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Genetics of carotenoids for provitamin A biofortification in tropical-adapted maize

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ABSTRACT

Yellow maize contains high levels of β-carotene (βC), making it an important crop for combating vitamin A deficiency through biofortification. In this study, nine maize inbred lines were selected at random from 31 provitamin A (PVA) maize inbred lines and crossed in a partial diallel mating design to develop 36 crosses. The crosses were evaluated in the field in two locations (Samaru and Kerawa) and their seed carotenoid content were determined by high-performance liquid chromatography. The modes of gene action, heritability, and correlations between agronomic traits and carotenoid content were estimated. Additive genetic variances (σ^2_a) were lower than non-additive genetic variances (σ^2_d) for all the carotenoids, plant height (PH), and grain yield (GY), suggesting a preponderance of non-additive gene action. Broad-sense heritability (H^2) was high ($H^2 > 60\%$) for zeaxanthin, days to anthesis, and PH, moderate ($30\% < H^2 < 60\%$) for lutein and GY, and low ($H^2 < 30\%$) for alpha carotene, beta cryptoxanthin, βC, and PVA. Genetic advance as a percentage of mean, considered with H^2, also suggests a preponderance of non-additive gene action for PVA carotenoids. Hybrid variety development is thus an appropriate approach to improving grain yield and PVA. GY showed no significant genotypic correlations with carotenoid content, suggesting that these traits can be improved concurrently. Thus, there is ample scope for improvement of PVA and GY in the sample of tropical-adapted maize.

1. Introduction

Maize (*Zea mays* L.) was introduced into Africa in the 1500s and has since become a dominant food crop [1]. In 2012, of 870 million metric tons (MT) of maize grains produced worldwide, Africa contributed about 70 million MT, with 25.7% of it produced in West Africa [2]. Nigeria accounted for more than half of the maize produced in the region, with a production of 9.4 million of 18 million MT [2]. Africa consumes 30% of maize produced worldwide, of which sub-Saharan Africa (SSA) consumes 21%. Almost all (95%) of the maize in Africa is used for human consumption, in contrast to other regions of the world that use most of their maize as animal feed [3,4]. However, the dominant role of maize in the African diet can lead to malnutrition and vitamin-deficiency diseases such as night blindness and kwashiorkor, as a result of its inability to provide the recommended daily allowance (RDA) of protein and essential

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micronutrients for the body. The prevalence of micronutrient malnutrition greatly exceeds the prevalence of protein energy malnutrition (PEM). While there are 180 million children with PEM, 3.5–5.0 billion persons are iron-deficient and 140–250 million persons experience vitamin A deficiency (VAD) [5–8]. The Standing Committee on Nutrition of the United Nations estimates at 160 million the number of preschool children in low-income countries who are affected by VAD. Physical symptoms of eye problems as a result of the deficiency have a worldwide prevalence of about 1–2%. Of this proportion, Africa accounts for 26.0% of all cases for preschool children, 4.0% of cases being in Nigeria. Of maternal night blindness cases, Africa accounts for 4.4% of cases, of which Nigeria accounts for 2.4% worldwide [8]. Approaches to combating VAD through use of regular supplementation and food fortification with vitamin A have not been sustainable in the developing world [9,10]. Hence, to alleviate VAD, maize has been targeted for biofortification. Biofortification is an endogenous method of fortifying crops such as rice, sorghum, and maize. After a one-time investment in developing biofortified seeds, recurrent costs are low and the approach is highly sustainable.

Yellow maize contains considerable levels of β-carotene, a source of vitamin A [11], and has high natural variation for carotenoids [12–15]. Typical yellow maize varieties have 0.5 to 1.5 μg g^{-1} provitamin A (PVA) [16] which is inadequate to prevent VAD in diets dominated by maize, so that the current international target of combating VAD by use of maize is the development of maize kernels with as much as 15 μg g^{-1} PVA [13]. Although natural genetic variation in carotenoids has been found in yellow maize lines and hybrid varieties in the temperate zone [17–20] and in the tropics [14,21,22], limited reports describing the genetics of carotenoids in yellow maize in SSA are available. Egesel et al. [20], Burt [21], and Suwarno et al. [22] represent some of the few studies on gene action and inheritance of carotenoids in maize.

This study was undertaken to (1) estimate the genetic diversity among 31 tropical-adapted provitamin A maize inbred lines, (2) create genetic variability for agronomic traits and carotenoids by hybridization, (3) determine the nature of gene action, heritability, and correlations for agronomic traits and carotenoids, and (4) recommend effective strategies for PVA maize biofortification programs in the tropical SSA.

2. Materials and methods

2.1. Genetic materials and their pedigrees

Yellow maize inbred lines developed at the International Institute of Tropical Agriculture (IITA) Ibadan, were used for this study. Table 1 shows the 31 inbred lines with their descriptions and pedigrees. The inbred lines were developed from biparental crosses and backcrosses involving some tropical inbred lines and temperate lines (9450, KU1409, 9071, KI21, KU1414-SR, and KVI43 as donors of high β-carotene). The materials have been tested across Nigeria and selected for medium to high PVA contents and adaptability to the SSA tropics [11].

