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ScienceDirect
Food Chemistry 109 (2008) 521–529
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Carotenoid diversity in tropical-adapted yellow maize inbred lines

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Received 12 July 2007; received in revised form 13 November 2007; accepted 2 January 2008

Abstract

Maize is a staple food for millions of people in sub-Saharan Africa where a significant number of people suffer from vitamin A deficiency. Yellow maize contains both pro-vitamin A and nonprovitamin A carotenoids with potential health benefits to humans. An improvement in the concentration of these compounds can have a positive impact on dietary intakes in areas where yellow maize is consumed. An essential first step in breeding yellow maize for enhanced carotenoid concentrations involves an assessment of the carotenoid diversity of adapted maize inbred lines. Trials were thus conducted (i) to explore the genetic variation in carotenoid concentrations among tropical-adapted yellow maize inbred lines, (ii) to assess the potential for concurrent improvement of different carotenoids and (iii) to determine the consistency of carotenoid concentrations in different locations. Seed samples of a large set of lines harvested from four trials grown in one location and a fifth trial grown in two locations were analyzed for carotenoid concentrations using HPLC. The analyses of variance revealed that carotenoid concentrations were not strongly affected by the differences in replications or locations. There were large differences among the tropical-adapted yellow maize inbred lines in lutein, zeaxanthin, β-carotene, β-cryptoxanthin, α-carotene and total pro-vitamin A contents. As significant correlations were observed among carotenoids sharing a single branch of the carotenoid biosynthetic pathway, it should be feasible to increase the levels of multiple carotenoids simultaneously. Principal component analysis on the carotenoid composition of the yellow inbred lines identified some lines with higher levels of all carotenoids formed across both major branches of the carotenoid biosynthetic pathway, and other lines having higher levels of those carotenoids formed under a single major branch of the carotenoid biosynthetic pathway. These indicate that the selection of parental lines with diverse carotenoid profiles may possibly be exploited for genetic improvement of carotenoids in tropical maize.

Keywords: Carotenoids; Tropical-adapted; Yellow maize inbred lines

1. Introduction

Maize is a staple food for millions of people in sub-Saharan Africa. Yellow maize is produced in at least one major maize production zone in the various countries in West Africa (CIMMYT, 1988) and its grain is converted into well-accepted local food products, including gruels, porridges, pastes and infant weaning food. Yellow maize is preferred, as green maize and is consumed boiled or roasted on the cob to bridge the "hunger gap" after a long dry season. Maize is an important dietary source of energy, protein, vitamins, minerals and lipids. Vitamin A intake is sub-optimal for a large number of people in Africa who rely heavily on cereal-based diets. Vitamin A deficiency predisposes an estimated 100 million Africans to a higher risk of visual impairment and blindness (African Union, 2005). Young children, pregnant women and lactating mothers are most vulnerable to vitamin A deficiency. An estimated 33 million preschool-age children in Africa are reported to

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doi:10.1016/j.foodchem.2008.01.002

be deficient in vitamin A (West, 2002), which contributes to predisposition to several major diseases, such as anemia, diarrhea, measles, malaria and respiratory infections (Shankar, Genton, & Semba, 1999; Sommer & West, 1996; Villamor & Fawzi, 2000; West, 2000). Nearly 20–24% of child mortality from diarrhea, measles and malaria and 3% mortality from infectious diseases can be ascribed to vitamin A deficiency (Rice, West, & Black, 2004). Also, vitamin A deficiency contributes to maternal death as well as poor pregnancy and lactation outcome (Rice et al., 2004). Thus, any effort directed to minimize vitamin A deficiency would have the potential to improve the health and well being of women and children.

In recent years, efforts are underway to improve the carotenoid levels in staple food crops to overcome vitamin A deficiency in areas with limited access to animal products, fruits and vegetables. Yellow maize contains three carotenoids, namely β-carotene, β-cryptoxanthin and α-carotene, that are precursors for vitamin A. Maize is also a good source of nonprovitamin A carotenoids, including lutein and zeaxanthin, which play beneficial roles in human health (Krinsky, Landrum, & Bone, 2003). The consumption of carotenoid-rich foods is associated with reduced risks of developing cancer (Agarwal & Rao, 2000; Gerster, 1993; Sies & Stahl, 1995;) and cardiovascular diseases (Dagenais, Marchioli, Yusuf, & Tognoni, 2000; McDermott, 2000), enhanced immune responses (Watzl, Bub, Briviba, & Rechkemmer, 2003; White, Kim, Kalkwarf, Bustos, & Roe, 1988), improved vision and prevention of night blindness (Combs, 1992; Granado, Olmedilla, & Blanco, 2003; Olmedilla, Granado, Blanco, & Vaquero, 2003) as well as maintenance of healthy skin and gastrointestinal and respiratory systems (Bendich, 1993). Increased dietary intake of lutein and zeaxanthin has been associated with lowering of the risk of cataracts, age-related macular degeneration and other degenerative diseases (Mares et al., 2006; McDermott, 2000). Since the various carotenoids have different roles in human health, further enhancement of their levels in staple food crops, such as maize, may have a positive health impact in areas where yellow maize is consumed.

