic traits and PVA carotenoid accumulation at different locations (Iseghohi *et al*., 2020; Menkir *et al*., 2021). The relative low GY and PVA content recorded at Ikenne (Tropical rainforest belt) compared to the yields and PVA contents obtained at Mokwa (Southern Guinea savannah) and Zaria (Sudan savannah) underscores the fact that climatic conditions, including cloud cover and soil nutrient status in Northern Nigeria support the development and GY of maize more than in the southern regions of the country, consistent with the report of Kamara *et al*. (2020). The relative decline of all carotenoids in 2019 compared to 2018 reaffirmed the instability of carotenoids as degradation occurs in storage, handling and processing. Study has revealed that biofortified endosperm maize kernel can lose up to 80% of PVA content after 12 months of storage at room temperature (Ortiz *et al*., 2018). Carotenoid degradation can

be minimized under cold storage at about -20°C which was used in this study. Therefore, the loses recorded in this study could be attributed to post harvest handling arising from harvesting, packaging, transport of harvested ears from fields to laboratory, and milling.

The significant improvement of PVA, βC and lutein (LUT) due to MARS in HGA but not in HGB indicated that MARS was efficient in improving the favourable alleles of beta carotene hydroxylase 1 (*crtRB1*) and lycopene epsilon cycles (*LYCE*) genes in HGA more than in HGB. This underscores the fact that effectiveness of MARS is genotype dependent. The associated decline in zeaxanthin (ZXT) and βcryptoxanthin (βCX) with increased PVA, βC and LUT in HGA accentuates the dichotomous activities in the alpha and beta branches of the carotenoid biosynthetic pathway as increased βC is predicted to decrease zeaxanthin while increased lutein is predicted to decrease βCX, due to increased hydroxylation of the alpha or beta carotene, consistent with Wurtzel *et al*. (2012). The marked enhancement in GY and some agronomic traits in HGB and the non-improvement of GY in HGA suggested that MARS had considerable effect on GY in HGB but not in HGA. The improvement in PVA in HGA and the concomitant none improvement of GY, and vice versa in HGB indicated an inverse relationship between PVA and GY in the respective MS. The result was similar to the report of Dhliwayo *et al*. (2014) who hypothesized that the negative correlated effect between GY and PVA could be as a result of high linkage disequilibrium for the traits and may have resulted in trade-off in GY for enhanced PVA level during selection.

Genetic gain in GY in HGB was lower than the results obtained by Vales *et al*. (2001) but higher than the gains in two open-pollinated maize cultivars studied by Dhliwayo *et al*. (2014). It is however difficult to compare the genetic gain in PVA obtained in this study with those of other studies as there is a dearth of reports of maize improved for PVA through high throughput PVA markers. Nevertheless, similar negative correlated effects have previously been reported between grain weight of maize and other nutritional contents such as oil content, protein content and starch content of maize kernels (Dudley and Lambert, 2004).

The *per se* mean performances of the selection cycles (SCs) of the MS buttress the effect of MARS for the various traits. The nonsignificant difference among the SCs of

HGA for GY indicated the non-effect of MARS on GY of HGA, whereas the enhanced yield performances of PVASYNHGBC$_1$ and PVASYNHGBC$_2$ underscores the gain in yield attributable to MARS in HGB, although significantly lower than the GY of PVASYN13. The advanced SC PVASYNHGAC$_2$ had the highest concentration of LUT, βC, PVA and TC among the SCs of HGA indicating the progress and effect of MARS in HGA. In HGB, the nonsignificant difference between PVASYNHGBC$_0$ and PVASYHGBC$_2$ signified the lack of genetic gain in LUT, βC, PVA and TC which may have been due to the inability to increase the favourable alleles of *crtRB1* and *LYCE* in HGB.

The *per se* performances of five VH which were not significantly different for GY and other agronomic trait but markedly outperformed the check variety signified that these VH harbour favourable genes which resulted in consistent performance in yield and agronomic traits. However, the outstanding agronomic performance in these VH which was associated with relative poor performance of the VH in PVA and βC emphasizes the linkage disequilibrium between enhanced PVA content and GY, following the pattern of the parental MS. Nonetheless, the two VH (PVASYNHGBC$_0$/PVASYNHGAC$_0$ and PVASYNHGBC$_0$/PVASYNHGAC$_1$) which had desirable GY and PVA content indicated that the VH could be simultaneously improved for both traits and commercialized for PVA maize seed delivery.

The mode of inheritance of a trait is important in any breeding programme as it has implications on the progress that can be made from selection (Njeri *et al*., 2017). One of the procedures used in determining genetic inheritance is estimation of combining ability of parental genotypes used in a breeding programme. Combining ability is the estimate of the performance of parental genotype in hybrid combination (Griffing, 1956; Fasahat *et al*., 2016). An inbred line or genotype could exhibit a General Combining Ability (GCA) or Specific Combining Ability (SCA) depending on the gene action of the trait (Sprague and Tatum 1942). In the hybridization process of the SCs of the two MS for the development of the VH, the proportional contribution for the expression of GY, DA and PH in the VH indicated that HGA contributed more gametes than HGB for these traits, whereas HGB effect was more in the expression of DS, PA, EA and most carotenoids. This result was consistent with the report of Derera *et al*. (2008) who stated that the contribution of GCA male or female to hybrids varied depending on the trait. Derera *et al*. (2008) reported equal contributions of male and

female parents for the inheritance of GY, whereas, in the present study, one parent (HGA) had more contribution than the other (HGB) for GY inheritance.

The significant GCA and SCA effects for GY and other agronomic traits in this study denoted that both additive and non-additive gene effects were relevant in the inheritance of these traits (Hallauer *et al*., 2010). On the other hand, the significant GCA but non-significant SCA effects for PVA and other carotenoids implied that there is preponderance of additive gene effects in the inheritance of maize carotenoids, consistent with previous studies on maize ILs (Muthusamy *et al*., 2016; Menkir *et al*., 2017; Azmach *et al*., 2021). However, the result is different from that of Suwarno *et al*. (2014) and Obeng-Bio *et al*. (2019) who reported the presence of additive and non-additive gene effects in the inheritance of PVA while Halilu *et al*. (2016) reported the preponderance of non-additive gene effects in the inheritance of PVA and other carotenoids. Although there are conflicting reports on the gene action of PVA and other carotenoids of diverse ILs, this study has provided additional information on the gene action and inheritance of maize carotenoids of MS. Furthermore, the moderate to high predictability ratio and narrow-sense heritability estimate for GY and agronomic traits underscores the presences of additive and nonadditive effects for these traits, while the high predictability ratio and narrow-sense heritability estimates for PVA and carotenoids, except αC reaffirms that these traits are governed by additive gene effects.

The knowledge of gene action of traits of interest in any breeding programme helps the researcher to choose suitable breeding procedure for genetic improvement, identify and select parents for crossing and estimate other relevant genetic parameters. The presence of both additive and non-additive gene effects for GY and most agronomic traits in this study implies these traits are predominantly governed by polygenes which are of continuous variation in nature and thus require appropriate breeding method such as RRS for population improvement. Meanwhile, the preponderance of the non-additive gene effect for GY suggests that the MS can further be enhanced through recurrent selection and targeted for heterosis breeding for hybrid commercialization. Nonadditive gene effect is described to be the main genetic base for heterosis breeding (Melchinger, 1999). On the other hand, the presence of mainly additive gene effect for PVA and other carotenoids indicates that MARS increased the frequency of favourable alleles for homozygosity. It also implies that genetic gain can be realized under selection as additive gene effect is the precondition for gain per SC; hence, is the

relative high genetic gain observed in LUT, βC, PVA and TC compared to GY and other agronomic traits in the present study. In intermating populations such as the SCs of the two MS, additive genetic variance is inexhaustible as continuous conversion of heterozygosity to homozygosity takes place (Fasoula and Fasoula, 1997). Furthermore, the preponderance of additive gene effect for PVA and most carotenoids provides useful information for the selection of suitable parents for hybridization. It enhances the efficiency of early generation testing as outstanding hybrids can be selected based on the prediction of the GCA effects of the parents (Melchinger *et al*., 1998; Badu-Apraku *et al*., 2015).

A significant or high GCA value whether positive or negative (depending on the trait) indicates that the mean of the parent is either higher or lower than the general mean of the crosses (Fasahat *et al*., 2016), while a low GCA value indicates the contrary. A high GCA value signifies transmission of desirable genes from parents to progenies. Therefore, the three SCs ($PVASYNHGAC_0$, $PVASYNHGAC_1$ and $PVASYNHGBC_2$) which had significant positive GCA effects for GY could serve as source populations for enhanced yield in hybrid development while $PVASYNHGBC_0$ which had positive GCA effect for the trait could further be improved for enhanced GCA through recurrent selection or targeted for synthetic breeding and composite variety development. In the same vein, $PVASYNHGAC_2$ and $PVASYNHGBC_0$ which had significant positive GCA effects for LUT, βC, PVA and TC could serve as source populations for ILs extractions for these carotenoid traits. They can also be used as potential testers for discriminating among ILs for possible topcross hybrid development. The two SCs ($PVASYNHGBC_0$ and $PVASYNHGBC_2$) which combined significant and positive GCA effects for GY with significant and positive GCA for βC and/or PVA can simultaneously be improved for these traits without the consequence of negative correlation effect. The negative correlation effects observed between GY and PVA carotenoids among some SCs could be attributed to linkage disequilibrium (Dhliwayo *et al*., 20014) during selection. The linkage can be broken through random mating of large population of each SC and subsequent selection.

The significant positive SCA effects observed in the four VH ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_2/PVASYNHGAC_0$, $PVASYNHGBC_2/PVASYNHGAC_1$ and $PVASYNHGBC_1/PVASYNHGAC_2$) and the positive SCA effect exhibited by $PVASYNHGBC_0/PVASYNHGAC_1$ for GY indicate

significant nonadditive gene effect in the VH. The significant positive/positive SCA effects of the five VH were derived from parents that exhibited significant positive/positive GCA effects, suggesting that the favourable SCA effects displayed by the VH for GY were derived from additive × additive gene action, consistent with Badu-Apraku *et al*. (2015). According to Fasahat *et al*. (2016), selection of parents based on SCA effect alone may not yield desired results in breeding programmes. Therefore, Badu-Apraku *et al*. (2015) suggested the use of SCA effect together with high *per se* performance of hybrid from atleast one parent with favourable GCA effect. In this study, the high SCA effects observed in the five VH were consistent with their high *per se* performances, indicating that these parents can be selected for hybrid development and commercialization to optimize heterosis for GY. The resultant significant Mid-parent Heterosis (MPH) for GY manifested in the five VH reaffirmed the relevance of nonadditive gene effect in the manifestation of heterosis.

The nonsignificant SCA effect for PVA carotenoids signified the absence of nonadditive gene effect, thus the lack of significant MPH for PVA content. However, the relatively low level MPH for PVA and βC observed in the crosses of $PVASYNHGBC_0$ i.e. ($PVASYNHGBC_0$/$PVASYNHGAC_0$, $PVASYNHGBC_0$/$PVASYNHGAC_1$ and $PVASYNHGBC_0$/$PVASYNHGAC_2$) indicates that $PVASYNHGBC_0$ harbours favourable alleles of *LYCE* and *CrtRB1* genes and thus can be improved through recurrent selection for high nonadditive gene effects and elevated magnitude of heterosis. According to Burt *et al*. (2011) heterosis for PVA carotenoids is rare in yellow and dent maize. They attributed the rarity to the influence of the QTLs controlling the flux of the pathway of the carotenoid biosynthesis, postulating that one branch of the pathway is usually favoured than the other. The MPH for PVA content observed in this study would have been higher if the pathway flux favoured the β-branch for enhanced βC and βCX as opposed to the α-branch which seems to have resulted in higher level of LUT. Furthermore, the lack of significant MPH for PVA content and other PVA carotenoids may have also resulted from hydroxylation of αC and βC to LUT and ZXT by *crtRB1* gene, thus the relative high levels of LUT and ZXT in the genotypes studied.

The proportion of the various carotenoids in the maize genotypes studied shows that the non-PVA content, xanthophylls (LUT and ZXT) makes up 50% of the total carotenoids, similar to previous reports (Menkir *et al*., 2014; Halilu *et al*., 2016;

Azmach *et al*., 2021) on the carotenoid profiles of tropical maize germplasm. The relatively high proportion of PVA (22%) attained in the endosperm of the maize genotypes under study compared to the PVA proportion of 16.9% (Menkir *et al*., 2014), 10.2% (Halilu *et al*., 2016), 13% (Azmach *et al*., 2019) of tropical maize germplasm and those of temperate origin (Egesel *et al*., 2003; Harjes *et al*., 2008; Senete *et al*., 2011; Muthusamy *et al.,* 2016), it is sufficient to say that appreciable level of genetic gain has been attained using MARS in this study. Therefore, the SCs identified as good combiners for GY and PVA and non-PVA carotenoids and the VH which exhibited significant MPH for GY and significantly outperformed the checks could be deployed in breeding programmes to combat VAD in SSA.

