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Genetic variability for total carotenoid concentration in selected tropical maize (*Zea mays*) inbred lines

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ABSTRACT

A panel of 111 Indian and exotic maize (*Zea mays* L.) inbred lines were analyzed for total kernel carotenoid concentration. The study revealed significant genetic variability for the target trait, with concentration ranging from 6.5 to 67.3 µg/g with a mean of 26.9 µg/g. Among the genotypes analyzed, HPLET-03-36 developed under Maize HarvestPlus program at CIMMYT-Mexico recorded the highest total carotenoid concentration of 67.3 µg/g, followed by HPLET-03-37 (63.2 µg/g), HPLET-03-35 (59.9 µg/g), BLSB-RIL17 (57.0 µg/g), BLSB-RIL43 (56.7 µg/g), HPLET-03-41 (56.1 µg/g) and BLSB-RIL95 (50.9 µg/g). While the mean total carotenoid concentration in the HarvestPlus lines was 34.1 µg/g, the mean value for the target trait in the Indian maize lines was 23.8 µg/g. Selected inbreds from RIL populations developed using CM140 and CA00106 showed transgressive segregation for total carotenoid concentration. The average total carotenoid concentration among the deep orange inbred lines were 31.1 µg/g, while the same was found to be 28.03 µg/g, 23.97 µg/g, and 16.13 µg/g among the orange, yellow and pale yellow kernel classes of inbred lines, respectively. However, the range for total carotenoid concentration in each of the coloured classes (excluding white) varied considerably. Positive correlation was found between kernel colour and total carotenoid concentration; deep orange kernel colour genotypes, in general, recorded higher levels of total carotenoid as compared to those with yellow or light yellow kernel colour. Identification of maize inbreds with high total kernel carotenoid concentration in the present study provides a strong opportunity for utilizing these promising genotypes in breeding for carotenoid-rich maize germplasm.

Key words: Biofortification, Carotenoids, Kernel colour, Maize

"Hidden hunger", a popular phrase to denote micronutrient malnutrition, has become one of the alarming problems afflicting an estimated three billion people around the world (UNSCN 2004). Micronutrient deficiencies are mainly concentrated in the semi-arid tropics, particularly in South and South East Asia and Sub-Saharan Africa (Reddy *et al*. 2005). In India, 230 million people were reported to be undernourished, accounting for more than 27% of the world's undernourished population (Lodha *et al.* 2005). An estimated 190 million pre-school children and 19 million pregnant women, mostly from Africa and South Asia, are affected due to vitamin A deficiency (WHO 2009). An early symptom of vitamin A deficiency is night blindness. Structural alterations of the conjunctiva and the cornea such as xerophthalmia and keratomalacia may follow, and subsequent inflammation and infection results in irreversible blindness (Mayer *et al*. 2008).

Carotenoids derived from plant products have been the major source of vitamin A in the human diet particularly in the developing countries, where access to animal food is restricted due to poor economic conditions. Carotenoids are lipid soluble polyenes, and function mainly to prevent photo-oxidative damage of chlorophyll, and other associated molecules in plants (Niyogi 2000). Plant sources provide provitamin A which serves as the precursor for vitamin A synthesis in the human body (Hess *et al*. 2005).

Maize (*Zea mays*) is the staple food of hundreds of millions of people, especially in the tropical and subtropical areas of the developing world. Together with rice and wheat, maize provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries. Maize provides over 20% of total calories in human diets in 21 countries, and over 30% in 12 countries that are home to a total of more than 310 million people (Shiferaw *et al*. 2011). White kernel maize lacks β-carotene in the endosperm due to the presence of *yellow1* (*y1*) recessive allele in homozygous condition (Buckner *et al*. 1990). The dominant version of this allele, *Y1* produces enough β-carotene and makes the kernel yellow in

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colour. Although yellow maize is rich in β carotene concentration as compared to other cereals, the concentration is below the prescribed daily requirement by human. Based on the factors such as carotenoid concentration, bioavailability, food matrix, amount of food consumed in the meal and nutrient status of the host, nutritionists have proposed a target of 15 µg of provitamin A concentration per unit of dry weight of maize kernel (www.harvestplus.org).