2.2. Parent selection and population development

The 31 lines were analyzed for genetic diversity using 14 polymorphic SSR markers as described by Adeyemo et al. [23] and Warburton et al. [24]. Leaf DNA was extracted by the cTAB method according to Dellaporta et al. [25] as modified by Halilu et al. [26]. The SSR data were used for cluster analysis as implemented in the PowerMarker 3.25 software [27] based on Euclidean genetic distance estimated from allele frequencies [28]. A dendrogram was constructed based on the unweighted pair group method with arithmetic averages (UPGMA) clustering using MEGA 5 software [29]. It revealed the patterns of genetic relationships among the 31 inbred lines in four clusters. Nine inbred lines were randomly selected from the four clusters (two inbred lines from each of clusters 1, 2, and 3 and 3 lines from cluster 4) (Fig. 1). The nine selected lines were used as parents in population development using Griffing's partial diallel mating design method 4 [30], in which the lines are used as both male and female parents. The lines were planted in pairs (male and female) as specified using Maize Fieldbook 8.5.1 software (http://www.cimmyt.cgiar.org/), under irrigation at IAR farm Samaru in 2011. They were planted twice with one-week interval to allow synchrony of anthesis and silking (flowering) among them. Thirty-six crosses were developed by hand pollination (with no selfs and no reciprocals), according to the formula $n(n-1)/2$, where n is the number of parents involved [30,31], with each parent performing male and female roles for each combination of crosses. The seeds produced were bulked for each cross combination. The crosses were harvested in May, 2012. The harvested crosses (F_1) were used for field evaluation in the 2013 rainy season.

2.3. Field evaluation

The 36 cross progenies and two commercial yellow maize hybrids designated as checks 1 and 2 (Obasuper2 and Obasuper4, respectively) were evaluated in the 2013 wet season for agronomic traits under field conditions in Samaru (11°11.297′ N, 07°37.078′ E, 673 m) and Kerawa (10°59.145′ N, 07°25.081′ E, 605 m). Each experiment was conducted using a randomized complete block design with two replications. Entries were grown in 1-row plots of 5.00 m × 0.75 m. Fertilizer (120 kg N, 60 kg P_2O_5, 60 kg K_2O) was applied in two split doses at planting and at 30 days after planting using NPK 15:15:15 and urea. The experiments were kept weed-free by application of five l/ha of a mixture of gramaxone (a contact herbicide) and atrazine (a pre-emergence herbicide). Manual weeding was performed four weeks after sowing. The first 2–3 plants per plot were self-pollinated by hand and their seeds harvested separately for carotenoid analyses. Field data were recorded for agronomic traits such as days to anthesis (DA) (number of days from planting to the time 50% of the plants tasseled), plant height (PH) (average height of 5 plants per plot in cm from the base of the plant to where tassel branching begins), and grain yield (GY) (t ha^{-1}). Mean performance of each entry was calculated from the replicated plot means for the traits.

Table 1 – Seed description and pedigree of maize inbred lines having medium to high provitamin A (PVA) contents received from IITA in 2009.

S/No.	Code name	Pedigrees	Seed type	Seed color	β-Carotene (μg g^{-1})	Provitamin A [a] (μg g^{-1})
1	E1	(9450 × CM 116 × 9450)-3_3-1-2-1-B-B-B-B-B-B-B	Dent	Orange	4.749	7.752
2	E2	9450 × KI 21-1-4-1-2-1-B-B-B-B-2-B-B-B	Flint	Orange	2.501	4.506
3	E3	9450 × KI 21-1-4-1-2-1-B-B-B-B-1-B-B-B	Dent	Orange	2.501	4.506
4	E5	TZMI214 × A619LPA × TZMI214-10-3-B-B-B-B-B-B	Flint	Orange	3.613	4.961
5	E6	SYN-Y-STR-34-1-1-1-1-2-1-B-B-B-B-B-B-B-B-B	Flint	Orange	8.277	9.792
6	E7	9450 × KI 21-7-3-1-2-5-B-B-B-B-B-B	Flint	Orange	3.071	5.085
7	E8	9450 × KI 21-7-2-2-1-1-B-B-B-B-B-B	Flint	Orange	3.838	5.877
8	E9	(9071 × 4058)-8-2-1-1-B-B-B-B-B-B	Dent	Orange	4.579	7.872
9	E10	KU1409/KU1414-SR/CI187-B-B-B-B-B-B	Flint	Orange	2.535	4.229
10	E11	KU1414-SR/KVI43-4-1-B-B-B-B-B-B	Flint	Orange	2.799	4.573
11	E12	KU1414-SR/KVI43-6-1-B-B-B-B-B-B	Flint	Orange	2.854	5.051
12	E14	(9450 × KI 28)-5-1-1-1-B-B-B-B-B-B	Flint	Orange	2.521	4.688
13	E16	KU1414-SR/KVI43-6-4-B-B-B-B-B-B	Flint	Orange	3.019	5.287
14	E18	KU1409/KU1414-SR/SC55-B-B-B-B-B-B	Flint	Orange	2.270	4.307
15	E19	ACR97TZL-CCOMP1-Y-S3-33-5-B-B-B-B-B-B-B-B	Flint	Orange	2.275	4.052
16	E20	ACR97TZL-CCOMP1-Y-S3-13-1-B-B-B-B-B-B-B-B	Flint	Orange	2.586	4.511
17	E21	9450 × KI 21-1-4-1-1-2-B-B-B-B-B-B-B	Flint	Orange	2.385	5.317
18	E22	POP 61-SR-11-2-3-3-1-B-B-B-B-B-B-B-B	Flint	Orange	3.027	4.703
19	E23	(MP420 × 4001 × MP420)-3-1-3-1-B-B-B-B-B-B-B-B	Flint	Deep orange	2.818	4.309
20	E24	9450 × KI21-1-5-3-2-1-B-B-B-B-B-B-B	Flint	Orange	3.006	5.484
21	E25	9450 × KI21-1-5-3-2-2-B-B-B-B-B-B-B	Flint	Orange	3.101	5.951
22	E26	KU1409 × MO17LPA × KU1409-11-4-1-B-B-B-B-B-B	Flint	Orange	2.058	4.178
23	E27	9450 × KI 21-3-1-1-2-1-B-B-B-B-B-B-B	Flint	Orange	1.867	4.157
24	E28	KU1414-SR/KVI11-7-1-B-B-B-B-B-B	Flint	Orange	3.243	5.736
25	E30	ACR97SYN-Y-S1-27-B-B-B-B-B-B-B-B	Flint	Orange	2.386	4.271
26	E31	ACR97SYN-Y-S1-38-B-B-B-B-B-B-B-B	Flint	Deep orange	2.567	4.044
27	E32	KU1409 × MO17LPA × KU1409-27-3-1-1-B-B-B-B-B-B-B-B-B	Flint	Orange	2.815	4.881
28	E33	KU1409 × MO17LPA × KU1409-27-3-1-1-B-B-B-B-B-B-B	Flint	Orange	2.815	4.881
29	E35	(GT-MAS:Gk × BABANGOYO × GT-MAS:Gk)-1-1-1-3-B-B-B-B-B-B-B-B-B	Flint	Orange	2.062	3.167
30	E36	(MP420 × 4001 × MP420)-3-1-3-1-B-B-B-B-B-B-B-B-B	Flint	Orange	2.148	3.520
31	E37	KU1409 × MO17LPA × KU1409-27-3-4-1-B-B-B-B-B-B-B	Dent	Orange	2.264	3.426