The non-significant correlation coefficients between GY and carotenoids including PVA content implied that the traits could silmutaneously be improved through MARS or phenotypic selection without marked decline in either trait. This is consistent with the results of Suwarno *et al*. (2014) but varied from the results of Ortiz-Covarrubias *et al*. (2019) who reported significant negative correlation between GY and PVA content. The perfect positive correlation between βC and PVA is consistent with previous results as βC is known to have the greatest PVA potential amongst maize carotenoids (Wurtzel *et al*., 2012; Menkir *et al*., 2014; Suwarno *et al*., 2014; Ortiz-Covarrubias *et al*., 2019). However, the strong positive correlation between LUT and βC/PVA was inconsistent with previous findings (Menkir *et al*., 2014; Suwarno *et al*., 2014; Ortiz-Covarrubias *et al*., 2019) as both carotenoids belong to different arms of the pathway that synthesize carotenoids (Wurtzel *et al*., 2012). This may have been due to activity of *LCYE* which facilitates high accumulation of LUT at the α-branch of the pathway at the expense of βC at β-branch with consequent effect of high hydroxylation of βC (Wurtzel *et al*., 2012). Linkage mapping and expression analyses showed that variation at *LCYE* locus altered pathway flux (Harjes *et al*., 2008). Nevertheless, Menkir *et al*. (2008) reported similar relationship between LUT and βC in maize inbred lines in two of five trials conducted.

The variations in global climatic conditions necessitate the need to develop climate-resilent crop varieties. Efforts to tackle the challenges posed by VAD in sub-Saharan Africa by developing PVA-enhanced maize varieties would be counter-productive if the varieties are highly susceptible to water stress. Therefore, evaluation of genotypes under varying stress conditions is important to select genotypes adapted to a wide

range of environments (Badu-Apraku *et al*., 2019). The significant mean squares of genotype (selection cycles, varietal-cross hybrids and the check) for GY and DS under MDS and WWC specified marked variation among the genotypes for these traits. This suggests that selection could be made under the different water regimes for enhanced yield. In addition, the significant year effects for grain yield and all or most agronomic traits in the two test conditions indicated that the seasons varied, possibly due to differences in soil and climatic conditions during field evaluation. The genotype × year interaction for grain yield under drought-stress and combined water regime suggested differential responses of the genotypes in each year of evaluation. It also inferred that a single-year evaluation for yield would not be sufficient. On the contrary, the absence of genotype × year interaction for all the agronomic traits measured under drought stress indicated that the genotypes were stable for these traits in the years of evaluation.

The higher GY recorded in 2018 than in 2019 under MDS could be ascribed to the relative high water deficit during field evaluation in 2019/2020 season, when compared to 2018/2019 season. Timing, intensity and uniformity of imposed moisture stress are key factors determining the effect of drought stress on grain yield and agronomic performance of maize (Bänziger et al. 2000; Zaidi 2019). Parental synthetics and varietal-cross hybrids which had anthesis-silking-interval of more than 3 days had significantly low yield, suggesting that drought stress caused delayed silking resulting in pollen asynchronization and subsequent kernnel abortion (Bänziger *et al*., 2000; Edmeades *et al*., 2000). Drought stress had little effect on days to anthesis, consistent with previous reports (Edmeades *et al*., 2000; Araus *et al*., 2012), but its effect on days to silking resulted in long anthesis-silking interval. Anthesis-silking interval longer than 3 days is likely to result in silk senescent, abortion following pollination, barreness, few grains per ear and general yield loss (Bänziger *et al*., 2000, Araus *et al*., 2012). In the present study, genotype with longer ASI had relatively lower yield than those that had shorter ASI, consistent with the findings of Ngugi *et al*. (2013).

The 56% yield reduction attributable to drought-stress in the present study indicated that the imposed drought stress targeted at flowering and grain filling stages was severe enough to discriminate among the genotypes. Previous studies showed that in maize, drought stress coinciding with flowering resulted in 17 to 60% yield losses

(Edmeades *et al*. 1999; Aslam *et al*. 2015), whereas drought stress at flowering and grain-filling stages caused yield losses of about 40 to 90% (Menkir and Akintunde 2001). In addition, adaptive mechanisms to moisture stress is said to be activated when imposed drought stress has the capacity to decrease GY by 30 – 50% (Edmeades *et al*. 2004). The percentage yield reduction observed in the present study is comparable to the yield loss reported in some previous studies (Menkir and Akintunde 2001; Adebayo and Menkir, 2014; Meseka *et al*., 2018) of tropical maize ILs and hybrids, but lower than the 78 and 79% yield losses reported among PVA maize hybrids (Manjeru 2017; Ortiz-Covarrubias *et al*. 2019). This suggested that some of the provitamin A-enriched maize genotypes included in this study exhibited improved tolerance to drought stress.

The significantly lower grain yield of the parental synthetics than the check variety, as well as their low to moderate STI indicated that the maize synthetics and their selection cycles, except PVASYNHGBC$_0$, did not exhibit high tolerance to drought stress, possibly because they were not originally selected for drought tolerance. However, they showed potentials for tolerance in their crosses, suggesting that they could be improved for drought tolerance through recurrent selection. The comparable performance of four varietal-cross hybrids (PVASYNHGBC$_0$/PVASYNHGAC$_0$, PVASYNHGBC$_2$/PVASYNHGAC$_0$, PVASYNHGBC$_0$/PVASYNHGAC$_1$ and PVASYNHGBC$_1$/PVASYNHGAC$_2$) with the check variety under drought stress, and their outstounding performances under well-watered condition suggested that these hybrids are adapted to different water regimes and could be explored for breeding drought tolerant maize lines. In addition, their moderate to high DTI and YSI indicated that the four varietal-cross hybrids were tolerant and stable across the test environments. Stress tolerance index has been used to identify and select drought tolerant genotypes in maize and other cereals (Oyekale *et al*., 2008; Anwar *et al*., 2011; Kondwakwenda *et al*., 2019; Santos *et al*., 2020). The DTI of the four varietal-cross hybrids in this study was similar to those reported by Oyekale *et al*. (2008) (DTI = 0.62), Kumar *et al*. (2016) (DTI = 0.64) and Kondwakwenda *et al*. (2019) (DTI = 0.58 - 0.77) among PVA maize inbred lines and tropical maize hybrids.

Maize genotypes of high and stable GY with desireable PVA concentration and adapted to multiple drought stresses of SSA are important for successful breeding programmes. The varietal-cross hybrids PVASYNHGBC$_0$/PVASYNHGAC$_0$ and

PVASYNHGBC$_1$/PVASYNHGAC$_2$ which were adapted to drought stress and PVASYNHGBC$_2$/PVASYNHGAC$_0$, PVASYNHGBC$_0$/PVASYNHGAC$_1$ and PVASYNHGBC$_2$/PVASYNHGAC$_1$ adapted to combined drought and well-watered conditions can be used as source populations for the development of inbred lines that are high in grain yield and provitamin A content and tolerant to drought stress. In addition, the relatively stable selection cycles, PVASYNHGAC$_0$, PVASYNHGAC$_1$, PVASYNHGAC$_2$ and PVASYNHGBC$_2$ can be further improved for increased grain yield and drought tolerance through recurrent selection. Recurrent selection in several diverse tropical maize populations for tolerance to drought at flowering over 2 to 10 cycles has increased grain yield under stress by about 100 kg /ha/cycle and reduced ASI by 0.6 days/ year (Edmeades *et al*., 2000).

The varying degrees of heterosis for grain yield under the different water regimes indicated the responses of the parents and varietal-cross hybrids in the two test conditions. The significant MPH of PVASYNHGBC$_0$/PVASYNHGAC$_0$, PVASYNHGBC$_0$/PVASYNHGAC$_1$ and PVASYNHGBC$_1$/PVASYNHGAC$_2$ for grain yield under the two water conditions indicated that these varietal-cross hybrids were well adapted to the two water regimes and can be used as sources of inbred lines to optimize heterosis under multiple water deficit environments. In addition, the varietal-cross hybrid PVASYNHGBC$_2$/PVASYNHGAC$_0$, which had relatively high MPH and BPH (> 30%) under drought stress and significant MPH, BPH and STH under well-watered condition, can be used as a commercial varietal-hybrid at an affordable cost for small-scale farmers. Under drought stress, the negative heterosis for grain yield expressed by varietal-cross hybrids (PVASYNHGBC$_1$/PVASYNHGAC$_0$ and PVASYNHGBC$_0$/PVASYNHGAC$_2$) reaffirmed their poor *per se* performances under moisture stress. Inbred-derived maize hybrids often exhibit higher heterosis under moisture deficit, specifically under serious drought, than under WW conditions (Betran *et al*., 2003; Naggar *et al*., 2016). This is because the differences in grain yield between hybrids and inbred lines increased with the intensity of drought stress, since inbred lines are more sensitive to environmental variations (Betran *et al*. 2003; Naggar et al. 2016). However, similar pattern was not observed for MPH and BPH for all the varietal-cross hybrids, probably because they were derived from synthetics, which are generally known to be more tolerant and adapted to drought stress than inbred lines (Kutka *et al*., 2011).

The significant association of GY with flowering traits under drought stress compared to the nonsignificant effect under well-water condition signified that drought stress imposed at flowering stage induced significant yield losses in the maize genotypes, consistent with the findings of Bänziger *et al*. (2000). Drought-stress on maize at flowering stage of the crop's life cycle delays silking and increases anhesis-silking interval, resulting in pollen-silk asynchronization (Edmeades *et al*., 2000). Anthesis-silking interval is a universal indicator of the level of drought stress, and a good predictor of grain yield and barrenness under stress (Edmeades *et al*., 2000). Several studies (Jensen 1971; Ribaut *et al*., 1996, 1997) have confirmed that indeed, a short ASI is a real measure of drought tolerance in maize. In addition, the significant positive correlation of GY with DTI and YSI indicated that the higher the drought tolerance and yield stability indices, the higher the grain yield, and vice versa. Therefore, selection for short ASI, earliness, and high DTI and YSI would be an indirect approach for selecting genotypes with high and stable yields under moisture deficit conditions.

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6. 1 **Summary and conclusion**

Vitamin A deficiency (VAD) and its associated effects are serious problems around the world, especially in sub-Saharan Africa (SSA) and South East Asia. Therefore, yellow and orange maize has been targeted for provitamin-A (PVA) enrichment to combat VAD because of its capacity to naturaly accumulate PVA carotenoids [αcarotene (αC), βcryptoxanthin (βCX) and βcarotene (βC)] in its kernel. Maize kernel also accumulates xanthophylls [lutein (LUT) and zeaxanthin (ZXT)], important dietary carotenoids which prevent or reduce cataracts in old people. Marker-assisted Recurrent (MARS) is a breeding procedure to enhance the frequency of favourable alleles.

In a study conducted at the International Institute of Tropical Agriculture (IITA), beta-carotene hydroxylase1 Kompetitive Allele Specific PCR (*crtRB1*-KASP) markers were used in a MARS of two maize synthetics (PVASYNHGA and PVASYNHGB). In the present study, the effects of MARS on the agronomic performance, carotenoid content, combining ability and heterosis of the maize synthetics were assessed. Sixteen maize genotypes including three selection cycles, each of the two maize synthetics, nine Varietal-cross Hybrids (VH) and a check variety (PVASYN13) were evaluated under Managed Drought Stress (MDS) and Well-watered Condition (WWC) to determine the effect of drought stress on the agronomic performance of the genetic materials. Summary and conclusion of the results are outlined below:

(i) The General Combining Ability (GCA) and Specific Combining Ability (SCA) effects of the maize synthetics and selection cycles were significant for GY and most agronomic traits measured, indicative that these traits were controlled by both additive and non-additive gene actions.

(ii) Only GCA effect of the maize synthetics and selection cycles was significant for PVA, βC, βCX, LUT and total carotenoids (TC), indicative that these traits were predominantly controlled by additive gene action.

(iii) The VH differed for GY, most agronomic traits, PVA content and most carotenoids, suggesting that improvement and selection can be made among the hybrids for increased PVA content and agronomic performance.

(iv) Marker-assisted recurrent selection (MARS) had little effect on GY and agronomic traits of the maize synthetics, probably because they were not targeted for agronomic improvement. However, MARS increased PVA, βC, LUT and TC by 15%, 25%, 26% and 8%, respectively in HGA, while ZXT and αC improved by 3% and 5%, respectively in HGB.

(v) Five varietal-cross hybrids ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_2/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$, $PVASYNHGBC_2/PVASYNHGAC_1$ and $PVASYNHGBC_1/PVASYNHGAC_2$) had GY that were significantly higher than the yield of the PVA-enriched check variety.