A number of methods such as food fortification and supplementation have been tried all over the world for alleviating micronutrient deficiencies. However, these measures in general have been either partly or largely unsuccessful for several reasons. Therefore, biofortification is proposed as a logical and cost-effective means for providing the required levels of micronutrients in the diets of the people and is a sustainable approach to alleviate malnutrition in humans (Long *et al*. 2004, Bouis 2002, Pfeiffer and McClafferty 2007). Ascertaining the genetic variability in kernel carotenoid concentration in the elite maize germplasm is the first step toward breeding for provitamin A-enriched maize germplasm. While intensive efforts are being undertaken by international research institutions, such as CIMMYT and IITA, under the Maize HarvestPlus Program, there has been no comprehensive analysis of genetic variability for carotenoids among the inbreds developed under the Indian maize breeding programme. Therefore, the present study was undertaken to analyze the genetic variability for total carotenoid concentration in large set of maize inbred lines developed at different breeding centres in India vis-à-vis selected CIMMYT maize lines developed under the HarvestPlus Program.

MATERIALS AND METHODS

A diverse panel of 111 inbred lines developed from the maize breeding programs in India as well as from CIMMYT was used in this study for analyses of variability for total carotenoid concentration. The inbreds were selected based on the kernel colour ranging from white (negative 'control') to deep orange. The panel comprised 69 inbreds developed by various maize breeding centers in India, and 42 inbred lines obtained from CIMMYT, Mexico, of which 36 were from the Maize HarvestPlus Program. All these inbred lines were grown at the IARI Experimental Farm, New Delhi during *kharif* 2009. The detailed characteristics of the selected inbred lines are presented in Table 1.

Total carotenoid concentration from each of the maize genotypes was estimated using quick carotenoid extraction protocol, as suggested by Rocheford (2004). After kernel maturation and plant dry down, ears with the husk were manually harvested and were dried under the shade to lower post-harvest grain moisture to 14%. Randomly sampled 50 kernels from each genotype, and the ground sample (0.5g) was further divided into three replicates per entry. Each step prior to and during biochemical analysis was carried out

Table 1 Mean total carotenoid concentration and kernel colour among the inbred lines

Inbreds	Source	Total carotenoid concentration (µg/g)	Kernel colour*
CM128	VPKAS, Almora	24.7	Y
CM129	VPKAS, Almora	14.1	PY
CM135	IARI, New Delhi	19.5	Y
CM136	IARI, New Delhi	22.8	O
CM137	IARI, New Delhi	21.3	DO
CM138	IARI, New Delhi	24.9	O
CM139	PAU, Ludhiana	35.3	DO
CM140	PAU, Ludhiana	34.8	O
CM142	IARI, New Delhi	15.8	O
CM143	VPKAS, Almora	15.1	Y
CM144	VPKAS, Almora	19.3	O
CM145	VPKAS, Almora	23.9	O
CM150	IARI, New Delhi	23.1	DO
CM151	IARI, New Delhi	10.2	Y
CM152	VPKAS, Almora	15.5	O
CM153	VPKAS, Almora	21.9	O
CM212	VPKAS, Almora	23.8	O
DMRQPM-28-03	DMR, New Delhi	13.0	PY
DMRQPM-58	DMR, New Delhi	30.2	DO
DMRQPM-60	DMR, New Delhi	20.0	O
DQPM-03-102	DMR, New Delhi	19.8	O
DQPM-03-118	DMR, New Delhi	20.0	DO
DQPM-03-121	DMR, New Delhi	23.3	DO
HKI295	CCS-HAU, Uchani	26.7	O
HKI586	CCS-HAU, Uchani	6.5	O
HKI335	CCS-HAU, Uchani	18.9	Y
LM13	PAU, Ludhiana	18.4	O
LM14	PAU, Ludhiana	20.5	Y
LM15	PAU, Ludhiana	22.3	DO
LM16	PAU, Ludhiana	22.3	DO
LM6	PAU, Ludhiana	17.2	Y
NAI125	UAS-Nagenahalli	25.8	O
NAI147	UAS-Nagenahalli	10.9	O
SE547	PAU, Ludhiana	24.4	O
V340	VPKAS, Almora	13.7	Y
V341	VPKAS, Almora	11.6	O
V348	VPKAS, Almora	23.5	O
VQL1	VPKAS, Almora	27.8	O
VQL5	VPKAS, Almora	16.2	PY
BAJIM-6-17	CSK-HPKV, Bajaura	13.3	PY
BAJIM-6-3	CSK-HPKV, Bajaura	19.7	O
BAJIM-8-10	CSK-HPKV, Bajaura	29.9	O
BAJIM-8-3	CSK-HPKV, Bajaura	20.1	Y
BAJIM-8-4	CSK-HPKV, Bajaura	15.5	O
BAJIM-8-7	CSK-HPKV, Bajaura	10.6	Y
BAJIM-8-8	CSK-HPKV, Bajaura	21.9	O
BLSB-RIL 106	IARI, New Delhi	22.4	DO