[a] Typical yellow maize varieties have 0.5 to 1.5 μg g^{-1} PVA content (Harjes et al.) [13]; medium 3.0 ≤ PVA ≤ 5.5 μg g^{-1}, high PVA > 5.5 μg g^{-1}.

2.4. *Carotenoid analysis using high-performance liquid chromatography (HPLC)*

Ten grams of random samples of 20 to 30 seeds of each of the self-pollinated ears were freeze-dried at –80 °C, ground to fine powder (0.5 μm) and used for carotenoid analysis by HPLC. The extraction protocol used was that of Granado et al. [32], as modified by Menkir et al. [11], for analysis of dried maize kernels. The carotenoids α-carotene (αC), β-carotene (βC) (*cis* and *trans* isomers), β-cryptoxanthin (βCX), lutein (LUT), and zeaxanthin (ZEA) contents were estimated. Total provitamin A content (PVA) was calculated for each sample as the sum of βC plus one half of βCX and one half of αC. This calculation was based on the molecular structures; αC and βCX are considered to have 50% of the provitamin A activity of βC [33].

2.5. *Data analysis*

Diallel analysis of variance and estimation of the general combining ability (GCA) of parents and specific combining ability (SCA) of crosses for the agronomic traits (DA, PH, GY) and for all the measured carotenoids were performed using Griffing's method 4 model II [30].

The analysis for each trait was based on the linear model.

$$X_{ijk} = \mu + r_k + g_i + g_j + s_{ij} + e_{ijk} \text{ for a single location.} \quad (1)$$

For a multilocation diallel test the model was.

$$X_{ijk} = \mu + r(l) + l + g_i + g_j + s_{ij} + l * g_i + l * g_j + l * s_{ij} + e_{ijk} \quad (2)$$

where μ is the grand mean, r_k is the replication effect, g_i and g_j are GCA effects, s_{ij} is the SCA effect, l^*g_i and l^*g_j are location by GCA effect interactions, l^*s_{ij} is location by SCA effect interaction, $r(l)$ is replication nested within location effect, l is location effect, and e_{ijk} is the experimental error for the X_{ijk} observation (k = 1, 2; $i = j$ = 1, 2, … , 9) [30].

The variance due to differences among parents (σ^2_{GCA}) is equal to the covariance among half-sib (HS) progenies (crosses with a common parent) and is, in turn, equal to half of additive variance (σ^2_a). The variance among crosses (σ^2_{SCA}) involving full-sib individuals is equal to dominant variance (σ^2_d) for F = 1 [30]. Heritability estimates were obtained using