The carotenoid biosynthetic pathway in maize and other plants has two major branches that occur after the biosynthesis of the linear carotenoid, all-*trans*-lycopene (DellaPenna & Pogson, 2006). Lycopene may be cyclized to form two beta rings, as found in β-carotene and its derivatives, β-cryptoxanthin and zeaxanthin. Alternatively, lycopene may be cyclized to form one beta ring and one epsilon ring, as found in α-carotene and its derivatives, zeinoxanthin and lutein. The two predominant pro-vitamin A carotenoids in maize, β-carotene and β-cryptoxanthin are produced by the β, β branch of the biosynthetic pathway, whereas the third common pro-vitamin A carotenoid, α-carotene, is produced by the β, ε branch.

The first step in breeding maize for enhanced carotenoid contents involves an assessment of the extent of genotypic variation existing in adapted germplasm, to achieve the desired improvement. A few studies have found significant genetic variation in carotenoids in yellow maize lines and hybrids adapted to temperate environments (Brunson & Quackenbush, 1962; Grogan, Blessin, Dimler, & Campbell, 1963; Kurilich & Juvik, 1999; Quackenbush, Firch, Brunson, & House, 1966; Weber, 1987). Furthermore, carotenoids are known to be heritable traits in temperate maize (Hauge & Trost, 1930; Wong et al., 1998). However, limited reports have been published on the range of variation of carotenoids in diverse tropical-adapted yellow maize inbred lines. Survey of such inbred lines, in which many genes for adaptation and resistance to the prevailing major diseases are already fixed with modern analytical tools, can facilitate the selection of suitable breeding materials for successful development of maize cultivars that combine high carotenoid levels with desirable agronomic performance. These trials were, therefore, conducted (i) to explore the genetic variation in carotenoid concentrations among tropical-adapted yellow maize inbred lines, (ii) to assess the potential for concurrent improvement of different carotenoids and (iii) to determine consistency of carotenoid concentrations in different locations.

2. Materials and methods

2.1. Genetic materials and field trial

Sets of 139, 89, 96 and 97 tropical-adapted yellow-endosperm maize inbred lines were included in four independent trials (Tables 1 and 2). The lines were developed from diverse adapted × adapted and adapted × exotic crosses and backcrosses, as well as broad-based populations and open-pollinated varieties (ACR97TZLCOMP1-Y, ACR97SYN-Y, TZE-COMP5-Y-C7, Z.diplo.BC4, Taraba-local and STR SYN-W/Y) with selection for resistance to the major diseases and adaptations to tropical lowlands at the various stages of inbreeding. The diverse inbred lines were selected for potential variability in carotenoid content based on differences in kernel colour, such as light yellow, bright yellow, light orange and dark orange. The first trial consisted of 139 inbred lines and was grown in single row plots without replication at Ibadan (7°22′N, 3°58′E, altitude 150 m) during the 2003 dry season. The remaining sets of 89, 96 and 97 inbred lines included in the second, third and fourth trials, respectively, were arranged in a randomized complete block design with two replications. These three trials were grown at Ibadan in Nigeria during the main rainy seasons in 2003, 2004 and 2005, respectively. A fifth trial, consisting of 22 inbred lines with 2.25 to 6.7 μg/g of pro-vitamin A contents selected from the first four trials was also evaluated at Saminaka (10°34′N, 8°39′E, altitude 760 m) and Zaria (11°7′N, 7°21′E, altitude 640 m) in Nigeria in 2005. The lines in this trial were also arranged in a randomized complete block design with two replications. Each inbred line in each trial was grown in a single row plot 5 m long with 0.75 m spacing between rows and 0.25 m spacing between plants within a row. A compound fertilizer was applied at the rates of 60 kg N, 60 kg P, and 60 kg K ha^{-1} at the time of sowing.

Table 1
Pedigrees of list of entries included in two trials evaluated for carotenoids at Ibadan in 2003 and 2004

Pedigree	Number of tested lines
Trial 1 (2003)	
9450	1
((4001 × 9848) × 4001)	3
((KU1414 × 9450) × 9450)	4
(9450 × CM 116 × 9450)	14
(CIM116 × TZMI302 × CIM116)	1
4001 × KI 21	6
9450 × KI 21	80
ACR97TZL CCOMP1-Y	4
(CIM 118 × TZMI102)	1
DMR 9450 × TROPHY MOISTURE	7
PIONEER SEEDS HYBRID	1
POOL 18 QPM	2
POP 61-SR	8
SYN-Y-STR	4
TZECOMP.5-Y	3
Trial 2 (2004)	
4205	1
9613	1
4001 × 9848 × 4001	6
((ATP SR × KU1414 SR/SR) × ATP SR)	1
((KU1414 × 9450) × 9450)	3
4001 × KI 21	18
9450 × KI 21	1
ACR97TZL CCOMP1-Y	6
ACR97SYN-Y	8
KU1409	1
KU1414 × B37(HI)-1 × KU1414	1
KU1414 × CIMA 21-1 × KU1414	1
KU1414 × Fla 2AT 116-1 × KU1414	1
KU1414 × ICAL 36-1 × KU1414	1
KU1414 × SC 43-1 × KU1414	1
MMB90	1
MOK PION.Y	1
Sam Inbred-Y	1
SYN-Y-STR	1
Taraba-Local	21
TZE-COMP5-Y-C7	11
Z.diplo.BC4	2