(*Contd.*)

Table 1 (*Contd.*)

Inbreds	Source	Total carotenoid concentration (µg/g)	Kernel colour*
BLSB-RIL107	IARI, New Delhi	28.5	O
BLSB-RIL108	IARI, New Delhi	23.0	O
BLSB-RIL15	IARI, New Delfhi	23.5	O
BLSB-RIL8	IARI, New Delhi	19.0	O
BLSB-RIL89	IARI, New Delhi	20.5	O
BLSB-RIL92	IARI, New Delhi	18.6	DO
BLSB-RIL15	IARI, New Delhi	40.4	O
BLSB-RIL16	IARI, New Delhi	42.7	O
BLSB-RIL17	IARI, New Delhi	57.0	O
BLSB-RIL33	IARI, New Delhi	43.9	O
BLSB-RIL41	IARI, New Delhi	27.7	O
BLSB-RIL43	IARI, New Delhi	56.7	O
BLSB-RIL7	IARI, New Delhi	42.5	O
BLSB-RIL76	IARI, New Delhi	49.9	O
BLSB-RIL95	IARI, New Delhi	50.9	O
DM-RIL47	IARI, New Delhi	21.8	O
DM-RIL48	IARI, New Delhi	25.3	O
DM-RIL52	IARI, New Delhi	17.5	O
DM-RIL60	IARI, New Delhi	23.1	Y
DM-RIL66	IARI, New Delhi	20.3	O
DM-RIL70	IARI, New Delhi	21.9	DO
DM-RIL74	IARI, New Delhi	22.3	DO
HPLET-03-8	CIMMYT-HarvestPlus	48.3	O
HPLET-03-41	CIMMYT-HarvestPlus	56.1	O
HPLET-03-11	CIMMYT-HarvestPlus	24.6	Y
HPLET-03-39	CIMMYT-HarvestPlus	38.4	O
HPLET-03-37	CIMMYT-HarvestPlus	63.2	DO
HPLET-03-26	CIMMYT-HarvestPlus	39.9	Y
HPLET-03-20	CIMMYT-HarvestPlus	26.0	Y
HPLET-03-44	CIMMYT-HarvestPlus	38.9	O
HPLET-03-23	CIMMYT-HarvestPlus	11.7	PY
HPLET-03-43	CIMMYT-HarvestPlus	48.8	O
HPLET-03-18	CIMMYT-HarvestPlus	33.7	Y
HPLET-03-33	CIMMYT-HarvestPlus	24.1	PY
HPLET-03-12	CIMMYT-HarvestPlus	34.2	Y
HPLET-03-35	CIMMYT-HarvestPlus	59.9	DO
HPLET-03-36	CIMMYT-HarvestPlus	67.3	DO
HPLET-03-38	CIMMYT-HarvestPlus	36.5	Y
HPLET-03-5	CIMMYT-HarvestPlus	39.3	Y
HPLET-03-24	CIMMYT-HarvestPlus	31.0	Y
HPLET-03-29	CIMMYT-HarvestPlus	32.4	Y
HPLET-03-28	CIMMYT-HarvestPlus	28.9	O
HPLET-03-22	CIMMYT-HarvestPlus	15.7	PY
HPLET-03-34	CIMMYT-HarvestPlus	45.8	O
HPLET-03-2	CIMMYT-HarvestPlus	17.3	O
HPLET-03-3	CIMMYT-HarvestPlus	40.9	O
HPLET-03-10	CIMMYT-HarvestPlus	29.3	Y
HPLET-03-31	CIMMYT-HarvestPlus	33.5	O
HPLET-03-4	CIMMYT-HarvestPlus	18.1	Y
HPLET-03-30	CIMMYT-HarvestPlus	19.4	DO
HPLET-03-1	CIMMYT-HarvestPlus	17.8	PY
HPLET-03-9	CIMMYT-HarvestPlus	27.8	O
HPLET-03-32	CIMMYT-HarvestPlus	37.4	O
HP-19-01	CIMMYT-HarvestPlus	23.6	Y
HP-19-33	CIMMYT-HarvestPlus	33.7	O
HP-34-18	CIMMYT-HarvestPlus	15.9	O
HP-10	CIMMYT-HarvestPlus	1.4	W
CML162	CIMMYT, Mexico	25.1	O
CML287	CIMMYT, Mexico	14.6	O
CML69	CIMMYT, Mexico	18.7	O
CA00106	CIMMYT, Mexico	36.0	DO
SKV18	UAS-Nagenahalli	15.6	O
SKV21	UAS-Nagenahalli	18.9	O
H16	CIMMYT-Africa	1.3	W
	Standard Error	0.53	