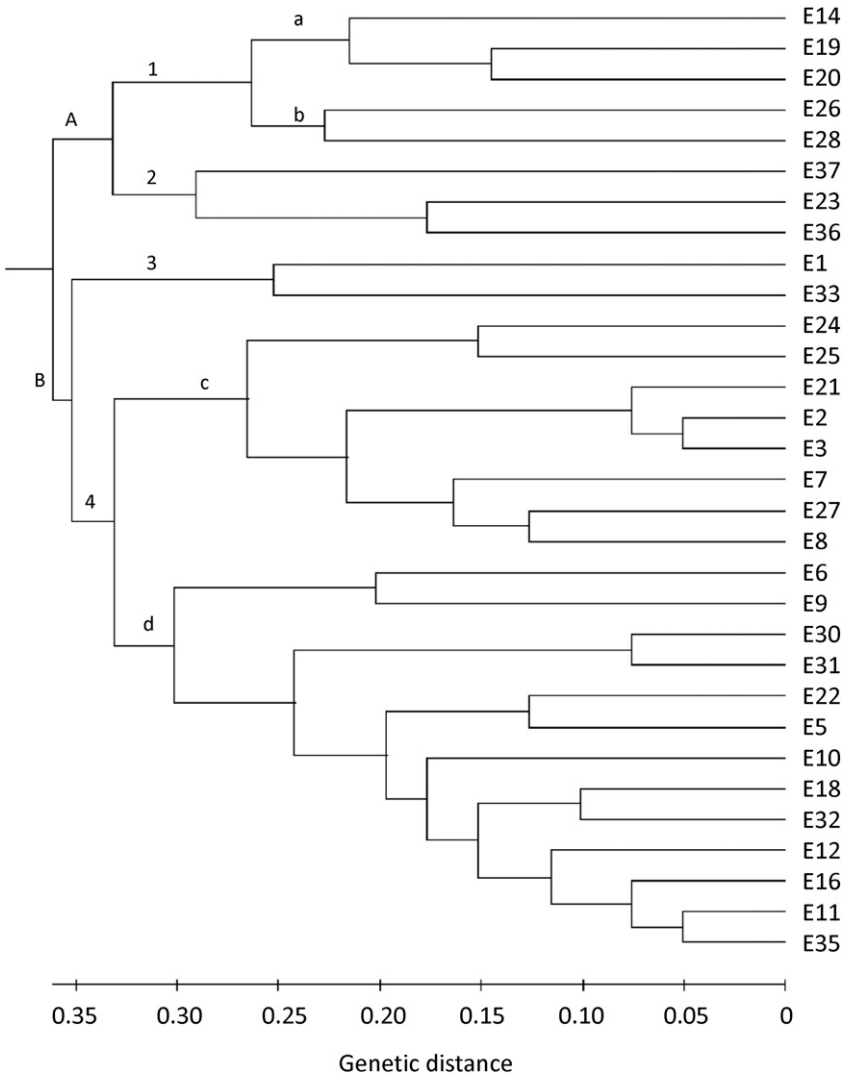

Fig. 1 – Dendrogram of 31 tropical-adapted maize inbred lines containing medium to high provitamin A content. Relationships were based on Euclidean genetic distances calculated using 14 polymorphic simple sequence repeat (SSR) markers. The dendrogram depicts two main groups, A and B, with each of the main groups divided into two clusters. The main group A consists of clusters 1 and 2 and group B consists of clusters 3 and 4. Although the lines are related by descent, they show considerable levels of genetic diversity, sufficient to create variation among crosses made between lines from different clusters. The nine selected lines used for inheritance studies were drawn from the 4 clusters.

the estimated variance components according to Griffing [30] and Hallauer et al. [31].

Genetic advance as percentage of mean (GAM), for better description and prediction for selection progress for the highly variable nutritional composition of maize seeds was calculated according to Allard [34].

Phenotypic and genotypic correlation coefficients to determine the degree of association between yield-related traits and carotenoids were estimated according to Singh and Chaudhary [35] and Evans et al. [36].

The mean squares and *F*-tests for random effects were obtained using the GLM procedure of SAS [37] and the appropriate error term on the basis of their respective type III estimated mean squares. The GCA and SCA variances were estimated using Griffing's method 4, model II [30] and the DIALLEL-SAS program developed by Zhang et al. [38] adapted to SAS software version 9.2 [37]. Correlation analyses were performed in SAS using the CORR procedures.

3. Results

3.1. *Analysis of variance*

A diallel analysis of variance across locations (Samaru and Kerawa) for the 36 crosses is presented in Table 2. The traits considered in the ANOVA were three major agronomic traits (DA, PH, GY) and all the measured carotenoids (βCX, αC, βC, PVA, LUT, ZEA). The mean squares (MS) for location, crosses, GCA, and SCA showed that there were significant differences for DA, PH, GY, LUT, and ZEA, with location showing significant effects for all the traits except PH, ZEA, and βCX

Table 2 – Mean squares for days to anthesis (DA), plant height (PH), grain yield (GY), lutein (LUT), zeaxanthin (ZEA), β-cryptoxanthin (βCX); α-carotene (αC), β-carotene (βC), and total provitamin A (PVA) contents for 36 provitamin A maize crosses evaluated across two locations (Samaru and Kerawa) in the 2013 wet season.

Source of variation	*df*	Agronomic traits			Carotenoid contents					
		DA	PH	GY	LUT	ZEA	βCX	αC	βC	PVA
Location (L)	1	162.56**	303.34	19.93**	42.07**	14.87	0.82	0.12*	2.27**	4.55**
Rep (Loc)	2	39.81**	1478.84**	0.71	2.11	2.15	0.30	0	0.26	0.57
Crosses (C)	35	29.59**	581.56*	4.78**	9.37**	18.83**	0.30	0.03	0.42	0.69
GCA	8	69.96**	578.36**	2.30*	13.58**	28.52**	0.43	0	0.27	0.50
SCA	27	17.63**	582.51**	5.52**	8.12**	15.96**	0.27	0.03	0.47	0.74
CxL	35	16.18**	483.00	1.45*	4.76	11.00	0.46	0.03	0.31	0.68
GCAxL	8	17.38**	849.85**	1.06	1.27	7.68**	0.60	0.01	0.29	0.47
SCAxL	27	15.82**	375.60**	1.57*	5.79**	11.98**	0.42	0.03	0.31	0.74
Error	70	7.57	329.31	0.76	3.82	8.28	0.41	0.02	0.31	0.70
GCA:SCA		4.0	1.0	0.4	1.7	1.8	1.6	0	0.6	0.7

df, degrees of freedom; GCA, general combining ability; SCA, specific combining ability.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.

(Table 2). The variances for cross-by-location interaction were not significantly different for any of the traits except DA and GY. However, variances for both GCA- and SCA-by-location interactions were significant for DA, PH, and ZEA with SCA-by-location interaction variances being significant also for GY and LUT.