Table 2
Pedigrees of list of entries included in two trials evaluated for carotenoids at Ibadan in 2004 and 2005

Pedigree	Number of tested lines
Trial 3 (2004)	
GT-MAS:Gk × KU1414SR × GT-MAS:Gk	2
(MP420 × 4001 × MP420)	3
1368 × GT-MAS-Gk	10
1368 × GH 34	4
1822 × B73LPA × 1822	3
4001 × B73LPA × 4001	30
5057 × B73LPA × 5057	1
9450 × KI 21	11
ACR97 TZL COMP1-Y	9
KU1409 × MO17LPA × KU1409	18
KU1414-SR × MO17LPA × KU1414-SR	2
MP420 × 9450 × MP420	1
STR SYN-W/Y	2
Trial 4 (2005)	
KU1414-SR/SC213	5
KU1414-SR/NC350	7
KU1414-SR/NC298	12
KU1414-SR/KVI43	15
KU1414-SR/KVI3	4
KU1414-SR/KVI11	5
KU1414-SR/KUI2007	1
KU1414-SR/CI187	6
KU1414-SR × MO17LPA × KU1414-SR	1
KU1414-SR	1
KU1409 × MO17LPA × KU1409	2
KU1409	1
ACR97TZL CCOMP1-Y	23
9450 × KI 21	3
4001 × KI 21	1
4001 × B73LPA × 4001	2
1823 × B73LPA × 1823	1
1368 × GH 34	1
9450 × CM 116 × 9450	1
9450 × KI 28	4
4001	1

An additional 60 kg N ha^{-1} was applied as top dressing four weeks later. In each trial, gramazone and atrazine were applied as pre-emergence herbicides at 5 l ha^{-1} each of Paraquat and Primextra. Subsequent manual weeding was done to keep the trials weed-free. At least 10 plants were selfed in each row and the harvested ears were threshed to make a composite sample. A 10 g sample from each line was sent to Iowa State University for carotenoid analysis.

2.2. Laboratory assay

Seed samples from the five trials were sent to Iowa State University for carotenoid analysis using HPLC. The extraction protocol used was the method of Granado, Olmedilla, Gil-Martinez, and Blanco (2001), as modified by Li, Tayie, Fox, Rocheford, and White (2007), for analysis of dried maize kernels. Each 10 g seed sample was ground to a fine powder. A 1.0 g aliquot was transferred to a 40 ml screw-capped test tube. Then 6 ml of MeOH (0.01% BHT) and a magnetic stir bar were added, and the tube was loosely capped. Multiple test tubes were placed in a beaker of water and incubated with stirring on a magnetic hotplate for 15 min at 50 °C. After cooling to room temperature, 6 ml of tetrahydrofuran (THF) were added to each test tube, and the tube was vortexed for 90 s. The test tubes were then allowed to stand for 5 min to allow the fine particles to settle. A 0.5 ml aliquot of the MeOH/THF extract was transferred to a 15 ml screw-capped test tube. Then 1.0 ml of 40% KOH in MeOH, containing 0.1 M pyrogallol, was added. The tube was flushed with argon, tightly capped, and vortexed for 3 min. To each tube, 2 ml of HPLC-grade water were added and the tube was vortexed for 30 s. An internal standard, β-apo-8′-carotenal, in MeOH was added. The carotenoids were partitioned into 4 ml of hexane/methylene chloride (5:1 by vol containing 0.01% BHT) by vortexing for 60 s. The sample was centrifuged for 5 min at 700×*g* to obtain visible phase separation, and the upper phase was dried under vacuum. The dried extract was dissolved in 100 μl of methyl-*tert*-butyl ether (MTBE), followed by 300 μl of

MeOH. A 100 μl aliquot was injected into the HPLC system for analyses of α-carotene, β-carotene (*cis* and *trans* isomers), β-cryptoxanthin, lutein, and zeaxanthin. The HPLC components included a 717 Plus autosampler with temperature control set at 5 °C, two 515 solvent-delivery systems, and the 2996 photodiode array detector (Waters Corporation). The system was operated with Empower 1 Software (Waters Corporation). Carotenoids were separated on a 5 μm C30 Carotenoid Column (4.6 × 250 mm; Waters Corporation) eluted by a mobile phase gradient from 100% methanol (containing 1 g ammonium acetate/l) to 100% MTBE over 60 min. The flow rate was 1.0 ml/min. Solvents were HPLC grade. To maximize detection of carotenoids, absorbance was measured at 453 nm.

2.3. Data analysis

The pro-vitamin A content of the maize was calculated by adding the amount of β-carotene (*cis* and *trans*) to one-half of the amounts of α-carotene and β-cryptoxanthin. On the basis of molecular structure, α-carotene and β-cryptoxanthin are considered to have 50% of the pro-vitamin A activity of β-carotene (U.S. Institute of Medicine, 2001). Therefore, we calculated the amount of pro-vitamin A activity obtained from α-carotene and β-cryptoxanthin in the maize to be half the amount obtained from β-carotene.