(vi) Three crosses of $PVASYNHGBC_0$ (i.e. $PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$ and $PVASYNHGBC_0/PVASYNHGAC_2$) and $PVASYNHGBC_2/PVASYNHGAC_2$ had similar PVA content as the check variety; while two of the hybrids ($PVASYNHGBC_0/PVASYNHGAC_2$ and $PVASYNHGBC_2/PVASYNHGAC_2$) had significant higher βC level than the check variety.

(vii) Four selection cycles ($PVASYNHGAC_0$, $PVASYNHGAC_1$, $PVASYNHGBC_0$ and $PVASYNHGBC_2$) had significant and positive GCA effects for GY. Two of the selection cycles ($PVASYNHGAC_2$ and $PVASYNHGBC_0$) also had significant and positive GCA effects for PVA, βC, LUT and TC.

(viii) Five varietal-cross hybrids ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_2/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$, $PVASYNHGBC_2/PVASYNHGAC1$ and $PVASYNHGBC_1/PVASYNHGAC_2$) had significant and positive SCA effects for GY and thus expressed significant and positive mid-parent heterosis for the trait.

(ix) Four varietal-cross hybrids ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_1/PVASYNHGAC_1$, $PVASYNHGBC_0/PVASYNHGAC_2$ and $PVASYNHGBC_2/PVASYNHGAC_2$) had positive SCA effects for PVA, βC and LUT. Two of these VH ($PVASYNHGBC_0/PVASYNHGAC_0$ and $PVASYNHGBC_0/PVASYNHGAC_2$) as well as

$PVASYNHGBC_0/PVASYNHGAC_1$ exhibited positive MPH for the carotenoid traits.

(x) The association between GY and PVA carotenoids was not significant. Therefore, both traits could be improved simultaneously in the genetic materials with no probable marked decline in either trait.

(x) Managed drought stress (MDS) targeted at the flowering and grain-filling stages of the maize genotypes resulted in a 56% reduction in GY, indicating that drought stress is a severe menace to maize farming in SSA.

(xi) The association between GY and the flowering traits was significant under MDS but not under WWC, suggesting that MDS targeted at flowering had significant impact on the GY. In addition, selection for early flowering and silking and short ASI under MDS is an indirect approach for selecting drought-tolerant maize genotypes.

(xii) Under MDS, three VH ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_2/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$ had similar GY, drought tolerance index (DTI) and yield stability index (YSI) as the drought-tolerant check, whereas one VH ($PVASYNHGBC_1/PVASYNHGAC_2$) had significant higher YSI and produced 12.5% more GY than the check. In addition, these hybrids had GY that were 37 to 46% higher than the check variety under WWC.

6.2 **Recommendations**

(i) Beta carotene hydroxylase-1 Kompetitive Allele Specific PCR (*crtRB1-KASP*) marker improved PVA, βC and LUT in PVASYNHGA, thus recommended for the improvement of PVA content in maize.

(ii) Three selection cycles ($PVASYNHGAC_0$, $PVASYNHGAC_1$ and $PVASYNHGBC_2$) had significant positive GCA effects for GY and thus recommended as source populations for inbred line developments for enhanced GY. $PVASYNHGAC_2$ and $PVASYNHGBC_0$ combined positive GCA effects for GY with PVA content and thus recommended as sources for ILs extractions for the combined traits.

(iii) Five VH ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_2/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$,

$PVASYNHGBC_2/PVASYNHGAC_1$ and $PVASYNHGBC_1/PVASYNHGAC_2$) are recommended for hybridization because they exhibited significant and/or positive SCA and MPH for for GY, and out-yielded the commercial check (PVASYN13). Four of the VH ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_2/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$ and $PVASYNHGBC_1/PVASYNHGAC_2$) also exhibited moderate to high DTI and YSI; thus, recommended for further testing under managed drought stress.

(iv) There was no significant SCA effect for PVA carotenoids, therefore significant heterosis was absent. However, three VH ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$ and $PVASYNHGBC_0/PVASYNHGAC_2$) exhibited positive SCA and MPH for PVA, βC and LUT, and thus recommended for improvement using reciprocal recurrent selection for larger magnitude of heterosis.

6.3 **Contributions to knowledge**

1. Marker-assisted Recurrent Selection (MARS) increased β-carotene and Provitamin-A (PVA) by 25.0% and 15.0%, respectively in maize synthetics (PVASYNHGA) and α-carotene by 5.0% in PVASYNHGB.

2. Both additive and nonadditive effects were important in the inheritance of grain yield and most agronomic traits of the maize synthetics.

3. Inheritance of PVA carotenoids in the maize synthetics was mainly controlled by additive gene effect.

4. Three varietal-cross hybrids ($PVASYNHGBC_0/PVASYNHGAC_0$, $PVASYNHGBC_0/PVASYNHGAC_1$ and $PVASYNHGBC_0/PVASYNHGAC_2$ were found to exhibit Mid-Parent Heterosis for α-carotene, β-cryptoxanthin, β-carotene and PVA content.

5. Drought stress reduced grain yield of the maize synthetics by 56.0%.

6. Simultaneous improvement of grain yield and PVA content could be achieved with no probable marked decline in either of them.

REFERENCES

Abdel-Aal, E.M., Akhtar, H., Zaheer, K. and Ali, R. 2013. Dietary sources of lutein and zeaxanthin carotenoids and their roles in eye health. *Nutrients 5*: 1169-1185, doi:10.3390/nu5041169

Abdel-Aal, E.S.M., Young, J.C., Akhtar, H. and Rabalski, I. 2010. Stability of lutein in whole grain bakery products naturally high in lutein or fortified with free lutein. *Journal of Agriculture and Food Chemistry 58*: 10109-10117

Abdulmalik, R.O., Menkir, A., Meseka, S.K., Unachukwu, N., Ado, S.G., Olarewaju, J.D., Aba, D.A., Hearne, S., Crossa, J. and Gedil, M. 2017. Genetic Gains in Grain Yield of a Maize Population Improved through Marker Assisted Recurrent Selection under Stress and Non-stress Conditions in West Africa. *Frontier in Plant Science* 8: 1-11, *doi:10.3389/fpls.2017.00841*

Abdulrahaman, A.A. and Kolawole, O.M. 2006. Traditional preparation and uses of maize in Nigeria. *Ethnobotany Leaflets* 10: 219-227

Adebayo, M.A. and Menkir, A. 2014. Assessment of hybrids of drought tolerant maize (*Zea mays* L.) inbred lines for grain yield and other traits under stress managed conditions. *Nigerian Journal of Genetics* 28: 19-23

Ahmed-Amal, O. and Mekki, B.B. 2005. Yield and yield components of two maize hybrids as influenced by water deficit during different growth stages. *Egyptian Journal of Applied Science* 20: 64-79

Ali, Q., Ashraf, M. and Anwar, F. 2010. Seed composition and seed oil antioxidant activity of maize under water stress. *Journal of the American Oil Chemist' Society* 87: 1179-187

Alfieri, M., Hidalgo, A., Berardo, N. and Redaelli, R. 2014. Carotenoid composition and heterotic effect in selected Italian maize germplasm. *Journal of Cereal Science* 59: 181-188

Allard, R.W. 1960. *Principles of Plant Breeding*. 2nd ed. New York. John Wiley and Sons Incorporation.

Analysis of Genetic Designs in R (AGD-R) 2015. International Maize and Wheat improvement Centre (CIMMYT), Mexico

Anjorin, O., Okpala, O. and Adeyemi, O. 2019. Coordinating Nigeria's micronutrient deficiency control programs is necessary to prevent deficiencies and toxicity

risks. *Annals of the New York Academy of Sciences* 446: 153-169, *doi: 10.1111/nyas.14055*

Anwar, J., Subhani, G., Hussain, M., Ahmad, J., Hussain, M. and Munir, M. 2011. Drought tolerance indices and their correlation with yield in exotic wheat genotypes. *Parkistan Journal of Botany* 43: 1527-1530

Araus, J.L., Serret, M.D. and Edmeades, G. 2012. Phenotyping maize for adaptation to drought. *Frontiers in Physiology* 3: 1-20, *doi:10.3389/fphys.2012.00305*

Aslam, M., Maqbool, M.A., Cengiz, R. 2015. Effects of drought on maize. *Drought stress in maize (Zea mays L.) Effects, resistance mechanisms, global achievements and biological strategies for improvement.* Aslam, M., Maqbool, M.A., Cengiz, R. Eds. *Springer*, New York. Chapter 2: 5-17

Astatke, D.K. 2018. Genetic gain in provitamin A and genetic diversity changes of two synthetic maize populations improved through marker assisted recurrent selection. M.Sc. Dissertation. Department of Agronomy, University of Ibadan; Life and Earth Institute, Pan African University, Nigeria. Xiv + 68

Azmach, G., Gedil, M., Spillane, C. and Menkir, A. 2021. Combining ability and heterosis for endosperm carotenoids and agronomic traits in tropical maize lines. *Frontiers in Plant Science* 12: 674089, doi: 10.3389/fpls.2021.674089.

Azmach, G., Menkir, A., Spillane, C. and Gedil, M. 2018. Genetic loci controlling carotenoids biosynthesis in diverse tropical maize lines. *Genes Genomics Genetics* 8: 1049-1065, doi: https://doi.org/10.1534/g3.117.300511

Azmach, G., Gedil, M., Menkir, A. and Spillane, C. 2013. Marker-trait association analysis of functional gene markers for provitamin A levels across diverse tropical yellow maize inbred lines. *Biomed Central Plant Biology* 13: 227-243

Baafi, E., Ofori, K., Carey, E.E., Gracen, V.E., Blay, T.E. and Manu-Aduening, J. 2017. Genetic control of βC, iron and zinc content in sweet potato. *Journal of Plant Studies* 6: 1-10, *doi:10.5539/jps.v6nlpl*

Babu, R., Rojas, N.P., Gao, S., Yan, J. and Pixley, K. 2013. Validation of the effects of molecular marker polymorphisms in LCYE and CrtRB1 on provitamin A concentrations for 26 tropical maize populations. *Theorical and Applied Genetics* 126: 389-399.

Badu-Apraku, B., Fakorede, M.A.B., Menkir, A. and Sanogo, D. Eds. 2012. Data collection in maize variety trals. *Conduct and management of maize field trials*. IITA, Ibadan, Nigeria. Chapter 6: 26-29

Badu-Apraku, B., Annor, B., Oyekunle, M., Akinwale, R. O., Fakorede, M. A. B., Talabi, A. O., Akaogu, I.C., Gedil, M. and Fasanmade, Y. 2015. Grouping of early maturing quality protein maize inbreds based on SNP markers and combining ability under multiple environments. *Field Crops Research* 183: 169-183, doi:10.1016/*j.fcr.2015.07.015*

Badu-Apraku, B., Fakorede, M.A.B., Talabi, A.O., Oyekunle, M., Aderounmu, M., Lum, A.F., Ribeiro, P.F., Adu, G.B. and Toyinbo, J.O. 2019. Genetic studies of extra-early provitamin-A maize inbred lines and their hybrids in multiple environments, *Crop Science* 1-20, *doi: 10.1002/csc2.20071*

Baker, R.J. 1978. Issues in diallel analysis. *Crop Science* 18:535-536

Bankole, F., Menkir, A., Olaoye, G., Crossa, J., Hearne, S., Unachukwu, N. and Gedil, M. 2017. Genetic gains in yield and yield related traits under drought stress and favourable environments in a maize population improved using marker-assisted recurrent selection. *Frontier in Plant Science* 1-10, *doi:10.3389/fpls.2017.00808*

Bänziger, M., Edmeades, G.O., Beck, D. and Bellon, M. 2000. *Breeding drought and nitrogen stress tolerance in maize: From Theory to Practice*. Mexico, DF CIMMYT. 69 pages

Bernstein, P.S., Li, B., Vachali, P.P., Gorusupudi, A., Shyam, R., Henriksen, B.S. and Nolan, J.M. 2016. lutein, zeaxanthin, and meso-zeaxanthin: The basic and clinical science underlying carotenoid-based nutritional interventions in ocular disease. *Progress in Retinal and Eye Research* 50: 34-66.

Below, F.E., Seebauer, J.R., Uribelarrea, M., Schneerman, M.C., and Moose, M.P. 2004. Physiological changes accompanying long-term selection for grain protein in maize. *Plant breeding reviews*. Janick, J. Ed. John Wiley & Sons, Hoboken, New Jersey. 133-152.

Bernardo, R. 1996. Testcross selection prior to further inbreeding in maize: mean performance and realized genetic variance. *Crop Science* 36:867-871.