(*Contd.*)

Table 1 (*Concluded*)

*DO, Deep orange; O, Orange; Y, Yellow; PY, Pale yellow; W, White

under the dim yellow light to prevent the photo-oxidation of carotenoid. The absorbance of total carotenoid was measured at 450 nm, and the total carotenoid concentration was calculated based on the formulae as suggested by Rodriguez-Amaya and Kimura (2004). ANOVA for completely randomised design was analyzed using SAS 6.12, while Pearson's simple correlation coefficients were estimated using Office-Excel.

RESULTS AND DISCUSSION

ANOVA revealed significant variation for total carotenoid concentration among the selected set of inbred lines (Table 2), indicating the possibility of genetic improvement for the target trait in the breeding program. The total carotenoid concentration ranged from 6.5 µg/g (HKI586) to 67.3 µg/g (HPLET-03-36), with a mean of 26.9 µg/g (Table 1). Daood *et al*. (2003) reported a range of 7.5 to 24.9 µg/g for total carotenoid concentration in a set of Hungarian maize landraces, while Mishra and Singh (2010) reported a range of 8.72 to 25.75 µg/g in a small set of 36 yellow/orange

Table 2 ANOVA for total carotenoid concentration among 111 selected maize inbreds

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value	Pr>F
Treatment	110	54269.254	493.35**	922.45	0.0001
Error	222	118.733	0.535		
Total	332	54387.987			

Indian maize composites and inbreds. Hulshof *et al*. (2007) recorded a range of 9.9 to 40.0 µg/g for the same trait among a set of maize inbred lines, while similar range (9.46 to 42.84 µg/g) of total carotenoid was also observed among 134 maize genotypes by Cardoso *et al*. (2009). In separate panels, Egesel *et al.* (2003), Weber (1987) and Harjes *et al*. (2008) reported ranges of 17.9 to 51.4 µg/g, 30 to 77 µg/g and 5.5 to 66.0 µg/g, respectively, for total carotenoid concentration.

Among the 111 lines analyzed, HPLET-03-36 was found to be the best genotype with 67.3 µg/g total carotenoid concentration, followed by HPLET-03-37 (63.2 µg/g), HPLET-03-35 (59.9 µg/g), BLSB-RIL17 (57.0 µg/g), BLSB-RIL43 (56.7 µg/g), HPLET-03-41 (56.1 µg/g) and BLSB-RIL95 (50.9 µg/g). As many as 10 inbreds, including 5 HPLET lines and 5 BLSB-RILs, recorded total carotenoid concentration in the range of 40 to 50 µg/g, while 5 HPLET lines, besides one CIMMYT line (CA00106) and two CM lines that were developed in PAU-Ludhiana which are also parental lines of a single-cross hybrid Parkash (CM139 and CM140) could be classified as moderate (30 to 40 µg/g) for total carotenoid concentration. The two white lines, used as check had extremely low total carotenoid concentration (HP10: 1.4 µg/g and H16: 1.3 µg/g). The distribution of the inbred lines in different ranges of total carotenoid concentration is depicted in Fig 1.