Since Trial 1 was not replicated, analyses of variance were computed for carotenoids measured only in Trail 2, Trial 3, Trial 4 and Trial 5. In the analysis of variance combined across locations (Trial 5), inbred lines were considered as fixed effects, while replications and locations were considered as random effects for each carotenoid. All analyses were performed with PROC GLM in SAS (SAS Institute 2000) using a RANDOM statement with the TEST option. Pearson's simple correlation coefficients between pairs of carotenoids were computed from the line values for Trial 1 and line means averaged over replications for Trial 2, Trial 3 and Trial 4 and line means averaged over replications and location for Trial 5 (SAS Institute 2000). Spearman's rank correlation coefficients between pairs of location means were calculated for lutein, α-carotene and pro-vitamin A contents to assess the consistency of ranking of the selected inbred lines across locations (SAS Institute 2000). Principal component analysis was performed using the correlation matrix of the different carotenoids, excluding pro-vitamin A content (SAS Institute 2000). The principal component scores for the first two axes (PC1 and PC2) were plotted to visualize the separation of the yellow-endosperm lines into groups in the first four trials. Simple correlation analysis was performed between mean pro-vitamin A contents of the lines and their respective PC1 scores in the first four trials (SAS Institute, 2000).

3. Results

Many yellow maize inbred lines with diverse genetic backgrounds were surveyed for variability in lutein, zeaxanthin, β-carotene, β-cryptoxanthin and α-carotene contents. As Trial 1 was not replicated, analyses of variance for the different carotenoids were computed for the remaining three replicated trials (Trail 2, Trial 3 and Trial 4). Trial 1 was included in this study to examine whether the trends observed in carotenoid composition in un-replicated trial would be similar to those detected in replicated trials. Although replication had a significant ($p < 0.05$, $p < 0.01$ and $p < 0.001$) effect on all carotenoids in two of the three trials grown at Ibadan, it represented less than 2% of the total variation in the concentrations of all carotenoids (Table 3). The variance among inbred lines was significant ($p < 0.01$ and $p < 0.001$) and represented 91 to 96% of the total variation in the concentrations of all carotenoids recorded in the three trials grown at Ibadan. To further assess the consistency of carotenoid levels in different locations, a trial consisting of selected inbred lines from the above four trials was re-evaluated at two locations. In the combined analysis of variance, the effect of location on concentrations of zeaxanthin, β-cryptoxanthin, α-carotene and pro-vitamin A was significant ($p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.05$, respectively) but not on lutein ($p = 0.09$) and β-carotene ($p = 0.22$). This component represented less than 5% of the total variation in the concentrations of all carotenoids (Table 3). Line × location interaction was significant only for lutein ($p < 0.05$), α-carotene ($p < 0.01$) and pro-vitamin A ($p < 0.05$) contents. Further analysis of line × location interaction, using rank correlation analysis between pairs of location means, revealed strong positive correlations for lutein ($r = 0.86$, $p < 0.0001$) and pro-vitamin A ($r = 0.75$, $p < 0.0001$) but not for α-carotene ($r = 0.42$, $p < 0.054$) content. Furthermore, the line × location interaction represented 4% to 13% of the total variation in the concentrations of all carotenoids, while the differences among lines accounted for 71% to 88% of the total variance in each carotenoid concentration (Table 3).

A broad range of variation in the concentrations of lutein, zeaxanthin, β-carotene, β-cryptoxanthin, α-carotene and pro-vitamin A contents was found within lines included in each trial (Tables 4 and 5). Lutein and zeaxanthin were the predominant carotenoids in each group of inbred lines included in the various trials. Among the remaining three carotenoids, which were those with pro-vitamin A activity, α-carotene had the lowest concentration in each line. The inbred lines in the various trials presented diverse carotenoid profiles, as observed in Tables 4 and 5. For example, the best-inbred line in the second trial had a higher concentration of β-carotene than that of β-cryptoxanthin, while the reverse was the case for the best-inbred lines in the first and third trials. On the other hand, the best-inbred line in the fourth trial had similar concentrations of both β-carotene and β-cryptoxanthin. Among all sets of lines included in the various trials, 4205 had the lowest concentration of zeaxanthin (Table 4). Several inbred lines with similar contents of pro-vitamin A but with marked differences in lutein and zeaxanthin concentrations

Table 3
Mean squares from the analysis of variance for the various carotenoids recorded in different trials evaluated at one or two locations