Bernardo, R. 2008. Molecular markers and selection for complex traits in plants: learning from the last 20 years. *Crop Science* 48: 1649-1664, *doi: 10.2135/ cropsci2008.03.0131*

Betran, F.J. Ribaut, J.M., Beck, D. and Gonzalez de Leon, D. 2003. Genetic diversity, specific combining ability, and heterosis in tropical maize under stress and non-stress environments. *Crop Science* 43: 797-806

Beyene, Y., Semagn, K., Mugo, S., Prasanna, B.M., Tarekegne, A., Gakunga, J., Sehabiague, P., Meisel, B., Oikeh, S., Olsen, M. and Crossa, J. 2016. Performance and grain yield stability of maize populations developed using marker-assisted recurrent selection and pedigree selection procedures. *Euphytica* 208: 285-297

Bhutia, D.N., Seth, T., Shende, D.V., Dutta, S. and Chattopadhyay, Arup. 2015. Estimation of Heterosis, dominance effect and genetic control of freshfruit yield, quality and leaf curl disease severity traits of chilli pepper (*Capsicum annuum* L.). *Scientia Horticulturae* 182: 47-55

Biesalski, H. 2013. First international conference on hidden hunger, Hohenheim, Stuttgart, Germany March 6 – 9, 2013. *Food Security* 5: 457-473.

Bloem, M.W.M., Semba, R.D.R., and Kraemer, K. 2010. Castel Gandolfo workshop: an introduction to the impact of climate change, the economic crisis, and the increase in the food prices on malnutrition. *Journal of Nutrition* 140: 132-135

Blum, A. 1985. Breeding crop varieties for stress environments. *Critical Reviews in Plant Sciences* 2: 199-238.

Bojikoba, H. and Cokojiob, B. 2017. KASP genotyping technology and its use in genetic-breeding programs (a study of maize). *Plant Varieties Studying and Protect*ion 13: 131-40.

Bouis, H.E. and Welch, R.M. 2010. Biofortification – A sustainable agricultural strategy for reducing micronutrient malnutrition in the Global South. *Crop Science* 50: 20-32.

Bouslama, M. and W.T. Schapaugh, 1984. Stress tolerance in soybean. Part 1: Evaluation of three screening techniques for heat and drought tolerance. *Crop Sci*ence, 24: 933-937.

Burt, A.J., Grainger, C.M., Shelp, B.J. and Lee, E.A. 2011. Heterosis for carotenoid concentration and profile in maize hybrids. *Genome* 1004: 993-1004.

Carena, M. J. and Hallauer, A. R. 2001. Expression of heterosis in Leaming and Midland Corn Belt dent populations. *Journal of Iowa Academy of Science* 108: 73-8.

Chandler, K., Lipka, A.E., Owens, B.F., Edward, H.L., Buckler, S., Rocheford, T. and Gore, M.A. 2013. Genetic analysis of visually scored orange kernel color in maize. *Crop Science* 53: 189-200.

Chapman, S.C. and Edmeades, G. O. 1999. Selection improves tolerance to mid/late season drought in tropical maize populations. II. Direct and correlated responses among secondary traits. *Crop Science* 39: 1315-1324.

Chen, Z., Tang, D., Ni, J., Li, P., Wang, L., Zhou, J., Li, C., Lan, H., Li, L. and Liu, J. 2021. Development of genic KASP SNP markers from RNA-seq data for map-based cloning and marker-assisted selection in maize. *BMC Plant Biology* 21: 157. *https://doi.org/10.1186/s12870-021-02932-8.*

Chude, V.O., Daudu, C., Olayiwola, S.O. and Ekeoma, A. 2012. Fertilizer use and management practices for crops in Nigeria. Federal Fertilizer Department, Federal Ministry of Agriculture and Rural Development, Abuja, Nigeria. 247 pages.

Crosbie, T.M., Eathington, S.R., Johnson, G.R., Edwards, M., Reiter, R., Stark, S., Mohanty, R.G., Oyervides, M., Buehler, R.E., Walker, A.K., Dobert, R., Delannay, X., Pershing, J.C., Hall, M.A. and Lamkey, K.R. 2006. Plant breeding: past, present, and future. *Plant breeding*. Lamkey, K.R. and Lee, M. Eds. The Arnel R. Hallauer international symposium Blackwell Publishing, Ames, IA. 3-50

Combs, G.F. 2012. The Vitamins: Fundamental aspects in nutrition and health. 4th Edition. Academic Press San Diego, USA. 598 pages

Cunningham, F. X. and Gantt, E. 2001. One ring or two? Determination of ring number in carotenoids by lycopene epsilon-cyclases. *Proceedings of National Academy of Science* 98: 2905-2910

Derera, J., Tongoona, P., Vivek, B.S. and Laing, M. 2008. Gene action controlling grain yield and secondary traits in southern African maize hybrids under drought and non-drought environments. *Euphytica* 162: 411-422

Dhliwayo, T., Palacios-Roja, N., Crossa, J. and Pixley, V. 2014. Effects of S_1 Recurrent selection for provitamin A carotenoid content for three open-pollinated maize cultivars. *Crop Science* 54: 2449-2460

Doorenbos, J. and Pruitt, W.O. 1977. *Guidelines for predicting* crop *water requirements.* FAO Irrigation and Drainage Paper No. 24. Food and Agriculture Organization of the United Nations, Rome, Italy.

Dudley, J.W. 1982. Theory for transfer of alleles. *Crop Science* 22: 631-637

Dudley, J.W., Saghai-Maroof, M.A. and Rufener, G.K. 1991. Molecular markers and grouping of parents in a maize breeding program. *Crop Science* 31: 718-723.

Dudley, J.W. and Lambert, R.J. 2004. 100 generations of selection for oil and protein in corn. *Plant Breeding Reviews* 24: 79-110

East, E. M. 1936. Heterosis. *Genetics* 26: 375-97

Eathington, S. 2005. Practical applications of molecular technology in the development of commercial maize hybrids. *Proceedings of the 60th Annual Corn and Sorghum Seed Research Conferences*. American Seed Trade Association, Washington, DC

Eathington, S.R., Crosbie, T.M., Edwards, M.D., Reiter, R.S. and Bull, J.K. 2007. Molecular markers in commercial breeding. *Crop Science,* 47: 154-163.

Eberhart, S.A. 1964. Least squares method for comparing progress among recurrent selection methods. *Crop Science* 4: 230-231

Eberhart, S.A., Debela, S. and Hallauer. A. R. 1973. Reciprocal recurrent selection in the BSSS and BSCB1 maize varieties and half-sib selection in BSSS. *Crop Science* 13: 451-456

Edmeades, G.O., Bolaños, J., Elings, A., Ribaut, J.M., Bänziger, M. and Westgate, M.E. 2000. The role and regulation of the anthesis-silking interval in maize. *Physiology and modeling kernel set in maize*. Westgate, M.E. and Boote, K. Eds. CSSA, Madison, 43-73.

Edmeades, G.O. 2013. Progress in Achieving and Delivering Drought Tolerance in Maize - An Update, ISAAA: Ithaca, NY. 44 pages

Efeoglua, B., Ekmekcib, Y. and Cicekb, N. 2009. Physiological responses of three maize cultivars to drought stress and recovery. *South African Journal of Botany* 75:34–42

Egesel, C.O., Wong, J.C., Lambert, R.J. and Rocheford, T.R. 2003. Combining ability of maize inbreds for carotenoids and tocopherols. *Crop Science* 43: 818-823.

Eisenhauer, B., Natoli, S., Liew, G. and Flood, M.V. 2017. Lutein and zeaxanthin-Food Sources, Bioavailability and Dietary Variety in Age-Related Macular Degeneration Protection. *Nutrient* 9: 120, *doi: 10.3390/nu9020120*

Ekweagwu, E., Agwu, A.E. ad Madukwe, E. 2008. The role of micronutrients in child health: A review of the literature. *African Journal of Biotechnology* 7: 3804-3810

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S. and Mitchell, S.E. 2011. A Robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One*. 6: e19379

Falconer, D. S. and Mackay, T. F. C. 1996. *Introduction to Quantitative Genetics*, 4th ed. Edinburgh, UK. Longman Group.

FAOSTAT, 2021. http://www.fao.org/faostat/en/#data/QC, Retrieved 25/07/2023.

FAOSTAT, 2016. http://www.fao.org/faostat/en/#data/QC, Retrieved 26/4/2018

FAO, 2013. Drought. FAO land and water. 4 pages. www.fao.org/nr/aboutnr/nrl. Retrieved 26/03/2019

FAO/WHO 2006. Micronutrient malnutrition: a public health problem. *Guidelines on Food Fortification with Micronutrients.* Allen, L., Benoist, B., Dary, O. and Hurrell, R. Eds. World Health Organization and Food and Agricultural Organisation; Geneva, 48-52

FAO/WHO, 2004. Vitamin and Mineral Requirements in Human Nutrition 2nd ed. World Health Organization: Geneva. 362 pages

Fasahat, P., Rajabi, A., Rad, J.M. and Derera, J. 2016. Principles and Utilization of Combining Ability in Plant Breeding. *Biometric and Biostatistics International Journal* 4: 1-24: 00085. DOI: 10.15406/bbij.2016.04.00085

Fasoula, D.A. and Fasoula, V.A. 1997. Gene action and plant breeding. *Plant Breeding Reviews*. Janick, J. Ed. John Wiley & Sons, Inc. 315-374

Fennema, O. 2008. Fat-soluble vitamins. *Fennema's Food Chemistry*. Damodaran, S., Parkin, K.L. Eds. CRC Press, Taylor & Francis. 454-455

Fernandez, G.C.J. 1992. Effective selection criteria for assessing plant stress tolerance. *Proceedings of the International Symposium on Adaptation of Vegetables and other Food Crops in Temperature and Water Stress*, Taiwan, 13-16 August 1992; 257-270

Filho, 1999. Inreeding and heterosis. *The Genetics and Exploitation of heterosis in crops*. Proceedings of the International Symposium on the Genetics and Exploitation of Heterosis in Crops, CIMMYT, Mexico City, Mexico, 17-22 August, 1997. *American Society of Agronomy, Crop Science Society of America and Soil Science Society of America*, Madison, Wisconsin

Fu, Z., Chai, Y., Zhou, Y., Yang, X., Warburton, M.L., Xu, S., Cai, Y., Zhang, D., Li, J. and Yan, J. 2013. Natural variation in the sequence of PSY1 and frequency of

favorable polymorphisms among tropical and temperate maize germplasm. *Theorical and Applied Genetics* 126: 923-935.

Galicia, L., Miranda, A., Gutierrez, M.G., Custodio, O., Rosales, A., Ruiz, N., Surles, R. and Palacios, N. 2012. Laboratory of nutritional quality of corn and analysis of plant tissue: Laboratory protocols 2012. *Determination of carotenoids in corn by liquid chromatography*. Mexico, DF., CIMMYT. 58 pages

Garcia-Casal, M.N. 2006 Carotenoids increase iron absorption from cereal-based food in the human. *Nutrition Research* 26: 340-344

Gazal, A., Z.A., Dar, A.A.., Lone, I. and Abidi, A.G. 2015. Molecular breeding for resilience in maize. *Journal of Applied and Natural Science* 7: 1057-1063

Gebremeskel, S., Garcia-Oliveira, A.L., Menkir, A., Adetimirin, V. and Gedil, M. 2018. Effectiveness of predictive markers for marker assisted selection of pro-vitamin A carotenoids in medium-late maturing maize (*Zea mays* L.) inbred lines. *Journal of Cereal Science* 79: 27-34

Gedil, M. and Menkir, A. 2019. An Integrated Molecular and Conventional Breeding Scheme for Enhancing Genetic Gain in Maize in Africa. *Frontiers in Plant Science 10: 1430, doi: 10.3389/fpls.2019.01430*

Giuliano, G. 2017. Provitamin A biofortification of crop plants: a golden rush with many miners. *Current Opinion in Biotechnology* 44: 169-180.

Global Nutrition Report, 2017. Nourishing the Sustainable Development Goals (SDGs). Development Initiatives Poverty Research Ltd, Bristol, UK. 121 pages

Gokidi, Y., Bhanu, A. and Singh, M.N. 2016. Marker assisted recurrent selection: An overview. *Advances in Life Sciences* 5:6494-6499

Golabadi, M., Arzani, A. and Maibody, S.A.M.M. 2006. Assessment of drought tolerance in segregating populations in durum wheat. *African Journal of Agricultural Research* 1: 162-171

Gopi, J.S.T., and Hampannavar, M.R. 2018. Concept of heterotic groups and reciprocal recurrent selection in hybrid breeding. *Journal of Pharmacognosy and Phytochemistry* 7: 2504-2507

Griffing, B. 1956. A generalized treatment of the use of diallel crosses, in quantitative inheritance Heredity. *Australian Journal of Biological Sciences* 10: 31-50.

Grogan, C.O., Blessing, C.W., Dimler, R.J. and Campbell, C.M. 1963. Parental influence on xanthophylls and carotenes in corn. *Crop Science* 3: 213-214.

Gupta, H.S., Hossain, F., Muthusamy, V. and Zunjare, R.U. 2019. Marker-Assisted Breeding for enrichment of provitamin A in maize. In: Qureshi, A.M.I., Dar, Z.A., Wani, S.H (Eds). *Quality breeding in field crops*. *Springer Nature*, Switzerland, *https://doi.org/10.1007/978-3-030-04609-5_6*

Halilu, A.D., Ado, G., Aba, D.A. and Usman, S.I. 2016. Genetics of carotenoids for provitmain A biofortification in tropical-adapted maize. *The Crop Journal* 4: 313-322

Hall, A.E. 1993. Is dehydration tolerance relevant to genotypic differences in leaf senescence and crop adaptation to dry environments? *Plant Responses to cellular dehydration during environmental stress*. Close, T.J. and Bray, E.A. Eds. The American Soc. Plant Pathologists, Rockville, Maryland. 1-10.