The CIMMYT-HarvestPlus lines were clearly superior in terms of total carotenoid concentration with the mean of 34.1 µg/g, while the mean value recorded for the selected maize inbred lines from Indian breeding programs was 23.8 µg/g. Although a few HarvestPlus inbred lines, namely HPLET-03-23, HPLET-03-22, HPLET-03-4, HPLET-03-1 and HPLET-03-2, recorded low total carotenoid concentration, the study reveals that breeding for carotenoid enrichment under HarvestPlus led to significantly biofortified lines. The low to moderate range of total carotenoid concentration reflected in the Indian maize inbred lines could be the result of non-inclusion of this trait in the breeding strategies so far.

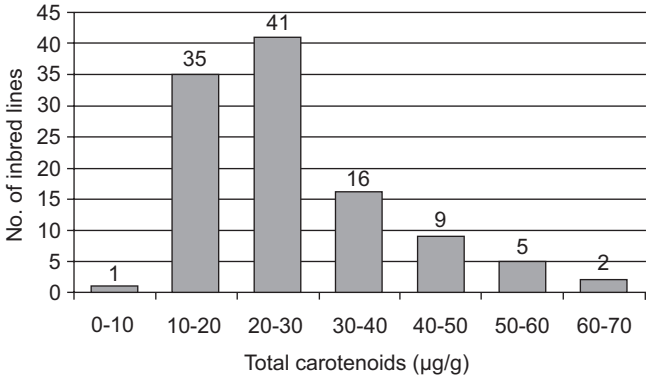

Fig 1 Frequency distribution of total carotenoid concentration in the maize panel. Numerical values on the top of the bar represent the number of inbred lines in the corresponding range.

Among the panel of 111 inbred lines, 16 lines were from a RIL population derived from the cross between CM140 (male parent of Parkash; susceptible to the disease Banded leaf and sheath blight, BLSB) and CA00106 (a CIMMYT-Asia inbred with resistance to BLSB). These lines were selected because of their deep orange kernel colour. The range of total carotenoid concentration among the selected 16 BLSB-RILs was 18.6 to 57.0 µg/g with a mean of 35.5 µg/g (Table 1). It is important to note that many of the RILs in the population exceeded parental mean values (CM140: 34.8 µg/g; CA00106: 36.0 µg/g) for the target trait. Although the selected RILs represent only a small proportion of miniscule of the entire RIL population, they showed large variation for the target trait. Wong *et al.* (2004) reported similar observation among 200 $F_{2:3}$ mapping population, where the range of total carotenoid concentration was 5.69 to 33.21 µg/g, with a few progenies in the mapping population performing better than the parental lines, W64a (17.99 µg/g) and A632 (11.56 µg/g). Chander *et al*. (2008) also reported the presence of trangressive segregants for total carotenoid among the RIL population developed by crossing By804 and B73. Thus, the clear demonstration of transgressive segregants for total carotenoid concentration in segregating populations like $F_{2:3}$ as well as 'immortal' mapping populations like RILs indicates the possibility of significant genetic improvement for the target trait as well as the utility of such populations in identification of marker-trait associations.

The dominant *Yellow1* (*Y1*) allele in maize controls the first rate-limiting step in the carotenoid biosynthesis by coding for the enzyme phytoene synthase, while the homozygous recessive allelic state (*y1y1*) results in almost no synthesis of carotenoids in the endosperm (Buckner *et al.* 1996). In the present study, white lines having *y1* allele in the endosperm produced almost no carotenoid (HP10: 1.4 µg/g and H16: 1.3 µg/g) in the endosperm. Presence of similar negligible amount of total carotenoids (1.14 µg/g) in the white maize endosperm (*y1y1y1*) was also reported by Buckner *et al*. (1996). The significant range for the total carotenoids (6.5 to 67.3 µg/g) in the yellow/orange inbred lines could be because of the control of carotenoid biosynthesis by other genes, besides the *Y1*.