Source	DF	Lutein	Zeaxanthin	β-Cryptoxanthin	α-Carotene	β-Carotene	Pro-vitamin A
Trial 2 (2004)							
REP	1	16.15***	25.04***	1.20***	0.02**	0.67***	2.07***
ENTRY	88	31.44***	44.25***	2.32***	0.03***	0.92***	2.72***
Error	88	1.31	1.71	0.07	0.00	0.06	0.11
Trial 3 (2004)							
REP	1	0.01	1.54	0.06	0.00	0.00	0.01
ENTRY	95	17.32***	48.61***	0.96***	0.03***	0.47***	1.18***
Error	95	0.87	2.49	0.07	0.00	0.04	0.10
Trial 4 (2005)							
REP	1	4.32**	29.77***	1.06*	0.01**	0.36*	1.21**
ENTRY	96	8.94***	72.85***	2.24***	0.01***	0.89***	2.63***
Error	96	0.43	2.47	0.16	0.00	0.06	0.17
Trial 5 (2005)							
LOC	1	2.75	24.83*	1.23**	0.02***	0.09	0.64***
REP(LOC)	2	6.46	3.49	0.18	0.00	0.11	0.24
ENTRY	21	27.60***	94.22***	2.83***	0.01***	0.71***	2.43***
LOC × ENTRY	21	1.73*	4.14	0.22	0.00**	0.10	0.28*
Error	42	0.89	3.54	0.12	0.00	0.06	0.15

*,**,***Corresponding mean squares significantly different from zero at $p < 0.05$, $p < 0.001$ and $p < 0.001$ levels, respectively.

Table 4
Mean carotenoids of the best six lines with at least 50% more pro-vitamin A than the trial average selected from two trials evaluated at Ibadan in 2003 and 2004

Pedigree	Lutein (μg/g)	Zeaxanthin (μg/g)	β-Cryptoxanthin (μg/g)	α-Carotene (μg/g)	β-Carotene (μg/g)	Pro-vitamin A (μg/g)
Trial 1						
(9450 × CM 116 × 9450)-3-3-1-2-1	18.2	24.5	5.5	0.5	4.7	7.8
9450 × KI 21-1-5-3-2-2	8.2	9.9	5.4	0.3	3.1	6.0
9450 × KI 21-3-2-2-1-3	8.9	6.5	2.7	0.5	3.9	5.5
9450 × KI 21-1-5-3-2-1	7.5	10.7	4.6	0.3	3.0	5.5
9450 × KI 21-1-4-1-1-2	7.0	13.8	5.3	0.5	2.4	5.3
POP 61-SR - 11-2-3-3-1-B	6.0	5.3	3.1	0.3	3.0	4.7
Minimum	0.1	0.6	0.4	0.0	0.7	1.1
Maximum	18.2	24.5	5.5	1.9	4.7	7.8
Mean	6.2	7.0	2.0	0.3	1.7	2.9
S.E.	0.25	0.28	0.09	0.02	0.05	0.10
Trial 2						
SYN-Y-STR-34-1-1-1-1-2-1-B × 2	6.9	12.7	2.0	0.2	4.3	5.4
4205	6.6	2.3	4.6	0.6	2.7	5.3
((KU1414 × 9450) × 9450)-5-1-1-B × 4	2.8	21.5	3.9	0.0	2.8	4.8
4001 × KI 21-4-1-1-1-1	6.4	17.2	3.8	0.2	2.8	4.8
4001 × KI 21-4-3-1-1-1	15.8	15.2	3.8	0.5	2.5	4.6
4001 × KI 21-4-3-2-1-1	8.4	13.5	3.8	0.3	2.4	4.4
Minimum	0.4	0.3	0.3	0	0.3	0.4
Maximum	19	21.5	4.6	0.6	4.3	19
Mean	6.9	7.8	1.6	0.2	1.5	2.4
S.E.	0.8	0.9	0.2	0.0	0.2	0.2
CV	17	17	17	29	16	14

were found in the different trials. Of the 421 yellow maize inbred lines, 41 had pro-vitamin A contents exceeding their respective trial average by 50% to 171%. A total of 15 promising yellow inbred lines with 5.0 to 7.8 μg/g of pro-vitamin A content were identified from the first, second and fourth trials (Tables 4 and 5).

Pearson's correlation coefficients between pairs of carotenoids were computed, to assess the potential for concurrent improvement of the various cartenoids and to maximize health benefits derived from yellow maize (Table 6). The observed correlation coefficients closely followed the carotenoid biosynthetic pathway (DellaPenna & Pogson, 2006). The correlations between lutein and zeaxanthin, which are typically the predominant carotenoids in yellow maize, were significant and positive in four of the five trials but were not strong ($r = 0.21$ to $r = 0.46$, $p < 0.05$).

Table 5
Mean carotenoids of the best six lines with at least 50% more pro-vitamin A than the trial average selected from two trials evaluated at Ibadan in 2004 and 2005