3.2. *Variance components, heritability and genetic advance*

The estimates of variance components, heritability, and GAM across the two locations (Samaru and Kerawa) in 2013 are presented in Table 3. The results show that GCA variance (σ^2_{gca}) ranged from 0 for PH to 15.02 for DA and from 0 for provitamin A-contributing carotenoids (i.e. αC, βCX, and βC) to 5.95 for ZEA. The SCA variance (σ^2_{sca}) ranged from 12.22 for DA to 1396.64 for PH and from 0 for αC and βCX to 26.87 for ZEA. The GCA-by-location interaction variance (σ^2_{gcaxl}) ranged from 0.17 for GY to 287.45 for PH and from 0 for all the carotenoids to 0.11 for βCX. The SCA-by-location interaction variance (σ^2_{scaxl}) ranged from 10.94 for GY to 624.92 for PH and from 0 for βC to 49.95 for ZEA. The additive variance (σ^2_a) ranged from 0 for PH to 30.05 for DA and the dominance variance (σ^2_d) ranged from 12.22 for GY to 1396.64 for PH. The σ^2_a for carotenoids ranged from 0 for provitamin A carotenoids with the exception of PVA (0.54) to 11.91 for ZEA and the σ^2_d ranged from 0 for provitamin A carotenoids with the exception of βC (0.15) to 26.87 for ZEA. The additive-by-location interaction variance (σ^2_{axl}) ranged from 0.34 for GY to 594.9 for PH and from 0 for all the carotenoids except βCX to 0.22 for βCX. The genotypic variance (σ^2_g) ranged from 27.37 for GY to 1397 for PH and ranged from 0 for αC and βCX to 38.77 for ZEA. The phenotypic variance (σ^2_p) ranged from 33.2 for GY to 2089 for PH and from 0.07 for αC to 65.82 for ZEA. Broad-sense heritability (H^2), ranged from 40.08% for DA to 82.44% for GY and from 0% for βCX and αC to 67.37% for βC. Narrow-sense heritability (h^2) ranged from 0% for PH to 28.49% for DA and from 0% for provitamin A carotenoids-αC, βCX, and βC to 19% for LUT. GAM for the combined locations ranged from 50.54 for GY to 11,655.99 for PH and from 0 to 105.95 for ZEA.

Table 3 – Estimates of variance components, heritability, and genetic advance for agronomic traits and carotenoid contents of tropical-adapted provitamin A maize crosses evaluated across locations in 2013.

Trait	σ^2_{gca}	σ^2_{sca}	σ^2_{gcaxl}	σ^2_{scaxl}	σ^2_a	σ^2_d	σ^2_{axl}	σ^2_{dxl}	σ^2_e	σ^2_g	σ^2_p	H^2	h^2	GAM
DA	15.02	12.22	5.61	111.38	30.05	12.22	11.21	111.38	7.57	42.26	105.50	40.08	28.49	510.07
PH	0	1396.64	297.45	624.92	0	1396.64	594.90	624.92	329.31	1397.00	2089.00	66.86	0	11,655.99
GY	0.35	26.66	0.17	10.94	0.71	26.66	0.34	10.94	0.76	27.37	33.20	82.44	2.13	50.54
LUT	3.52	15.73	0	26.60	7.03	15.73	0	26.60	3.82	22.76	37.01	61.49	19.00	47.72
ZEA	5.95	26.87	0	49.95	11.91	26.87	0	49.95	8.28	38.77	65.82	58.91	18.09	105.95
βCX	0	0	0.11	0.14	0	0	0.22	0.14	0.41	0	0.28	0	0	0
αC	0	0	0	0.14	0	0	0	0.14	0.02	0	0.07	0	0	0
βC	0	0.16	0	0	0	0.16	0	0	0.31	0.16	0.24	67.37	0	1.41
PVA	0.01	0	0	0.54	0.02	0	0	0.54	0.70	0.02	0.46	3.71	3.71	0.18

σ^2_{gca}, GCA variance; σ^2_{sca}, SCA variance; σ^2_a, additive variance; σ^2_d, dominance variance; σ^2_e, error variance; σ^2_{gcaxl}, GCA variance; σ^2_{scaxl}, SCA variance; σ^2_g, genetic variance; σ^2_p, phenotypic variance; H^2 = broad-sense heritability; h^2 = narrow-sense heritability; GAM, genetic gain as percentage of mean; DA, days to anthesis; PH, plant height; GY, grain yield ; LUT, lutein; ZEA, zeaxanthin; βCX, β-cryptoxanthin; αC, α-carotene; βC, β-carotene; PVA, total provitamin A.

Table 4 – Estimates of genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients among agronomic and carotenoid traits in 36 provitamin A maize crosses tested across two locations in 2013 wet season.

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

DA, days to anthesis; PH, plant height; GY, grain yield; LUT, lutein; ZEA, zeaxanthin; βC, β-carotene; βCX, β-cryptoxanthin; αC, α-carotene; PVA, total provitamin A.

* Indicates significance at the 0.01 probability level.

** Indicates significance at the 0.05 probability level.

3.3. *Correlations among grain yield, days to 50% anthesis, plant height, and carotenoids*

Grain yield (GY) showed significant positive phenotypic correlations (rP) with βC, βCX, and αC (Table 4). The phenotypic correlation between DA and αC was negative, but the genotypic correlations for the corresponding traits were nonsignificant and in the reversed direction.

The correlation between PH and ZEA was highly significant and negative at the genotypic level, whereas at the phenotypic level, though in the same direction, it was very low (Table 4). PVA was phenotypically positively correlated with βC, βCX, and ZEA and the genotypic correlation of PVA with βC was positive. The phenotypic correlations between PVA and βC, βCX, and ZEA were positive and significant. βC was phenotypically positively correlated with βCX, αC, and ZEA and βCX was positively correlated (rP) with αC and ZEA. However, most of the genotypic correlation coefficients (rG) showed marked reduction in magnitude and change in direction for all the corresponding phenotypic correlations (rP) between GY and each of the studied variables, indicating masking by environmental factors and suggesting that the associated traits were not under the influence of the same gene(s).

