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Analysis of genetic variability for kernel carotenoid concentration in selected maize inbred lines

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Abstract

A selected set of 24 maize (*Zea mays* L.) inbred lines were analyzed to study genetic variability for total kernel carotenoid concentration. The results revealed significant genetic variation among the selected maize inbreds for total carotenoid concentration with values ranging from12.20-30.10 µg/g, and a mean of 21.87 µg/g. One of the CIMMYT-HarvestPlus maize inbreds, HP19-33 recorded the highest (30.10 µg/g) carotenoid concentration, followed by DMRQPM-58 (28.40 µg/g), NAI115 (26.80 µg/g), LM16 (25.87 µg/g), HP20-37 (25.80 µg/g), VQL1 (25.30 µg/g) and HP20-53 (24.40 µg/g). No significant difference in total carotenoid concentration was observed among the QPM and non-QPM inbred lines, although positive correlation was found between kernel colour and total carotenoid concentration. Deep orange kernel coloured genotypes, in general, recorded higher levels of total carotenoids as compared to those with yellow or pale yellow kernel colour. Evaluation of different generations derived from a cross DM QPM-58 x DMR QPM-28-03 at Delhi and Pantnagar revealed polygenic control for total kernel carotenoid concentration with some genes having major effect. The study also indicated high heritability for total carotenoids at both Delhi (96.9%) and Pantnagar (95.6%). The carotenoid concentration of the same set of genotypes across different environments was reasonably stable, implying lesser influence of G x E for the trait.

Key words: Carotenoids, genetic variation, heritability, *Zea mays* L.

Introduction

Carotenoids are the lipid-soluble polyenes that are abundant in fruits, vegetables, and green plants [1]. In photosynthetic organisms, they function to prevent photo-oxidative damage of chlorophyll and other associated molecules, besides acting as accessory pigments for absorbing visible light [2]. Carotenoids also help in protecting membranes from lipid peroxidation and could provide tolerance to heat and light stress [3-5]. Plants also contain carotenoids in non-photosynthetic tissues, such as flowers and fruits, which might help in enhancement of pollination and seed dispersal under different ecosystems [6].

Carotenoids also have significant nutritional value for humans by providing precursor molecules required for the synthesis of vitamin A. An early symptom of vitamin A deficiency is night blindness. Vitamin A deficiency driven structural alterations in conjunctiva and cornea may cause xerophthalmia and keratomalacia, and subsequent inflammation and infection that could result in irreversible blindness [7]. Depression of the immune system due to vitamin A deficiency increases the severity of disease like measles and diarrhoea, leading to an increase in child mortality, which may be apparent even before the appearance of xerophthalmia [7]. As per the FAO/WHO recommendation, an adult male and female (between age of 25 and 50 years) require 1000 and 800 µg vitamin A, respectively, in their daily diet [8]. Medical supplements and fortification of food products have been tried in several countries for decades to ameliorate the devastating problem of micronutrient deficiencies [9, 10]. However, these measures have their own limitations such as ineffective distribution system and lack of purchasing power among poor people.

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Development of micronutrient-enriched staple plant foods either through conventional breeding or via molecular biological techniques holds great promise for sustainable food based solutions [11-14]. Maize provides over 20% of total calories in human diet in 21 countries, and over 30% in 12 countries that are home to a total of more than 310 million people [15].While, the white grained maize has no carotenoids, the yellow or orange grained maize contains carotenoids in the maize endosperm [16]. Recent advances in molecular biology have also led to the elucidation of the carotenoid biosynthetic pathway in plants and identification of candidate genes and favourable alleles influencing the carotenoids [17, 18]. However, very little is known about the levels of total carotenoids in the maize inbred lines that are used in the Indian maize breeding programs vis-a-vis advanced maize lines selected under the Maize HarvestPlus Program of CIMMYT. Therefore, the major objectives of the present study were to analyse the genetic variability for total carotenoids in a selected set of Indian maize inbred lines, and to study the heritability underlying this important trait.

Materials and methods

Genetic material

The experimental material consisted of 24 maize genotypes, including 11 inbred lines developed under the CIMMYT-Maize HarvestPlus Program; three inbred lines (two from Almora and one from Delhi centre) developed under the All-India Coordinated Maize Improvement Programme (AICMIP); two inbred lines from Punjab Agricultural University (PAU), Ludhiana; two inbreds from Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora; one inbred from University of Agricultural Sciences (UAS)-Nagenahalli Research Centre, Karnataka; and five inbred lines developed by the Directorate of Maize Research (DMR), New Delhi, India (Table 1). These inbred lines were selected based on their kernel colour ranging from deep orange to pale yellow.

Field experiments

The inbred lines were planted in a randomized complete block design (RCBD) at the Indian Agricultural Research Institute (IARI) Experimental Farm during *Kharif* (Monsoon season)-2007 with two replications per entry, and with one row (5 m length) per replication. Standard agronomic practices were followed during crop growth period. Two inbred lines, DMRQPM-58 and DMRQPM-28-03, contrasting for total carotenoid concentration, were crossed (hand-pollinated) to generate F_1 progenies; the F_2, BC_1P_1 and BC_1P_2 generations were subsequently generated at Maize Winter Nursery, Hyderabad during *Rabi* 2007-08. All the six generations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2) were planted in randomized complete block design (RCBD) with two replications at two locations: (i) IARI Experimental Farm, New Delhi, and (ii) Crop Research Centre, GB Pant University of Agriculture & Technology (GBPUAT), Pantnagar, during *Kharif* 2008. Plants in each entry, avoiding the border plants, were selfed to exclude the possibility of xenia effects. Representative plants from each replication were selected randomly, and ten individual plants each for P_1, P_2, F_1 generations, 110 plants for F_2 generation, and 65 plants each from BC_1P_1 and BC_1P_2 generations, were analyzed for total carotenoid concentration from each of the experimental locations (Delhi and Pantnagar).