Pedigree	Lutein (μg/g)	Zeaxanthin (μg/g)	β-Cryptoxanthin (μg/g)	α-Carotene (μg/g)	β-Carotene (μg/g)	Pro-vitamin A (μg/g)
Trial 3						
KU1409 × MO17LPA × KU1409-27-3-1-1	4.0	14.8	4.0	0.1	2.8	4.9
4001 × B73LPA × 4001-13-1-2	5.9	15.1	2.8	0.1	2.3	3.7
(MP420 × 4001 × MP420)-2-1-1-1-B-B	3.9	12.8	2.6	0.1	2.3	3.6
4001 × B73LPA × 4001-27-2-1	5.1	15.2	2.9	0.2	1.9	3.4
KU1409 × MO17LPA × KU1409-27-2-3-1	3.3	11.6	2.9	0.1	2.0	3.4
KU1409 × MO17LPA × KU1409-27-3-4-1	4.3	14.0	2.2	0.1	2.3	3.4
Minimum	0.6	0.5	0.4	0	0.5	0.6
Maximum	15.6	20.1	4	0.6	2.8	15.6
Mean	5.2	9.0	1.6	0.2	1.3	2.2
S.E.	2.9	4.9	0.7	0.1	0.5	0.8
CV	18	18	17	22	15	15
Trial 4						
KU1414-SR/KVI43-4-2	4.8	12.8	4.2	0.4	4.5	6.7
KU1414-SR/KVI11-7-1	3.5	32.4	4.8	0.4	3.2	5.7
KU1414-SR/KVI11-7-2	3.5	28.1	4.4	0.4	3.2	5.4
KU1414-SR/KVI43-6-4	7.4	18.6	4.3	0.3	3.0	5.3
(9450 × KI 28)-1-2-1-1	3.5	10.8	4.8	0.4	2.6	5.1
KU1414-SR/KVI43-6-1	9.0	19.3	4.1	0.3	2.9	5.1
Minimum	0.9	1.4	0.4	0.1	0.5	0.8
Maximum	11.3	32.4	4.8	0.4	4.5	6.7
Mean	4.9	13.4	2.7	0.2	1.9	3.4
S.E.	2.1	6.0	1.1	0.1	0.7	1.1
CV	13	12	15	12	13	12

Table 6
Correlation between pairs of carotenoids for four trials consisting of diverse sets of yellow inbred lines evaluated at Ibadan in 2003, 2004 and 2005

Correlation coefficients	Carotenoid combination				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Lutein with zeaxanthin	0.21*	0.31**	0.31**	0.46***	0.37
Lutein with β-cryptoxanthin	0.17*	0.12	0.12	0.18	0.09
Lutein with α-carotene	0.45***	0.62***	0.62***	0.10	0.37
Lutein with β-carotene	0.43***	−0.09	−0.09	0.23*	−0.08
α-Carotene with β-carotene	0.02	−0.10	−0.10	0.73***	−0.14
α-Carotene with zeaxanthin	0.14	−0.16	−0.16	0.50***	0.20
α-Carotene with β-cryptoxanthin	0.08	−0.06	−0.06	0.82***	0.16
Zeaxanthin with β-cryptoxanthin	0.69***	0.70***	0.70***	0.49***	0.60***
Zeaxanthin with β-carotene	0.62***	0.43***	0.43***	0.46***	0.35
β-Cryptoxanthin with β-carotene	0.70***	0.69***	0.69***	0.80***	0.70***

*,**,***Significantly different from zero at $p < 0.05$, $p < 0.01$ and $p < 0.001$ levels, respectively.

α-Carotene is the biosynthetic precursor of lutein in the β, ε-ring branch of the carotenoid biosynthetic pathway. Thus it is not surprising that lutein was significantly correlated with α-carotene ($r = 0.45$ to $r = 0.62$, $p < 0.0001$) in three of the five trials (Table 6). Similarly, the correlations of α-carotene with the β, β-ring pro-vitamin A carotenoids, β-cryptoxanthin and β-carotene, were weak and significant only in one trial (Table 6). As expected, zeaxanthin, a β, β-ring carotenoid, was significantly correlated with β-cryptoxanthin and β-carotene ($r = 0.43$ to $r = 0.70$, $p < 0.0001$) in at least four trials. Also, the correlation between β-cryptoxanthin and β-carotene was significant and positive ($r = 0.69$ to $r = 0.80$, $p < 0.0001$) in all the trials.

Principal component analysis was done to examine grouping of the yellow maize inbred lines on the basis of their carotenoid composition. The first two principal component axes (PC1 and PC2) accounted for 77% to 83% of the total variation in carotenoid content among the yellow maize inbred lines in all the trials (Table 7). The first PC1 accounted for 45% to 61% of the total variation and its high component scores were associated with increased concentrations of zeaxanthin, β-cryptoxanthin and β-carotene in all trials and with increased levels of lutein and α-carotene in three trials (Table 7). Also, increased concentrations of lutein and α-carotene contributed significantly to high PC2 scores in almost all of the trials. It thus appeared that

Table 7
Eigenvectors of the first two principal component axes for different trials evaluated in 2003, 2004, and 2005

	Trial 1 (2003)		Trial 2 (2004)		Trial 3 (2004)		Trial 4 (2005)	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Lutein	0.34***	0.59***	0.31**	0.64**	0.12	0.71**	0.22**	0.82**
Zeaxanthin	0.51***	−0.29***	0.48**	−0.31**	0.56**	0.08	0.41**	0.41**
β-Cryptoxanthin	0.54***	−0.23***	0.54**	−0.25**	0.62**	0.00	0.52**	−0.23*
α-Carotene	0.19***	0.71***	0.33**	0.62**	−0.08	0.68**	0.50**	−0.28**
β-Carotene	0.55***	−0.10	0.53**	−0.23*	0.53**	−0.15	0.50**	−0.17
Variance	0.51	0.26	0.53	0.30	0.45	0.33	0.61	0.22

*,**,***Corresponding eigenvector significantly different from zero at $p < 0.05$, $p < 0.001$ and $p < 0.001$ levels, respectively.