Hallauer, A.R. and Filho, J.B.M. 1995. *Quantitative genetics in maize breeding*. 2nd edition. Iowa State Univ. Press, Ames

Hallauer, A. R. and Carena, M. J. 2009. Maize breeding. *Handbook of Plant Breeding: Cereals*. Carena, M.J. Ed. Pp. 3-98. *Springer*, New York, NY.

Hallauer, A.R., Filho, J.B.M. and Carena, M.J. 2010. Heterosis. *Quantitative Genetics in Maize Breeding.* Prohens, J., Nuez, F. and Carena, M.J. Eds. 3rd edition. *Springer* Science+Business Media, New York. Chapter 10:477-481

Hanson, B.R., Orloff, S. and Peters, D. 2000. Monitoring soil moisture helps refine irrigation management, *California Agriculture* 54: 38-43

Harjes, C.E., Rocheford, T.R., Bai, L., Brutnell, T.P., Vallabhaneni, R., Williams, M., Wurtzel, E.T., Kandianis, C.B., Sowinski, S.G., Stapleton, A.E., Yan, J. and Buckler, E.S. 2008. Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. *Science* 319: 330-333.

Hasanuzzaman, M., Hakeem, K.R., Nahar, K., Alharby, H. Eds. 2019. Water deficit stress effects and responses in maize. *Plant Abiotic stress tolerance: agronomic, molecular and biotechnological approaches*. *Springer Nature*, Switzerland. 129-144

Hauge, S.M. and Trost, J.F. 1928. An inheritance study of the distribution of vitamin A in maize. *Journal of Biological Chemistry* 80: 107-114.

He, C., Holme, J., Anthony, J. 2014. SNP Genotyping: The KASP Assay. *Crop Breeding: Methods and protocols*, *Methods in molecular Biology*. Fleury, D. and

Whiteford, R. Eds. 1145: 75-86, *Springer*, New York, USA. *DOI 10.1007/978-1-4939-0446-4_7*

Hefferon, K.L. 2015. Nutritionally Enhanced Food Crops; Progress and Perspectives. *International Journal of Molecular Science* 16: 3895-3914, *doi: 10.3390/ijms16023895*

Hensley, D. and Deputy, J. 1999. Using Tensiometer for measuring soil water and scheduling irrigation, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, pp. 1-4

Hentschel, V., Kranl, K., Hollmann, J., Lindhauer, M.G., Böhm, V. and Bitsch, R. 2002. Spectrophotometric determination of yellow pigment content and evaluation of carotenoids by high-performance liquid chromatography in durum wheat grain. *Journal of Agricultural and Food Chemistry* 50: 6663-6668

Howe, J.A. and Tanumihardjo, S.A. 2006. Carotenoid-biofortified maize maintains adequate vitamin A status in Mongolian gerbils. *Journal of Nutrition* 136: 2562-2567.

Ihsan, M.Z., El-Nakhlawy, F.S., Ismail, S.M., Fahad, S. and Daur, I. 2016. Phenological development and growth studies as affected by drought and late season high temperature stress under arid environment. *Frontiers in Plant Science* 7: 1-14

IITA, 2016. First generation provitamin A rich open-pollinated maize varieties released. http://www.iita.org/news-item/first-generation-pro-vitamin-rich-open-pollinated-maize-varieties-released/. Retrieved 8/05/2018

Kumar, B., Karjagi, C.G., Jat, S.L., Parihar, C.M., Yathish, K.R., Singh, V., Hooda, K.S., Dass, A.K., Mukri, G., Sekhar, J.C., Kumar, R. and Kumar, R.S. Eds. 2012. *Maize biology: An Introduction*. Indian Council of Agricultural Research (ICAR), New Delhi. 32 pages.

IPCC, 2013. Summary for policy makers. *Climate change 2013: the physical science basis*. Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V. and Midgley, P.M. Eds. Cambridge University Press, Cambridge, UK. 2-30.

Iseghohi, I., Abe, A., Meseka, S., Mengesha, W., Gedil, M. and Menkir, A. 2020. Assessing effect of marker-based improvement of maize synthetics on agronomic performance, carotenoid content, combining ability and heterosis. *Agronomy* 10: 1625. Doi: 10.3390/agronomy10111625

Ishida, B.K. and Chapman, M.H. 2012. Effects of a hydrodynamic process on extraction of carotenoids from tomato. *Food Chemistry* 132: 1156-1160

Ismail, A.M.A. 1991. Soil properties and moisture characteristics and their relationship with crop mid-day stress in the Sudan Gezira. *GeoJournal* 23: 233 – 237.

Jagosz, B. 2012. Combining ability of carrot (*Daucus carota* L.) lines and heritability of yield and its quality components. *Folia Horticultrae* 24: 115-122

Kaeppler, S. 2012. Heterosis: Many genes, many mechanisms-End the search for an undiscovered unifying theory. *International Scholarly Research Network-Botany*: 1-12, *doi:10.5402/2012/682824*

Kamara, A.Y., Kamai, N., Omoigui, L.O., Togola, A., Ekeleme, F. and Onyibe, J.E. Eds. 2020. Production and importance of maize in Nigeria. *Guide to maize production in Northern Nigeria*. IITA, Ibadan, Nigeria. 1-5.

Kandianis, B.C., Stevens, R., Liu, W., Palacios, N., Montgomery, K., Pixley, K., White, W.S. and Rocherford, T. 2013. Genetic architecture controlling variation in grain carotenoid composition and concentrations in two maize populations. *Theoritical Applied Genetics* 126: 2879-2895

Katepa-Mupondwa, F.M., Christie, B.R. and Michaels, T.E. 2002. An improved breeding strategy for autotetraploid alfalfa (*Medicago sativa* L.). *Euphytica* 123: 139-146.

Kaur, K., Dhall, R.K. and Chawala, N. 2016. Heterosis and combining ability for quality attributing traits in cucumber (*Cucumis sativus* L.). *International Journal of Agricultural Research* 53: 475-479

Kean, E.G., Hamaker, B.R. and Ferruzzi, M.G. 2008. Carotenoids bio-accessibility from whole grain and degermed maize meal products. *Journal of Agriculture and Food Chemistry 56*: 9918-9926

Khachik, F., Spangler, C.J., Smith, J.C., Canfield, L.M., Steck, A. and Pfander, H. 1997. Identification, quantification, and relative concentration of carotenoids and their metabolites in human milk and serum. *Analytical Chem*istry 69: 1873-1881

Khamkoh, W., Ketthaisong, D., Lomthaisong, K., Lertrat, K. and Suriham, B. 2019. Recurrent selection method for improvement of lutein and zeaxanthin in orange waxy corn populations. *Australian Journal of Crop Science* 13: 566-573

Kiesselbach, T. A. 1980. *The structure and reproduction of corn*. Historical Research

Bulletins of the Nebraska Agricultural Experiment Station, vol 161. Lincoln, Nebraska: University of Nebraska Press. Pp 93. http://digitalcommons.unl.edu/ardhistrb/284. Retrieved 16/03/2021.

Kolawole, A.O., Menkir, A., Gedil, M., Blay, E., Ofori, K. and Kling, J.G. (2017). Genetic divergence in two tropical maize composites after four cycles of reciprocal recurrent selection. *Plant Breeding* 136: 41-49

Kondwakwenda, A., Sibiya, J., Zengeni, R., Musvosvi, C. and Tesfay, S. 2019. Screening of provitamin-A maize inbred lines for drought tolerance using βC content: morpho-physiological and biochemical traits, *Agronomy* 9: 1-17, *doi: 10.3390/agronomy9110692*

Kopsell, D.A. and Kopsell, D.E. 2008. Genetic and environmental factors affecting plant lutein/zeaxanthin. *Agro Food Industry Hi-tech* 19: 44–46

Kurilich A. and Juvik J. 1999. Quantification of carotenoid and tocopherol antioxidants in *Zea mays*. *Journal of Agriculture and Food Chemistry* 47: 1948-1955.

Kutka, F. 2011. Open-pollinated vs. Hybrid maize cultivars. *Sustainability* 3: 1531-1554, *doi: 10.3390/su3091531*

LGC 2015. KASP genotyping technology. LGC Limited, London. 8 pages

Liu, K., Goodman, M., Muse, S.J., Smith, J.S., Buckler, E. and Doebley, J. 2003. Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. *Genetics* 165: 2117-2128

Liu, J., Qu, J., Yang, C., Tang, D., Li, J., Lan, H. and Rong, T. 2015. Development of genome-wide insertion and deletion markers for maize, based on next-generation sequencing data. *BMC Genomics* 16:601. *https://doi.org/10.1186/s12864-015-1797-5*

Lonnquist, J.H., 1949. The development and performance of synthetic varieties of corn. *Agronomy Journal* 41: 153-156

Lynch, M. and Milligan, B.G. Analysis of population genetic structure with RAPD markers 1994. *Molecular Ecology* 3: 91-99.

Manjeru, P. 2017. The influence of abiotic stress on CIMMYT provitamin-A elite maize germplasm. Diss. Plant Science, Natural and Agiricultural Sciences. University of the Free State, South Africa. Xii +178

Mares-Perlman, J.A., Millen, A.E., Ficek, T.L. and Hankinson, S.E. 2002. The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease: Overview. *The Journal of Nutrition* 132: 518S-524S.

Mbagwu, J.SC. 1985. Estimating dry-season crop water requirements from climatological and soil available water capacity data in the sedimentary and basement complex areas of Southern Nigeria. *Catena* 12: 201-209

McDermott, J.H. 2000. Antioxidant nutrients: current dietary recommendations and research update. *Journal of American Pharmacists Association*. 40: 785-799

Melchinger, A.E. and Gumber, R.K. 1998. Overview of heterosis and heterotic groups in agronomic crops. *Concepts and Breeding of Heterosis in Crop Plants*. Lamkey, K.R. and Staub, J.E. Eds. CSSA, Madison, Wisconsin. 29-44.

Melchinger, A. E. 1999. Genetic diversity and heterosis. *The genetics and exploitation of heterosis in crops*. Coors, J. G. and Pandey, S. Eds. American Society of Agronomy-Crop Science Society of America, Madison, WI. 99-118.

Melchinger, A., Schmit, W. and Geiger, H.H. 1998. Comparison of testcrosses from F_2 and first backcross populations in maize. *Crop Science* 28(5): 743-749.

Mengesha, W. and Maru, J. 2017. Facts on provitamin A (PVA) maize in Nigeria. Building Nutritious food baskets, IITA publication. 4 pages

Menkir, A. and Akintude, A.O. 2001. Evaluation of the performance of maize hybrids, improved open-pollinated and farmer's local varieties under well-watered and drought stress conditions. *Maydica* 46: 227-238.

Menkir, A. and Maziya-Dixon, B. 2004. Influence of genotype and environment on βC content of tropical yellow-endosperm maize genotypes. *Maydica* 49: 313-318

Menkir, A. and Kling, J.G. 2007. Response to recurrent selection for resistance to *striga hermonthica* (Del.) Benth in a tropical maize population. *Crop Science* 47: 674-684

Menkir, A., Liu, W., White, W.S., Maziya-Dixon, B. and Rocheford, T. 2008. Carotenoid diversity in tropical-adapted yellow maize inbred lines. *Food Chemistry* 109: 521-529

Menkir, A., Gedil, M., Tanumihardjo, S., Adepoju, A. and Bossey, B. 2014. Carotenoid accumulation and agronomic performance of maize hybrids involving parental combinations from different marker-based groups. *Food Chemistry* 148: 131-137

Menkir, A., Rocherford, T., Maziya-Dixon, B. and Tanumihardjo, S. 2015. Exploiting natural variation in exotic germplasm for increasing provitamin-A carotenoids in tropical maize. *Euphytica* 205:203-217

Menkir, A., Maziya-Dixon, B., Mengesha, W., Rocheford, T. and Alamu, E.O. 2017. Accruing genetic gain in pro-vitamin A enrichment from harnessing diverse maize germplasm. *Euphytica* 213:105

Menkir, A., Dieng, I., Mengesha, W., Meseka, S., Maziya-Dixon, B., Alamu, O.E., Bossey, B., Muhyideen, O., Ewool, M. and Coulibaly, M.M. 2021. Unravelling the effect of provitamin A enrichmentment on agronomic performance of tropical maize hybrids. *Plants* 10, 1580.https://doi.org/10.3390/plants10081580.