3.4. *Performance and ranking of crosses for grain yield and provitamin A carotenoids*

Simultaneous selection for many desirable traits based on the mean performances of crosses (progenies) for grain yield and carotenoids and their rankings, according to a selection direction (for traits of interest) are presented in Table 5. Selection direction with a "+" sign indicates that a higher value of the trait is desirable (e.g. GY, βC, PVA), whereas a "–" sign indicates that a lower value of the trait is desirable (e.g. DA, LUT). For savanna ecologies showing year-to-year variability of rains with intermittent dry spells of 1–3 weeks during the rainy season, the use of early to intermediate maize cultivars (82–105 days from planting to maturity) has been recommended for the region [39]. Thus, the selection direction for DA was "–" in sign. Tall plants and plants with long ear heights tend to show excessive root and stalk lodging [39], so that selection direction is "–" in sign. GY and provitamin A carotenoids (βCX, αC, βC, PVA) have "+" selection directions. ZEA showed significant positive association with PVA active carotenoids and accordingly has a "+" selection direction as well. Although LUT has other important health benefits, it is required in low amounts because of its negative correlations with provitamin A carotenoids. The six top-performing crosses for GY and PVA were E6/E9, E3/E16, E6/E7, E9/E7, E8/E7, and E8/E6. The combination E6/E9 ranked fourth for PVA (4.0 μg g^{-1}), sixth for βC 2.36 μg g^{-1}, fourth for βCX (2.81) and second for yield (7.16 t ha^{-1}). The cross E3/E16 ranked first for PVA (4.42 μg g^{-1}) and fourteenth for GY (5.49 t ha^{-1}). Crosses E6/E7 and E9/E7 with GY 4.86 t ha^{-1} and 5.12 t ha^{-1} ranked third and fifth, respectively, for PVA, whereas crosses E8/E6 (4.58 t ha^{-1}) and E8/E7 (5.82 t ha^{-1}), ranked second and tenth, respectively. The six highest-yielding combinations among the crosses had the following PVA rankings: E26/E6 (7.33 t ha^{-1}) eleventh (3.68 μg g^{-1}), E6/E9 (7.16 t ha^{-1}) fourth (3.998 μg g^{-1}), E9/E28 (6.75 t ha^{-1}); twelveth (3.663 μg g^{-1}), E3/E28 (6.63 t ha^{-1}) nineteenth (3.48 μg g^{-1}), E37/E7 (6.62 t ha^{-1}) twenty-eighth (3.15 μg g^{-1}), and E9/E3 (6.37 t ha^{-1}) twentieth (3.45 μg g^{-1}). Lines E6, E7, E8, and E9 were the common parents in the best crosses for GY and PVA.

4. Discussion

All of the crosses evaluated in this study showed provitamin A contents within the recommended range for first-generation medium to high provitamin A maize genotypes (3–8 μg g^{-1}), with 15 μg g^{-1} PVA being the final target [14]. Crosses that showed a combination of desirable agronomic traits and carotenoids across locations were E6/E9, E3/E16, E6/E7, E9/E7, E8/E7, and E8/E6. The lines E6, E7, E8, and E9 were the common parents among the top-performing crosses. The non-provitamin A carotenoid (LUT and ZEA) content of 77.74% of the total carotenoids agreed with the results of Egesel et al. [20] for hybrids and those of Menkir et al. [11] for inbred lines. ZEA was the major carotenoid, with a value of about 50% in agreement with earlier reports of Wong [40], Egesel et al. [20], and Suwarno et al. [22], though its value was only 20.40% in Suwarno et al. [22]. The level of LUT was the second highest of the carotenoids (28.40%), a finding in agreement with Egesel

Table 5 – Mean performance and ranking for agronomic traits and carotenoid contents of provitamin A maize crosses evaluated across Samaru and Kerawa locations in 2013 wet season.