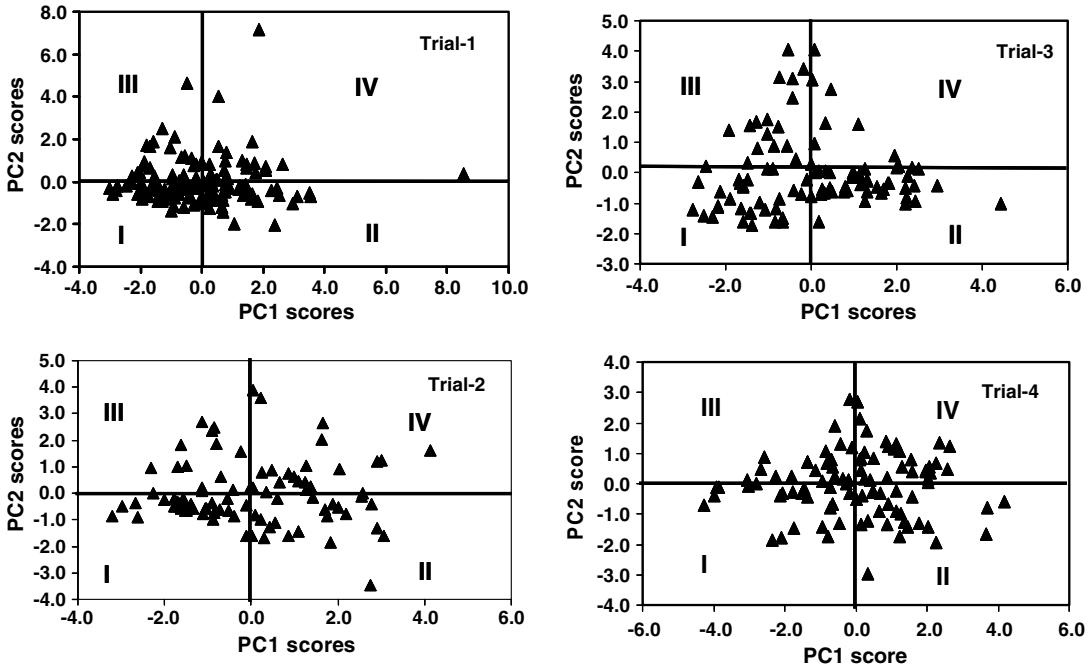

Fig. 1. Scatter plot of PC1 and PC2 axes scores for different sets of tropical-adapted yellow maize inbred lines with diverse carotenoid profiles.

carotenoids formed under the β, β branch of the carotenoid biosynthetic pathway (DellaPenna & Pogson, 2006) had the largest positive weights on PC1, whereas those formed under the β, ε branch of the pathway had the greatest positive weights on PC2 (Table 7). As shown in Fig. 1, the first group of inbred lines (Quadrant 1) in each trial had negative PC1 and PC2 scores and thus had low concentrations of all carotenoids. The second group of inbred lines (Quadrant 2) combined positive PC1 with negative PC2 scores, representing those with higher concentrations of zeaxanthin, β-cryptoxanthin and β-carotene. The third group of inbred lines (Quadrant 3) possessed higher concentrations of lutein and α-carotene but lower concentrations of the other carotenoids. The last group of inbred lines (Quadrant 4) had higher levels of all carotenoids. The correlation between pro-vitamin A content and PC1 scores was positive and significant ($r = 0.92$ to $r = 0.95$, $p < 0.0001$) in all the trials. PC2 scores had a weak negative correlation with pro-vitamin A content only in the second ($r = -0.26$, $p < 0.05$) and fourth ($r = -0.21$, $p < 0.05$) trials.

4. Discussion

The results of our studies revealed that replication, location and line × location interaction represented a small fraction of the total variation in the concentration of each carotenoid when compared with the variation among lines. Since the relative ranking of the lines was consistent across locations for the major pro-vitamin A carotenoids and for the major xanthophylls, lutein and zeaxanthin, the observed significant line × location interaction for lutein and pro-vitamin A content was mainly due to changes in magnitude of differences among lines within each location. Similar small effects of replication and year on carotenid concentration were reported in tropical open-pollinated varieties (Menkir & Maziya-Dixon, 2004) as well as temperate inbred lines (Brunson & Quackenbush, 1962; Kurilich & Juvik, 1999; Quackenbush et al., 1966) and hybrids (Egesel, Wong, Lambert, & Rocheford, 2003). These results indicate that major changes in carotenoids may not occur in yellow maize kernel samples harvested in different replications and

locations. The similarity of the trend observed for carotenoid composition in un-replicated and replicated trials in our studies has provided additional support for this conclusion. Other studies also indicate that inbred lines can have specific amounts of the different carotenoids consistently maintained in different replications and seasons (Brunson & Quackenbush, 1962; Egesel et al., 2003; Kurilich & Juvik, 1999; Quackenbush et al., 1966). As measurement of carotenoids in maize using HPLC is a relatively tedious and expensive procedure, it may thus be possible to identify promising parental materials from among a large number of lines grown in single row plots without replication. Further assessment of consistency of carotenoid concentrations across locations and seasons may be carried out using promising yellow maize inbred lines selected, based on the results of preliminary assessment in un-replicated trials.