Meseka, S., Menkir, A. and Obeng-Antwi, K. 2015. Exploitation of beneficial alleles from maize (*Zea mays* L.) landraces to enhance performance of an elite variety in water stress environments. *Euphytica* 201: 149-160

Meseka, S., Menkir, A., Bossey, B. and Mengesha, W. 2018. Performance assessment of drought tolerance maize hybrids under combined drought and heat stress. Agronomy 8: 1-17, *doi: 10.3390/agronomy8120274*

Meyers , K.J., Mares, J.A., Igo, R.P., Truitt, B., Liu, Z., Millen, A.E., Klein, M., Johnson, E.J., Engelman, C.D., Karki, C.K., Blodi, B., Gehrs, K., Wallace, L.R., Robinson, J., LeBlanc, E.S., Sarto, G., Bernstein, P.S., SanGiovanni, J.P. and Iyengar, S.K. 2014. Genetic evidence for role of carotenoids in age-related macular degeneration in the Carotenoids in Age-Related Eye Disease Study (CAREDS). *Investigative OPHhamology and Visual Science* 55: 587-599.

Mitra, J. 2001. Genetics and genetic improvement of drought resistance in crop plants. *Current Science*, 80: 758-762.

Moreau, L., Charcosset, A. and Gallais, A. 2004. Experimental evaluation of several cycles of marker-assisted selection in maize. *Euphytica* 137: 111-118.

Muthayya, S., Hyu Rah, J., Sugimoto, J., Roos, F., Kraemer, K. and Black, R. 2013. The global hidden hunger indices and maps: An advocacy tool for action. *PLoS ONE 8: E67860, doi:10.1371/ journal.pone.0067860*

Muthusamy, V., Hossain, F., Thirunavukkarasu, N., Saha, S. Agrawal, P.K. and Gupta, H.S. 2016. Genetic analyses of kernel carotenoids in novel maize genotypes possessing rare allele of *β*-carotene hydroxylase gene. *Cereal Research Communications* 44: 669-680.

Naggar, A.M., Atta, M.M., Ahmed, M.A. and Younis, A.S. 2016. Heterosis and combining ability of maize (*Zea mays* L.) grain protein, oil and starch content and yield as affected by water stress. *Archives of Current Research International* 4: 1-15.

NASC, 2017. 2017 Annual Report of the National Agricultural Seeds Council of Nigeria. *NASC*, Abuja, 54 pp.

Neelam, K., Brown-Guedira, G., Huang, L. 2013. Development and validation of a breeder-friendly KASPar marker for wheat leaf rust resistance locus Lr21. *Molecular Breeding* 31:233-237

Ngugi, K., Cheserek, J., Muchira, C. and Chemining'wa, G., 2013. Anthesis to Silking Interval Usefulness in Developing Drought Tolerant Maize. *Journal of Renewable Agriculture* 1:84-90, doi:10.12966/jra.08,03.2013.

Njenga, P., Richard, E. and Joseph, K.2014. Combining ability for βC and important quantitative traits in a cassava F_1 population. *Journal of Plant Breeding and Science* 6: 24-30

Njeri, S.G., Makumbi, D., Warburton, M.L., Diallo, A., Jumbo, M. and Chemining'wa, G. 2017. Genetic analysis of tropical quality protein maize (Zea mays L.) germplasm. *Euphytica* 213:1-19, *doi.org/10.1007/s10681-017-2048-4*

Nuss, E. T. and Tanumihardjo, S. A. 2011. Quality protein maize for Africa: closing the protein inadequacy gap in vulnerable populations. *Advances in Nutrition*. 2: 217–224, *doi: 10.3945/an.110.000182*

Obeng-Bio, E., Badu-Apraku, B., Ifie, B.E., Danquah, A., Blay, E.T. and Annor, B. 2019. Genetic analysis of grain yield and agronomic traits of early provitamin A quality protein maize inbred lines in contrasting environments. *The Journal of Agricultural Science* 1-21, *https://doi.org/10.1017/S0021859619000753*

Obeng-Bio, E., Badu-Apraku, B., Ifie B.E., Danquah, A., Blay, E.T. and Dadzie, M.A. 2020. Phenotypic characterization and validation of provitamin a functional genes in early maturing provitamin A-quality protein maize (*Zea mays*) inbred lines. *Plant Breed*ing 139: 575–88. *https://doi.org/10.1111/pbr.12798.*

Ogunkunle, A.O. 1998. An appraisal of the capacity of Nigerian soils to sustain food Self-sufficiency. *Nigeria Agricultural Journal* 29: 13-33

Okoruwa, A.E. 1997. *Utilization and processing of maize*. IITA Research Guide 35, 4th Edition. Ibadan: IITA press, 29 pp.

Olaniyan, A.B. Maize: Panacea for hunger in Nigeria. *African Journal of Plant Science* 9: 155-174

Olmedilla, B., Granado, F., Gil-Martinez, E., Blanco, I. and Rojas-Hidalgo, E. 1997. Reference values for retinol, tocopherol, and main carotenoids in serum of control and insulin-dependent diabetic spanish subjects. *Clinical Chemistry* 43:1066-1071

Ortiz, D., Ponrajan, A., Bonnet, J.P., Rocheford, T. and Ferruzzi, M.G. 2018. Carotenoid stability during dry-milling, storage and extrusion processing of biofortified maize genotypes. *Journal of Agricultural and Food Chemistry* 9: 4683-4691. DOI: 10.1021/acs.jafc.7b05706

Ortiz-Covarrubias, Y., Dhliwayo, T., Palacios-Rojas, N., Ndhlela, T., Magoroskosho, C., Aguilar-Rincon, V.H., Cruz-Morales, A. and Trachsel, S. 2019. Effects of drought and low nitrogen stress on provitamin A carotenoid content of biofortified maize hybrids. *Crop Science* 59: 1-12

Owens, B.F., Mathew, D., Diepenbrock, C.H., Tiede, T., Wu, D., Mateos-Hernandez, M., Gore, M.A. and Rocheford, T. 2019. Genome-Wide Association Study and Pathway-Level Analysis of Kernel color in maize. *Genes*| *Genomes*| *Genetics* 9:1945-1955

Oyekale, K.O., Daniel, I.O., Kamara, A.Y., Akintobi, D.C.A., Adegbite, A.E. and Ajala, M.O. 2008. Evaluation of tropical maize hybrids under drought stress. *Journal of Food, Agriculture and Environment* 6: 260-264

Pandey, S., SK, V., De, L.C., Ortega, A., Granados, G. and Villegas, E. 1984. Development and improvement of maize populations. *Genetika* 16: 23 - 42

Pandey, S., Jha, A., Kumar, S. and Rai, M. 2010. Genetics and heterosis of quality and yield of pumpkin. *Indian Journal of Horticulture* 67: 333-338

Park, S.J., Kwon, K.E., Choi, N.K., Park, K.H. and Woo, S.J. 2015. Prevalence and incidence of exudative age-related macular degeneration in South Korea: A nationwide population-based study. *OPHhalmology* 122: 2063-2070

Perry, A., Rasmussen, H. and Johnson, E.J. 2009. Xanthophyll (lutein, zeaxanthin) content of fruits, vegetables and corn and egg products. *Journal of Food Composition and Analysis 22*: 9-15

Pfeiffer, W.H. and McClafferty, B. 2007. Biofortification: breeding micronutrient-dense crops. *Breeding major food staples*. Kang, M.S. and Priyadarshan, P.M. Eds. Blackwell Publishing. 61-91.

Pixley, K., Palacios, N.R., Babu, R., Mutale, R., Surles, R. and Simpungwe, E. 2013. Biofortification of maize with provitamin A carotenoids. *Carotenoid and Human Health*. Ed. Tanumihardo, S.A. Humana Press, London. 271-292. *DOI: 10.1007/978-1-62703-203-2_17*

Ponta, K.M. 2001. Response to S_1 recurrent selection and estimation of genetic parameters in effective population sizes of the BS11 maize populations. Ph.D. Thesis. Faculty of Agriculture, Iowa State University, USA. ix+204pp.

Prasanna, B.M., Palacios-Rojas, N., Hossain, F., Muthusamy, V.H., Menkir, A., Dhliwayo, T., Ndhlela, T., Vicente, F.S., Nair, S.K., Vivek, B.S., Zhang, X., Olsen, M. and Fan, X. 2020. Molecular breeding for nutritionally enriched maize: status and prospects. *Frontiers in Genetics* 10.1392, doi: *10.3389/fgene.2019.01392*

Purseglove, J.W. 1972. *Tropical Crops: Monocotyledons*. New York: Longman Scientific and Technical. 603 pages.

Qu, J. and Liu, J. A. 2013. Genome-wide analysis of simple sequence repeats in maize and the development of polymorphism markers from next-generation sequence data. *BMC Research Notes* 6:403. *https://doi.org/10.1186/1756-0500-6-403*

Ranum, P., Peña-Rosas, J.P., Garcia-Casal, M.N. and Pe, J.P**.** 2014. Global maize production, utilization, and consumption. *Annals of the New York Academy of Science* 1312: 105-112.

Rein, D.B., Wittenborn, J.S., Zhang, X., Honeycutt, A.A., Lesesne, S.B., Saaddine, J. 2009. Forecasting age-related macular degeneration through the year 2050: The potential impact of new treatments. *Archives of OPHhalmol* 127: 533-540.

Ribaut, J.M., Hoisington, D.A., Deutsch, J.A., Jiang, C., Gonzalez-de-Leon, D. 1996. Identification of quantitative trait loci under drought conditions in tropical maize. I. Flowering parameters and the anthesis-silking interval. *Theoretical and Applied Genetics* 92: 905-914

Ribaut, J.M., Jiang, C., Gonzalez-de-Leon, D., Edmeades, G.O. and Hoisington, D.A. 1997. Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. *Theoretical and Applied Genetics* 94: 887-896

Ribaut, J. M., Vicente, M. C. and Delannay, X. 2010. Molecular breeding in developing countries: challenges and perspectives. *Current Opinion in Plant Biology*, 13: 1-6.

Rice, A.L., K.P. West, Jr. and R.E. Black. 2004. Vitamin A deficiency. *Comparative quantification of health risks— Global and regional burden of disease attributed to selected major risk factors*. Ezzati, M., Lopez, A.D., Rodgers, A. and Murray, C.J.L. Eds. Volume 1. The World Health Organization, Geneva, Switzerland.

Richard, R.A. 1996. Defining selection criteria to improve yield under drought. *Plant Growth Regulation* 20: 157-166.

Rivera, S.M., Canela-Garayoa, R. 2012. Analytical tools for the analysis of carotenoids in diverse materials. *Journal of Chromatography A*, 1224:1-10

Rodriguez, F., Alvarado, G., Pacheco, A., Burgueno, J. and Crossa, J. 2018. User's guide manual for Analysis of Genetic Designs in R (AGD-R). CIMMYT, Mexico. 46 pages.

Rosielle, A.A. and J. Hamblin. 1981. Theoretical aspects of selection for yield in stress and non- stress environment. *Crop Science* 21:943-946.

Sagare, B.D., Shetti, P., Surender, M., Reddy, S.S., Pradeep, T. and Anuradha, G. 2018. Maize: Potential crop for provitamin A biofortification. *Maydica* 63:1-11

Sansaloni, C., Petroli, C., Jaccoud, D., Carling, J., Detering, F., Grattapaglia, D. and Kilian, A. 2011 Diversity Arrays Technology (DArT) and next-generation sequencing combined: genome-wide, high throughput, highly informative genotyping for molecular breeding of Eucalyptus. *BMC Proceedings* 5 (Supplementary 7):54. *DOI:10.1186/1753-6561-5-s7-p54*

Santos, A., Pinho, R., De Souza, V., Guimarães, L.M., Balestre, M., Pires, L.P. and Da Silva, C. 2020. Grain yield, anthesis-silking interval and drought tolerance indices of tropical maize hybrids. *Crop Breeding and applied Biotechnology* 20: 1-9

SAS Institute 2012. SAS system for windows. Release 9.4. SAS Institute Inc. Cary, North Carolina, USA.

Scott, K.J., Thurnham, D.I., Hart, D.J., Bingham, S.A. and Day, K. 1996. The correlation between the intake of LUT, lycopene and beta-carotene from vegetables and fruits, and blood plasma concentrations in a group of women aged 50-65 years in the UK. *British Journal of Nutrition* 75: 409-418

Seif, G. and Link, W. 2007. Agronomic Performance and the Effect of Self-Fertilization on German Winter Faba Beans. *Journal of Central European Agriculture* 8: 121-128

Semagn, K., Babu, R., Hearne, S. and Olsen, M. 2013. Single nucleotide polymorphism genotyping using Kompetitive allele specific PCR (KASP): overview of the technology and its application in crop improvement. *Molecular breeding* 33:1–14. *https://doi.org/10.1007/s11032-013-9917-x*

Sen, S. and Chakraborty, R. 2011. The role of antioxidants in human health. *Oxidative stress, diagnostics, prevention and therapy.* Andreescu, S., Hepel, M. Eds. Oxford University Press, New York. 1-37

Senete, C.T., Guimarães, P.E.D.O., Paes, M.C.D. and De Souza, J.C. 2011. Diallel analysis of maize inbred lines for carotenoids and grain yield. *Euphytica* 182: 395-404.