Crosses	DA[a]		PH		GY		LUT		ZEA		αC		βCX		βC		PVA	
	day	(–)	cm	(–)	t ha^{-1}	(+)	μg g^{-1}	(–)	μg g^{-1}	(+)	μg g^{-1}	(+)	μg g^{-1}	(+)	μg g^{-1}	(+)	μg g^{-1}	(+)
E6/E9	62	28	178	12	7.16	2	12.21	28	7.60	6	0.46	7	2.81	4	2.36	6	4.00	4
E3/E16	57	2	183	17	5.49	14	14.87	35	4.43	34	0.61	2	2.61	9	2.81	1	4.42	1
E6/E7	63	29	196	28	4.86	22	14.39	33	7.51	7	0.48	3	2.65	7	2.65	3	4.22	3
E9/E7	59	10	177	11	5.12	20	9.58	12	6.88	10	0.42	12	2.48	17	2.50	5	3.95	5
E8/E7	57	2	176	9	5.82	10	12.43	30	8.01	5	0.40	14	2.83	2	2.12	15	3.74	10
E8/E6	59	10	174	6	4.58	24	12.23	29	9.04	3	0.39	16	2.72	5	2.74	2	4.30	2
E7/E16	61	23	179	13	4.93	21	9.56	11	5.6	22	0.47	5	2.53	13	2.06	19	3.56	15
E8/E9	60	18	186	20	6.26	7	6.78	2	4.23	36	0.42	12	2.50	15	2.12	15	3.58	13
E28/E7	66	34	183	17	4.06	30	11.70	26	8.27	4	0.44	8	2.23	24	2.55	4	3.88	6
E28/E37	66	34	183	17	5.35	17	8.77	4	4.84	30	0.47	5	1.84	36	2.33	8	3.49	18
E9/E3	56	1	170	3	6.37	6	12.50	31	4.24	35	0.43	10	2.22	25	2.12	15	3.45	20
E8/E16	59	10	196	28	5.44	15	6.04	1	6.44	12	0.44	8	2.11	32	2.25	9	3.53	16
E26/E6	60	18	174	6	7.33	1	14.61	34	4.88	29	0.37	20	2.69	6	2.15	13	3.68	11
E9/E28	63	29	191	23	6.75	3	9.87	16	6.41	13	0.35	24	2.61	9	2.18	12	3.66	12
E26/E9	59	10	165	1	3.67	33	10.74	19	5.35	25	0.38	18	2.91	1	2.14	14	3.78	8
E26/E37	60	18	176	9	4.05	31	15.49	36	5.57	23	0.64	1	2.39	19	1.71	28	3.23	26
E3/E28	58	6	186	20	6.63	4	9.19	7	6.55	11	0.33	27	2.42	18	2.11	18	3.48	19
E26/E7	58	6	171	5	4.81	23	11.30	23	5.72	21	0.39	16	2.56	11	1.92	27	3.40	22
E26/E3	57	2	179	13	4.19	29	12.16	27	5.93	16	0.36	23	2.49	16	2.34	7	3.76	9
E8/E26	59	10	199	32	5.59	13	7.61	3	5.77	18	0.37	20	2.17	27	2.04	21	3.31	24
E28/E16	64	32	194	26	4.36	26	11.38	24	7.45	8	0.37	20	2.31	22	2.22	11	3.56	14
E26/E28	59	10	166	2	3.5	35	9.30	8	5.84	17	0.43	10	2.12	30	1.68	30	2.95	32
E26/E16	63	29	205	34	5.37	16	9.79	15	5.27	27	0.38	18	2.18	26	2.05	20	3.33	23
E8/E37	61	23	179	13	5.25	18	11.68	25	5.76	19	0.34	25	2.52	14	1.98	23	3.40	21
E9/E37	58	6	198	30	5.67	12	10.70	18	6.07	15	0.33	27	2.56	11	1.63	35	3.08	30
E8/E3	58	6	212	36	4.20	28	9.39	9	10.9	1	0.33	27	2.16	29	2.04	21	3.28	25
E37/E7	61	23	179	13	6.62	5	10.83	20	9.21	2	0.32	32	2.11	32	1.94	25	3.15	28
E8/E28	59	10	195	27	6.05	9	9.16	6	4.73	31	0.33	27	2.25	23	1.58	36	2.87	34
E37/E16	60	18	170	3	6.17	8	11.01	22	5.36	24	0.30	34	2.12	30	1.97	24	3.19	27
E9/E16	60	18	188	22	3.94	32	8.84	5	4.9	28	0.33	27	2.17	27	1.68	30	2.93	33
E3/E37	57	2	205	34	5.74	11	9.62	13	5.75	20	0.40	14	2.35	20	1.71	28	3.09	29
E3/E7	61	23	175	8	2.98	36	9.43	10	7.32	9	0.34	25	1.93	35	1.65	34	2.79	36
E6/E28	67	36	202	33	4.56	25	10.93	21	5.32	26	0.48	3	2.62	8	2.25	9	3.80	7
E6/E37	59	10	198	30	3.56	34	13.69	32	4.72	32	0.30	34	2.82	3	1.93	26	3.49	17
E6/E3	64	32	191	23	5.20	19	9.90	17	6.38	14	0.32	32	1.96	34	1.66	32	2.80	35
E6/E16	61	23	193	25	4.35	27	9.74	14	4.64	33	0.30	34	2.34	21	1.66	32	2.98	31

[a] Parenthesis = weight assigned to trait; DA, days to 50% anthesis; DS, days to 50% silking; ASI, anthesis–silking interval (DS-DA); PH, plant height; EH, ear height; EPH, ear-to-plant height ratio; EPP, ears per plant; GY, grain yield; LUT, lutein; ZEA, zeaxanthin; βCX, β-cryptoxanthin; αC, α-carotene; βC = β-carotene; PVA, total provitamin A; RSI, rank summation index; (+), higher value preferred; (–), lower value preferred.

et al. [20] but differing from Suwarno et al. [22], who reported LUT as the least abundant (12.21%) and βCX as the second most abundant (16.85%) carotenoid. The least abundant carotenoid with provitamin A activity was αC, a finding similar to that of Menkir et al. [11]. βC comprised 9.42%, greater than the value of Egesel et al. [20] (4.70%) but lower than that (13.43%) of Suwarno et al. [22]. The results differed probably owing to the differences in the germplasm used in the studies. The inbred lines were products of introgression of different βC-enhancing alleles discovered in some maize variants [13,41]. The maize mutants/variants showed decreased cyclization of βC to βCX and βCX to ZEA [13]. They also showed reduced conversion of αC to LUT resulting in accumulation of βC, βCX, and αC [41].