A single biosynthetic pathway generates all carotenoids in maize and other plants (Gallagher, Matthews, Li, & Wurtzel, 2004). In our studies, most of the correlations between pairs of carotenoids were not significant, consistent with the findings of Kurilich and Juvik (1999). This suggests that the genes regulating the concentrations of these carotenoids were independent, and thus selection to increase the levels of these compounds simultaneously should be possible in tropical maize. The remaining correlations were significant but not very strong ($r < 0.75$), indicating that the inbred lines had a diverse array of alleles among genes that regulate the various steps in the biosynthetic pathway of carotenoids (Kurilich & Juvik, 1999). Palaisa, Morgante, Williams, and Rafalski (2003) found considerable allelic variation at the Y1 locus that regulates the synthesis of phytoene synthase, an essential enzyme that catalyzes the first step in the carotenoid biosynthetic pathway in maize. The polymorphic sites found at the Y1 locus were significantly associated with the endosperm colour phenotype (Palaisa et al., 2003).

The observed broad range of variation among the yellow maize inbred lines in the accumulation of carotenoids indicates the potential to select parental lines for genetic improvement of these compounds. Principal component analysis on the carotenoid composition of the diverse yellow inbred lines revealed that some inbred lines had higher concentrations of pro-vitamin A carotenoids, as well as the nonprovitamin A xanthophylls, lutein and zeaxanthin, while others could be distinguished on the basis of their high concentrations of carotenoids formed under the separate branches of the carotenoid biosynthetic pathway. There were also some yellow inbred lines with low concentrations of lutein, zeaxanthin, β-carotene, β-cryptoxanthin and α-carotene. Two breeding strategies can thus be pursued to enhance the concentrations of carotenoids in yellow maize adapted to the tropics. The first strategy can focus on genetic improvement of pro-vitamin A carotenoids (β-carotene, β-cryptoxanthin and α-carotene) and nonprovitamin A xanthophylls (lutein, zeaxanthin) by inter-crossing parental lines with high concentrations of these compounds. The second strategy can exploit parental lines with complimentary carotenoid profiles for making crosses to significantly increase the concentrations of provitamin A carotenoids and nonprovitamin A xanthophylls, representing both branches of the biosynthetic pathway.

Even though lutein and zeaxanthin were the two predominant carotenoids in the different sets of inbred lines included in our trials, considerable differences in these fractions were found in several inbred lines that can be exploited for making crosses to develop inbred lines with higher levels of pro-vitamin A carotenoids and acceptable levels of lutein and zeaxanthin. The absence of very strong correlations among carotenoids in all the trials indicates the potential that exists to utilize the diverse array of alleles present in the different sets of tropical-adapted inbred lines for regulating higher levels of synthesis of pro-vitamin A carotenoids under the biosynthetic pathway through hybridization and inbreeding.

Genetic enhancement in carotenoid accumulation should go hand in hand with gains in productivity and adaptation of maize cultivars to their production environments. The differential accumulation of carotenoids in tropical-adapted yellow maize inbred lines can thus form the basis for developing high-yielding hybrids and synthetics with high pro-vitamin A content, well-adapted to the current growing conditions and more profitable to farmers. Progress in breeding maize for increased carotenoid content could be rapid, as suggested by the high heritability estimates (Wong et al., 1998) and the preponderance of additive genetic variance for carotenoid content in maize (Brunson & Quackenbush, 1962; Egesel et al., 2003; Grogan et al., 1963). Although the combining abilities of the best-inbred lines selected for high pro-vitamin A content from these trials have not been determined, crosses between pairs of diverse lines among them will likely generate hybrids having higher levels of pro-vitamin A in their grain. The work of Brunson and Quackenbush (1962) demonstrated clearly that the pro-vitamin A content of all single-cross hybrids among high-pro-vitamin A inbred lines averaged 4.4 times more than that of all single-cross hybrids among lines with low pro-vitamin A content. As the best-inbred lines represent diverse genetic backgrounds, they can also be used for making diallel or factorial crosses to determine their combining ability that will be useful for developing productive hybrids and synthetics as well as improved inbred lines. Grogan (1964) successfully developed inbred lines with significantly higher levels of xanthophylls in their grain through hybridization and inbreeding. The best yellow-endosperm maize inbred lines selected from this study can also provide the genetic basis for introgressing temperate germplasm with high levels of pro-vitamin A carotenoids to increase their concentrations to much higher levels in tropical-adapted maize.

Acknowledgements

This research was conducted at the International Institute of Tropical Agriculture and financed by the HarvestPlus

challenge programme. The authors express their appreciation to all staff who participated during planting, harvesting and management of the trials at the three locations.

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