All the 111 inbred lines were categorized into five colour classes, namely white, pale yellow, yellow, orange, and deep orange. Genotypes with deep orange or yellow colour are usually expected to show higher level of total carotenoid concentration as compared to those with light yellow colour. In the present study, significant and positive correlation (r = 0.36**) was recorded between total carotenoid concentration and kernel colour. Tiwari *et al.* (2012) also reported positive correlation between kernel colour and total carotenoid concentration among a set of 24 diverse maize inbred lines. The average total carotenoid concentration among the deep orange inbred line was 31.1 µg/g, while the same was found to be 28.0 µg/g, 24.0 µg/g, 16.1 µg/g and 1.4 µg/g among the

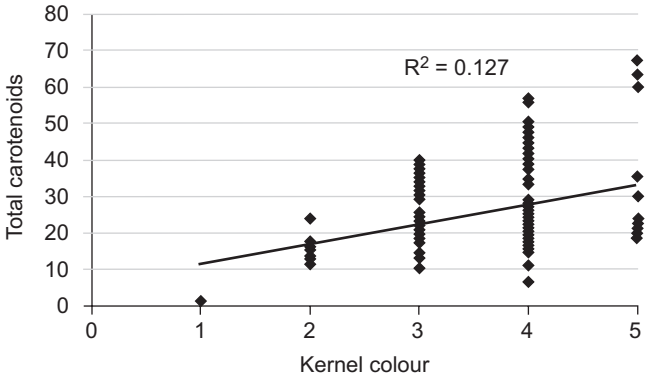

Fig 2 Relationship between kernel colour and total carotenoid concentration in the maize panel. Scale 1-5 represents kernel colour class, where, 1: white, 2: pale yellow, 3: yellow, 4: orange, 5: deep orange.

orange, yellow, pale yellow and white class, respectively. Thus, in a breeding program where a very large number of genotypes/breeding materials need to be screened, preliminary selection based on the deep orange kernel colour may be useful. However, the range for total carotenoid in each of the class varied drastically. For example, the range was found to be 18.6 to 67.3 µg/g in the deep orange class, while the ranges among the orange, yellow and pale yellow class were 6.5 to 56.1 µg/g, 10.2 to 39.9 µg/g and 11.7 to 24.1 µg/g, respectively. The study, thus, demonstrated that in spite of the significant positive correlation between deep orange/ yellow colour with the total carotenoid concentration, there could be sizable variation in each classes, resulting in a low R^2 value (Fig 2). Harjes *et al*. (2008) also observed low R^2 value between total carotenoid and kernel colour among a set of 228 diverse maize inbred lines.

It is important to note that the present analysis was undertaken using colorimetric based quick extraction method as per Rocheford (2004). The precise estimation of individual components of total carotenoids requires usage of HPLC or UPLC. However, the cost and time involved for analysis using HPLC is very high, and thus HPLC cannot be utilized for screening of large number of samples within a short period of time. Recent studies (Rocheford 2004, Hulshof *et al.* 2007, Harjes *et al.* 2008) on selected set of maize seed samples using both HPLC and colorimetric based methods for estimation of total carotenoid have reported high positive correlation between the two protocols, and thus the reliability of the colorimetric based analysis. Thus, colorimetric method can be effectively used in maize biofortification programs of the national agricultural research systems (NARS) in the developing countries, for quick screening and grouping of a large set of genotypes on the basis of low, medium, or high levels of total carotenoids, and the genotypes with high values for total carotenoid can be further subjected to HPLC analysis for precise estimation of individual components, especially β-carotene. In conclusion, the present study led to identification of some highly promising genotypes for total carotenoid concentration in both the Indian and exotic (HarvestPlus) tropical maize lines that can be potentially utilized for developing and deploying carotenoid-enriched maize cultivars.

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