High genetic variation for agronomic and carotenoid traits across locations was observed among the crosses. This variation was created by hybridization of lines drawn from different clusters on the basis of genetic diversity revealed using 14 SSR markers. This result corroborates the finding of Liu et al. [42] that 9–12 SSR markers are sufficient to genotype maize inbred lines. The observed variation for carotenoids is in agreement with the reports of Egesel et al. [20], Menkir and Maziya-Dixon [18], Chander et al. [43], and Menkir et al. [11], which propose that there is a scope for genetic improvement of provitamin A carotenoids in maize adapted to tropical savanna. The GCA and SCA mean squares suggest that both additive and non-additive gene actions control the agronomic and carotenoid traits, with a preponderance of additive gene action for DA, PH, LUT, ZEA, and βCX and of non-additive gene action for GY, βC, αC, and PVA, as indicated by the GCA:SCA mean square ratios. These findings are in agreement with those of Egesel et al. [20], Chander et al. [43] and Suwarno et al. [22] who reported that both additive and non-additive gene actions control carotenoid inheritance in maize endosperm. The preponderance of non-additive gene action for the traits was further indicated by the additive variances (σ^2_a), which

were generally lower than the dominance variances (σ^2_d) for all the carotenoids, PH, and GY, but not for DA and PVA. This finding indicates an increase in importance of non-additive gene action as inbreeding increases, because more homozygotic loci emerge [44]. Vasal et al. [45] observed similar non-additive genetic effects for PH, and Egesel et al. [20] and Burt [21] reported similar results for βC and βCX.

The results also showed that DA, GY, LUT, βC, αC, and PVA were responsive to location, probably owing to heterogeneity of soil nutrients and their availability. These findings, though, in contrast to those of reports by Menkir et al. [11] that carotenoid concentrations were not strongly affected by replications or locations or genotype by environment interactions, agree with those of Egesel et al. [20], who reported location effects for all carotenoids except ZEA in yellow hybrid maize. ZEA is downstream in the biosynthetic pathways of βC and βCX, and thus depends on βCX as its precursor [46,47]. Hence, the strong association among the provitamin A carotenoids and ZEA as indicated by the significant phenotypic correlation coefficients among them. Thus, the observed location effects on the carotenoids imply that they are quantitative traits controlled by a few major genes i.e. they are oligogenic. The phenotypic correlations between GY and the carotenoids were positive, but the corresponding genotypic correlations were negative and also very low, indicating masking by environmental effects. There is thus scope to concurrently improve GY and the provitamin A carotenoids for biofortification. PH was negatively correlated with ZEA at both the genotypic and phenotypic levels, indicating that taller plants tend to use up more of the carotenoid being already committed to the synthesis of abscisic acid (ABA), a phytohormone that modulates developmental and stress processes [48], such that the taller the plant, the lower is the ZEA carotenoid that will be available for storage in yellow seeds.

The results for the genotypic correlations were similar to those of the phenotypic correlations for GY and the carotenoids reported in Suwarno et al. [22]. This finding is likely due to the large number of entries (df = 154) considered in Suwarno et al. [22] in contrast to the 36 entries (df = 34) in the present study, causing the phenotypic correlation estimates to approach the genotypic correlations [31]. The differences might also be explained on the basis that correlations are defined by the evaluated genotypes and the test environments [49]. The high GAM and heritability estimates for DA, ZEA, and LUT, when considered for predicting effective selection as in Johnson et al. [50], indicate a preponderance of additivity of gene action, indicating that predominantly genetic factors were responsible for the expression of DA, ZEA, and LUT, in agreement with previous researches in maize [43,45]. Therefore, mass selection in the breeding program will be effective for improving DA, LUT, and ZEA in desired selection directions initiated early at S_1 using families as selection units. For the other traits PH, GY, βCX, βC, and PVA, hybrid development will be most effective, as they show a prevalence of non-additive gene action. This inference is in agreement with the results of Chander et al. [43] who reported high broad-sense heritability estimates for ZEA and LUT, and those of Wong et al. [40] who reported medium heritability for all the carotenoids. Inbreeding–selection–recombination–inbreeding as in recurrent selection to improve βCX, βC, and PVA in the base population constructed from the 31 inbred lines presents a quite adequate strategy and breeding methods for short- and long-term breeding goals [51–53]. Recurrent selection will also permit recycling of the inbred lines and improving their population mean for short and long-term goals, for higher βCX, βC, and PVA contents than can be obtained by pedigree and backcrossing methods of inbred-line development [54]. The presence of higher SCA than GCA variances for the grain yield and carotenoid traits further indicates the scope of gain through recurrent selection to increase additive gene action [54] and also harness both additive non-additive gene actions. This approach will be necessary for developing higher PVA maize inbred lines for further exploitation of heterosis in order to improve GY, other agronomic traits, and provitamin A carotenoid contents. Thus, an appropriate strategy for developing high-yielding genotypes for grain yield and provitamin A carotenoids is hybrid development [55], especially of synthetic varieties, using improved inbred lines having higher provitamin A contents to broaden adaptability and recyclability of seeds, a common practice of farmers in developing countries.

5. Conclusions

This study showed that there is genetic diversity among the 31 tropical-adapted provitamin A maize inbred lines that enabled the creation of genetic variation through hybridization of lines between different clusters for agronomic traits and carotenoids, despite their common ancestry. Provitamin A concentration and grain yield in the maize population were controlled by both additive and non-additive gene actions with a preponderance of non-additive gene action. Hybrid development will thus be an appropriate breeding method for the development of maize varieties with high provitamin A contents adaptable to the tropics. Grain yield (GY) and the carotenoids showed no significant genotypic correlations and can thus be improved concurrently. ZEA had positive correlations with the provitamin A carotenoids (PVA, βC, βCX, αC), making it a promising secondary target trait for indirect selection for βC and βCX.

The best cross combination was E6/E9, which yielded 7.16 t ha^{-1} and 4.0 μg g^{-1} PVA. Line E6 was involved in most of the crosses with the highest PVA concentrations. Other best-performing crosses for GY and PVA were E3/E16, E6/E7, E9/E7, E8/E7, and E8/E6 with lines E7, E8 and E9 being the common parents. Thus, there is ample scope for improvement of provitamin A carotenoids and grain yields in SSA for people who depend on maize as a staple diet in the region.

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