Sherwin, J.C., Reacher, M.H., Dean, W.H. and Ngondi, J. 2012. Epidemiology of vitamin A deficiency and xerophhalmia in at-risk populations. *Transaction of Royal Society of Tropical Medicine and Hygiene* 106: 205-214.

Schussler, J.R. and Westgate, M.E. 1995. Assimilate flux determines kernel set at low water potential in maize. *Crop Science* 35: 1074-1080.

Singh, M. 2004. Role of micronutrients for physical growth and mental development. *Indian Journal of Pediatrics* 71: 59-62.

Singh, B.D. 2012. *Plant breeding principles and methods*. 12th Edition. New Delhi: Kalyani Publishers.

Singh, B.D., Singh, A.K., 2015. Marker-assisted selection. *Marker-Assisted Plant Breeding: Principles and Practices*. Singh, B.D. and Singh, A.K. Eds. *Springer*, New Delhi, India. Chapter 9: 279-279, *doi 10.1007/978-81-322-2316-0*

Smith, O. S. 1979. A model for evaluating progress from recurrent selection. *Crop Science* 19:223-226

Sommer, A and West, K.P. 1996. Vitamin A deficiency: Health, survival and vision. Oxford University Press, New York. 19-250.

Souza, L.V., Miranda, G.V., Galvao, J.C., Guimaraes, L.M. and Santos, I.C. 2009. Combining ability of maize grain yield under different levels of environmental stress. *Pesquisa Agropecuária Brasileira* 44: 1297-1303

Sprague, G.F. and Tatum, L.A. 1942. General versus specific combining ability in single crosses of corn. *Journal of the American Society of Agronomy* 34: 923-932.

Steven, G.A., Beal, T., Mbuya, M.N., Luo, H. and Neufeld, L.M. 2022. Micronutrient deficiencies among preschool-aged children and women of reproductive age worldwide: a pooled analysis of individual-level data from population-representative surveys. *Lancet Global Health.* 10: e1590–99. DOI:https:doi.org/10.1016/S2214-109X(22)00367-9.

Suwarno, W.B., Pixley, K.V., Palacios-Rojas, N., Kaeppler, S.M. and Babu, R. 2014. Formation of heterotic groups and understanding genetic effects in a provitamin A biofortified maize breeding program. *Crop Science* 54: 14-24

Suwarno, W.B., Pixley, K.V., Palacios-Rojas, N., Kaeppler, S.M. and Babu, R. 2015. Genome-wide association analysis reveals new targets for carotenoid biofortification in maize. *Theoretical and Applied Genetics* 128: 851-864

The International Maize and Wheat Improvement Center (CIMMYT) Maize Program 1999. A User's Manual for Fieldbook 5.1/7.1 and Alpha. Mexico: D.F. CIMMYT

Tian, F., Bradbury, P. J., Brown, P. J., Hung, H., Sun, Q., Flint-Garcia, S., Rocheford, T.R., McMullen, D., Holland, J.B. and Buckler, E. 2011.Genome-wide association study of leaf architecture in the maize nested association mapping population. *Nature Genetics* 43: 159-162

Tucker, K.L., Chen, H., Vogel, S., Wilson, P.W., Schaefer, E.J. and Lammi-Keefe, C.J. 1999. Carotenoid intakes, assessed by dietary questionnaire, are associated with plasma carotenoid concentrations in an elderly population. *The Journal of Nutrition* 129: 438-445

Tulu, D., Tesso, B. and Azmach, G. 2018. Heterosis and combining ability analysis of quality protein maize (*Zea mays* L.) inbred lines adapted to mid-altitude sub-humid agro-ecology of Ethiopia. *African Journal of Plant Science* 12: 47-57

United Nations Standing Committee on Nutrition (UNSCN) 2010. Progress in Nutrition, 6th Report on the World Nutrition Situation, Geneva. 134 pages

US Institute of Medicine 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum,

Nickel, Silicon, Vanadium, and Zinc. Washington, DC, USA: *The National Academies Press*

Van Berloo, R. and Stam, P. 2001. Simultaneous marker-assisted selection for multiple traits in autogamous crops. *Theoretical and Applied Genetics,* 102: 1107-1112

Vales, M.I., Malvar, R.A., Revilla, P., Ordas, A. 2001. Recurrent selection for grain yield in two Spanish maize synthetics populations. *Crop Science* 41: 15-19

Velasco, P., Malvar, R.A., Revilla, P., Butron, A., Ordas, A. 1999. Ear Resistance of Sweet Corn Populations to *Sesamia nonagrioides (Lepidoptera: Noctuidae) and Ostrinia nubilalis (Lepidoptera: Pyralidae)*. *Journal of Economic Entomology* 92:732-739

Vignesh, M., Hossain, F., Nepolean, T., Saha, S., Agrawal, P.K., Guleria, S.K., Prasanna, B.M., and Gupta, H.S. 2012. Genetic variability for kernel βC and utilization of *crtRB1 3'TE* gene for biofortification in maize (*Zea mays* L.). *Indian Journal of Genetics and Plant Breeding* 72: 189-194.

Watzl, B., Bub, A., Briviba, K. and Rechkemmer, G. 2003. Supplementation of a low-carotenoid diet with tomato or carrot juice modulates immune functions in healthy men. *Annals of Nutrition and Metabolism* 47:255-261

Wessells, K.R. and K.H. Brown. 2012. Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. *PLoS One* **7**: 1-11

West J.R., Keith, Darnton-Hill, I. 2008. Vitamin A deficiency. *Nutrition and Health in Developing Countries.* Semba, R.D. and Bloem, M.W. Eds. Nutrition and Health Series, Humana Press: Totowa, New Jersey. 377-433.

Westgate, M.E. 1997. "Physiology of flowering in maize: identifying avenues to improve kernel set during drought." *Developing Drought and Low-N Tolerant Maize.* Edmeades, G.O., Bänziger, M., Mickelson, H. R. and Peña-Valdivia, C.B. CIMMYT, Mexico, DF: 136-141.

WHO, 2009. Global prevalence of vitamin A deficiency in populations at risk 1995-2005: *WHO global database on vitamin A deficiency*, World Health Organization, Geneva. 68 pages

WHO, 2015. The global prevalence of anemia in 2011. World Health Organization, Geneva. 43 pages

Wurtzel, E.T., Cuttriss, A. and Vallabhaneni, R. 2012. Maize provitamin A carotenoids, current resources, and future metabolic engineering challenges. *Frontiers in Plant Science* 3: 1-12

Yan, W. 2001. GGE Biplot-A windows application for graphical analysis of multi-environment trial data and other types of two-way data. *Agronomy Journal 93*: 1111-1118, *doi.org/ 10.2134/agronj2001.9351111x*

Yan, J., Kandianis, C.B., Harjes, C.E., Bai, L., Kim, E.H., Yang, X., Skinner, D.J., Fu, Z., Palacios, N., Li, J., DellaPenna, D., Brutnell, T., Buckler, E.S., Warburton, M.L. and Rocheford, R.T. 2010. Rare genetic variation at *Zea mays* crtRB1 increases b-carotene in maize grain. *Nature Genetics* 42: 322-329, *doi:10.1038/ng.551*

Zaidi, P.H. 2019. Management of drought stress in field phenotyping. CIMMYT, Mexico. 40 pages.

Zhang, Y., Kang, M.S. and Lamkey, R.K. 2005. DIALLEL-SAS05: A comprehensive program for Griffing's and Gardner-Eberhart analyses. *Agronomy Journal 97*: 1097-1106.

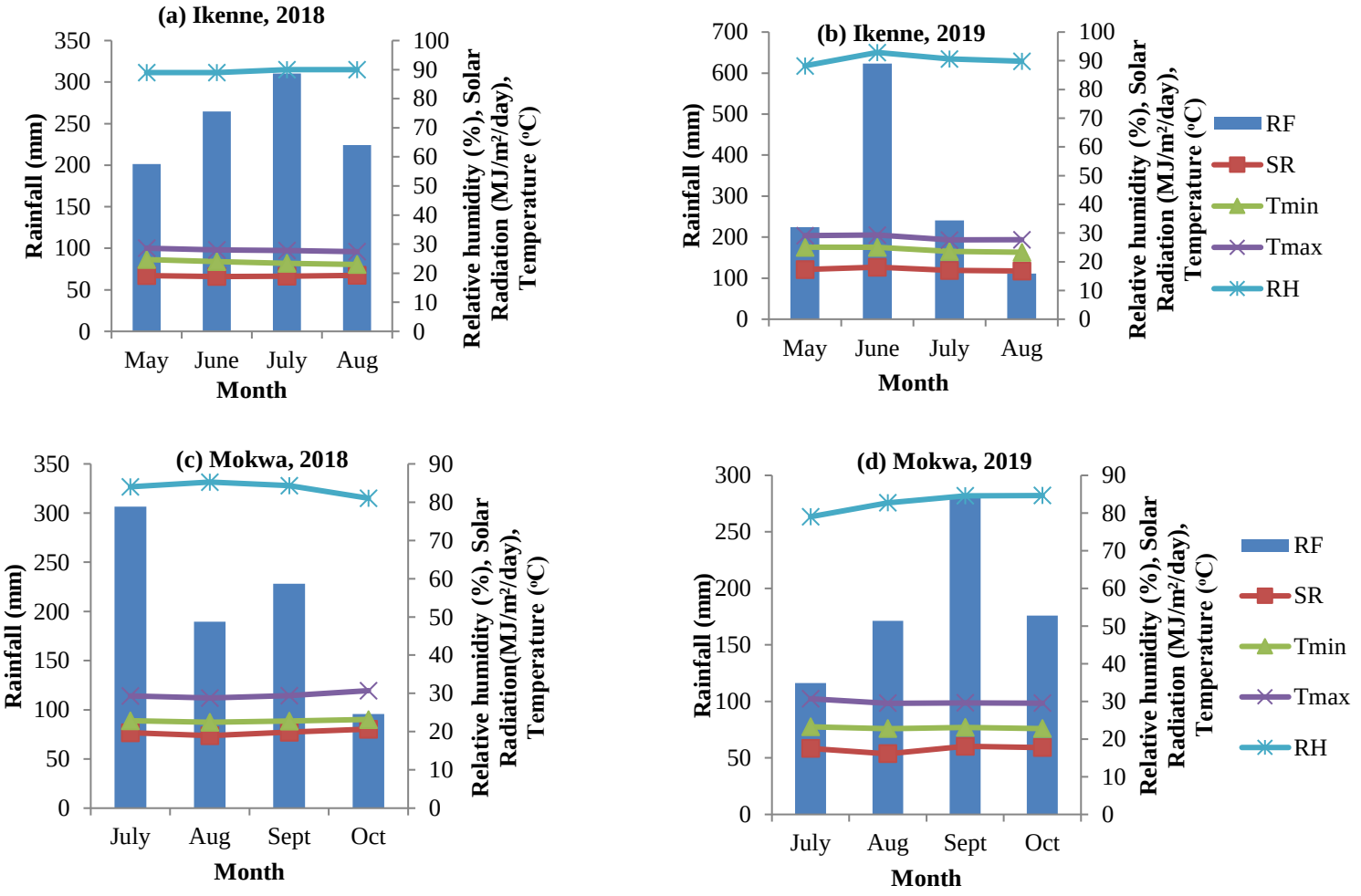
APPENDICES

APPENDIX 1: *crtRB1* Kompetitive Allele Specific PCR (KASP) assays used to improve provitamin-A carotenoids in two maize synthetics (HGA and HGB) evaluated across eight environments in Nigeria.

S/N	SNP ID	Source	Intertek ID	Favourable Allele	Unfavourable Allele
1	S10_134583972	CIMMYT	SnpZM0013	GG	CC
2	S10_134655704	CIMMYT	SnpZM0014	CC	TT
3	SYN11355	CIMMYT	SnpZM0015	AA	GG
4	PZE-110083653	CIMMYT	snpZM0016	GG	AA
5	S10_136072513	CIMMYT	SnpZM0017	TT	GG
6	S10_136840485	CIMMYT	SnpZM0018	CC	TT
7	S10_137904716	CIMMYT	SnpZM0019	CC	TT

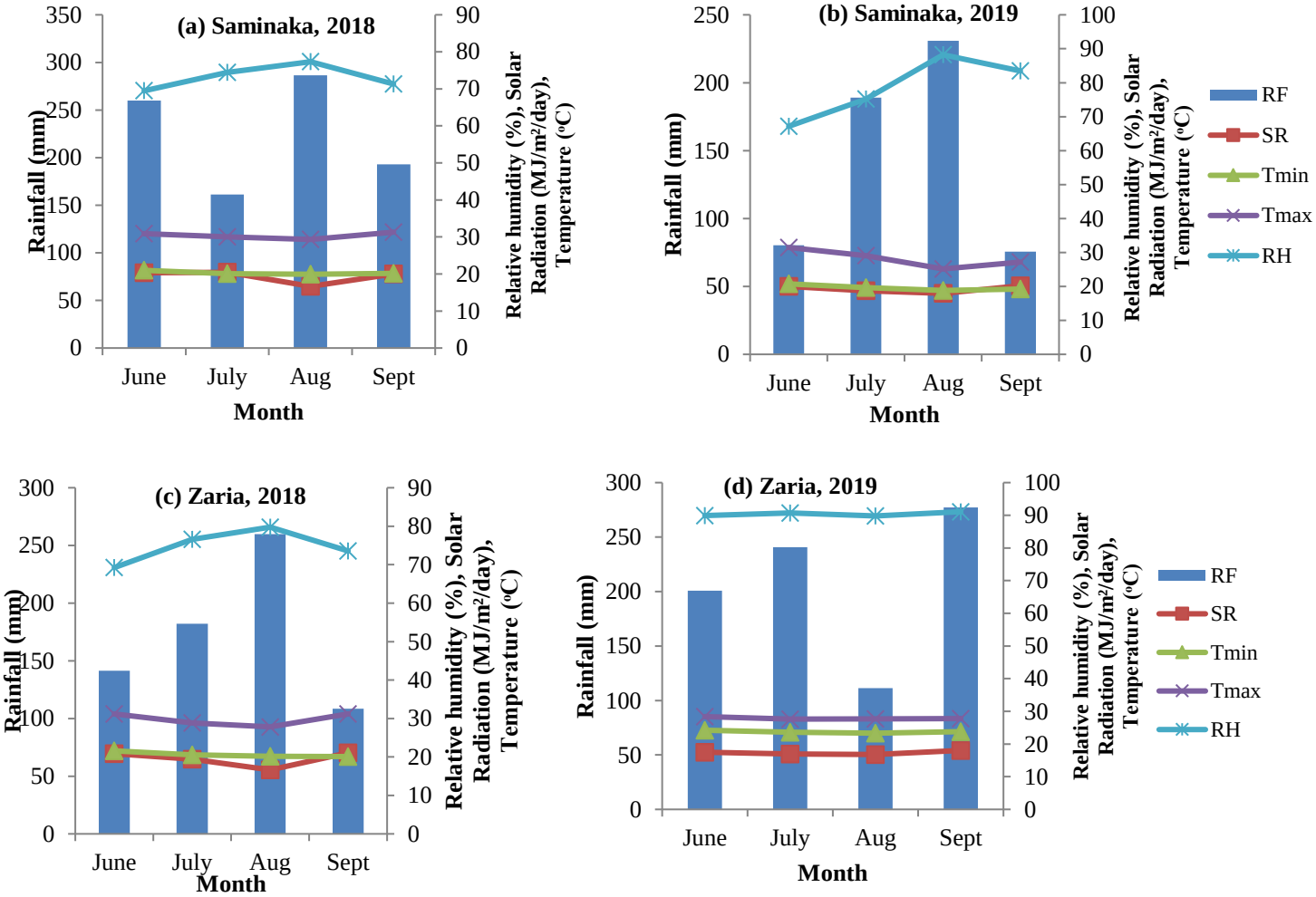
crtRB1: βcarotene hydroxylase1

APPENDIX 2: Rainfall, relative humidity, solar radiation, minimum and maximum temperature of Ikenne and Mokwa during the rainy season trials of 2018 and 2019

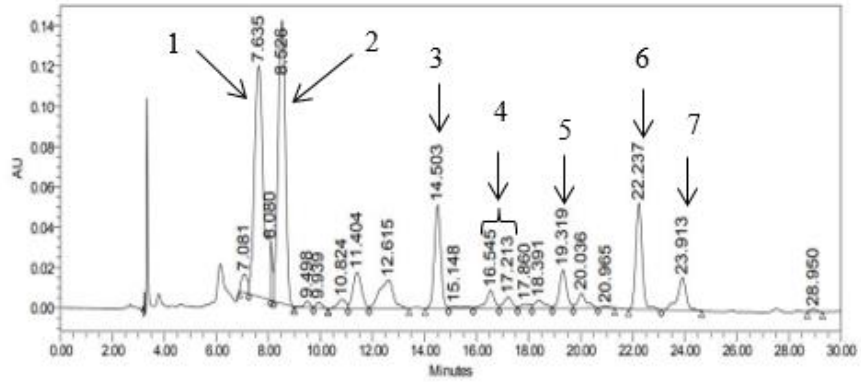


APPENDIX 3: Rainfall, relative humidity, solar radiation, minimum and maximum temperature of

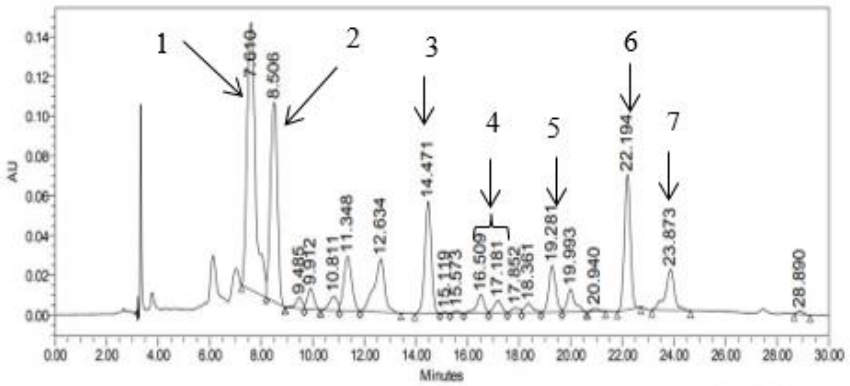
Saminak and Zaria during the rainy season trials of 2018 and 2019



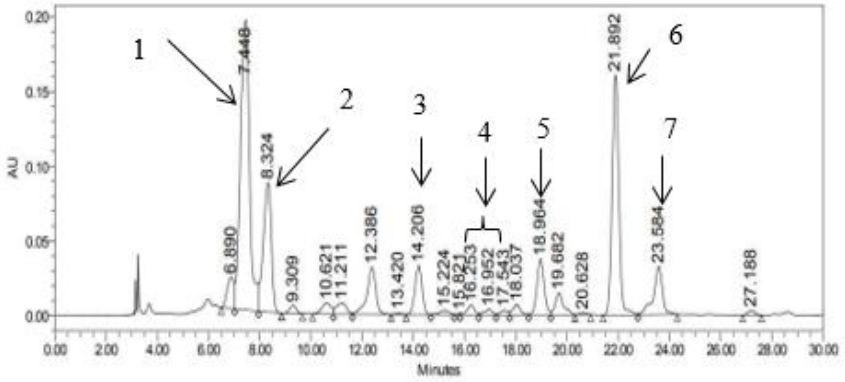
APPENDIX 4: Chromatograms for carotenoids of some SCs of two MS quantified using HPLC at IITA, Ibadan, Nigeria. Peaks are (1) LUT (2) ZXT (3) βCX (4) αC (5) 13-*cis* βC (6) *trans* βC (7) 9-*cis* βC



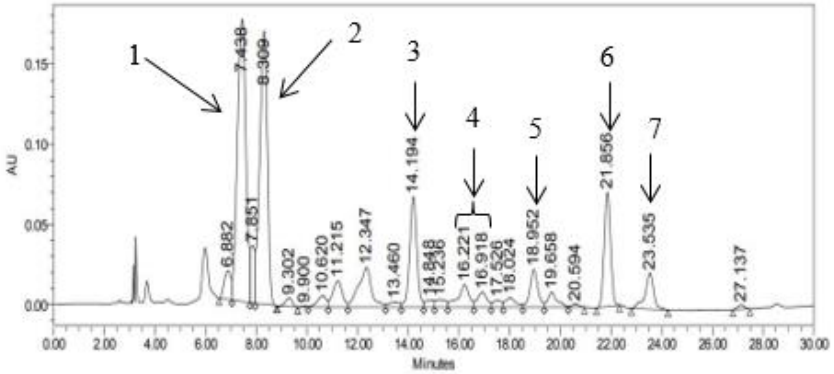
(A) PVASYNHGAC0



(B) PVASYNHGBC0

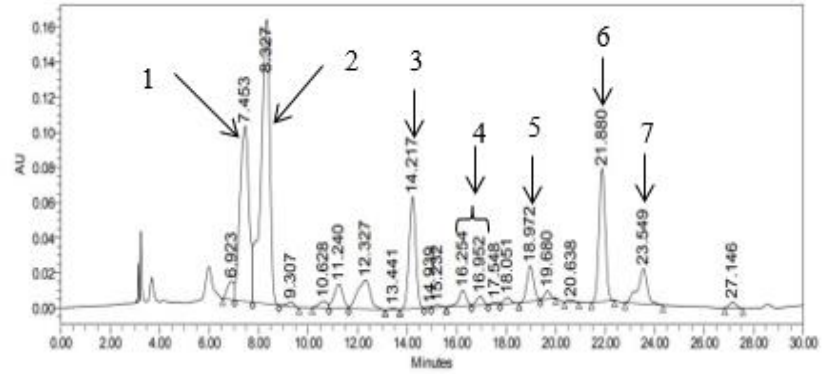


(C) PVASYNHGAC2

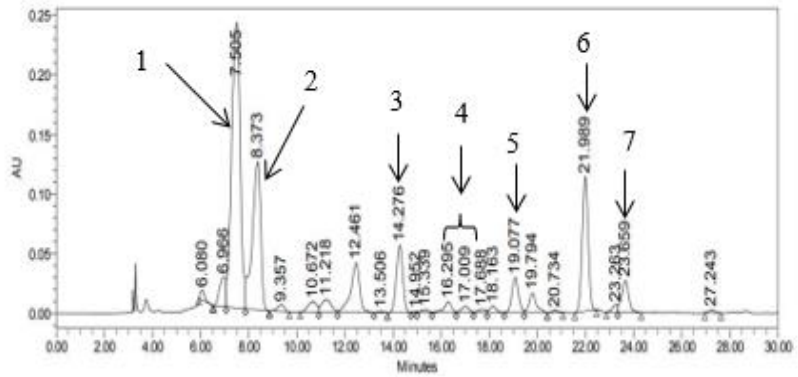


(D) PVASYNHGBC2

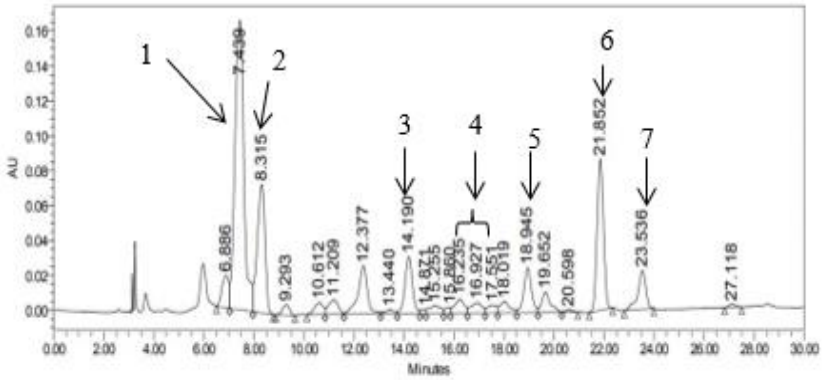
APPENDIX 5: Chromatograms for carotenoids of some maize VHand a check (PVASYN13) quantified using HPLC at IITA, Ibadan, Nigeria. Peaks are (1) LUT (2) ZXT (3) βCX (4) αC (5) 13-*cis* βC (6) *trans* βC (7) 9-*cis* βC (7) 9-*cis* βC



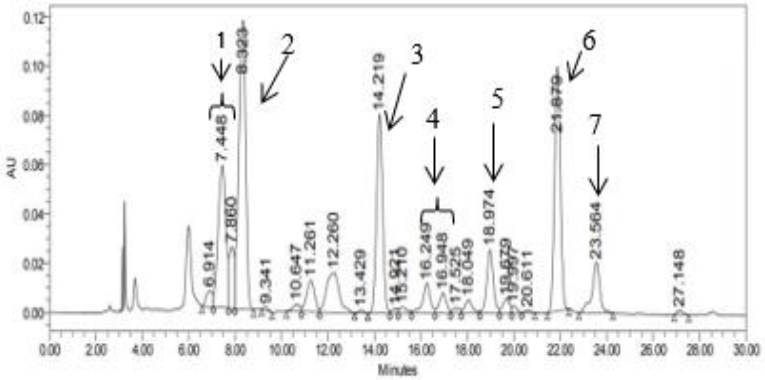
(E) PVASYNHGBC0/PVASYNHGAC0



(F) PVASYNHGBC2/PVASYNHGAC2



(G) PVASYNHGBC1/PVASYNHGAC1



(H) PVASYN13

APPENDIX 6: Proportion of carotenoids in sixteen provitamin-A maize genotypes evaluated across four locations in two years in Nigeria