Carotenoid analysis

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 per cent. Randomly sampled 50 seeds were ground into fine powder and 0.5g of sample (with three replicates per entry) was used for estimation of total carotenoid concentration. Each step prior to and during biochemical analysis was carried out at temperature below 10^oC and under the dim yellow light to avoid any degradation due to its sensitivity to light. Total carotenoid concentration was estimated using a protocol suggested by Kurilich and Juvik [19] with minor modifications, and the optical density (OD) at 450 nm was measured using a spectrophotometer (Spectronic20).

Statistical analysis

The data were analyzed for analyses of variance (ANOVA) and comparison of means was done using PROC GLM of SAS Version 9.1 (SAS Institute, 2005). Pearson's simple correlation coefficient was calculated using MS-Office Excel. Heritability and number of gene blocks affecting carotenoids (using Burton's formula) were estimated, as described by Sharma [20].

Results and discussion

ANOVA revealed significant variation among the selected maize genotypes for total carotenoids (table not presented), indicating wide genetic variability for total carotenoid concentration. The total carotenoids ranged from 12.20-30.10 μg/g, with a mean of 21.87 μg/g (Table 1). Daood *et al.* [21] reported a range of 7.54-24.94 μg/

Table 1. Total kernel carotenoid concentrations in the selected Indian and HarvestPlus maize inbred lines

S.No.	Inbreds	Pedigree/source	*Kernel colour	Mean carotenoids (µg/g)
1	CM137	IPA 9-7	DO	19.56
2	CM145	Pop 31 C4 HS bulk (Alm)-70	O	23.60
3	CM212	USA/Acc. No.2132 (Alm)	O	22.34
4	LM15	JS 2 x J3022 HS 80	DO	23.50
5	LM16	JS 4-30 x (Tarun x MS-1)	DO	25.87
6	NAI125	EV 25CD (Y) Alm-C7	O	26.80
7	V340	CM128 x CM129	Y	16.50
8	VQL1	CM212 x CML180-BC3	O	25.30
9	DMRQPM-03-102	Shakti-1	O	15.30
10	DMRQPM-03-118	Shakti-1	DO	21.45
11	DMRQPM-03-121	Shakti-1	DO	19.20
12	DMRQPM-28-03	Shakti (SO) HE-25 # CC Bulk	PY	12.20
13	DMRQPM-58	Shakti-1	DO	28.40
14	HP19-01	KUI Carotenoid Syn. FS1	Y	21.30
15	HP19-17	KUI Carotenoid Syn. FS3	Y	20.90
16	HP19-33	KUI Carotenoid Syn. FS5	O	30.10
17	HP19-54	KUI Carotenoid Syn. FS11	Y	21.87
18	HP19-79	KUI Carotenoid Syn. FS17	O	22.17
19	HP20-14	Carotenoid Syn3-FS4	Y	14.50
20	HP20-37	Carotenoid Syn3-FS8	O	25.80
21	HP20-53	Carotenoid Syn3-FS10	O	24.40
22	HP20-58	Carotenoid Syn3-FS10	O	23.00
23	HP34-01	Illinois Orange Syn-FS1	O	18.00
24	HP34-11	Illinois Orange Syn-FS16	O	22.76

SE = 0.64, LSD (1%) = 1.32; LSD (5%) = 1.79; *DO: Deep Orange; O: Orange; Y: Yellow; PY: Pale Yellow

g of total carotenoids among the Hungarian maize landraces, while Wong *et al*. [22] and Hulsof *et al*. [23] reported ranges from 5.69-33.21 µg/g and 9.90-39.96 µg/g, respectively. Thus, the range of total carotenoids observed in the present study was comparable to those earlier reported for the target trait. However, Weber [24] and Harjes *et al*. [17] reported much broader ranges of 30-77 µg/g and 5.5-66.0 µg/g, respectively. The differences with regard to kernel carotenoids could be due to the presence/absence of favourable gene combinations in specific germplasm.

Among the 24 inbred lines analyzed in the present study, HP19-33 was found to be the best inbred line with total carotenoid concentration of 30.10 µg/g (Table 1). Other promising inbreds include DMRQPM-58 (28.40 µg/g), NAI115 (26.80 µg/g), LM16 (25.87 µg/g), HP20-37 (25.80 µg/g), VQL1 (25.30 µg/g) and HP20-53 (24.40 µg/g). Some of the inbred lines, namely CM145 (23.60 µg/g), LM15 (23.50 µg/g), HP20-58 (23.00 µg/g), HP34-11 (22.76 µg/g), CM212 (22.34 µg/g), HP19-79 (22.17 µg/g), HP19-54 (21.87 µg/g), DMRQPM-03-118 (21.45 µg/g), HP19-01 (21.30 µg/g) and HP19-17 (20.90 µg/g), recorded moderate levels of total carotenoids. In contrast, DMRQPM-28-03 (12.20 µg/g) and HP20-14 (14.50 µg/g) were found to have relatively lower levels of carotenoids. Majority of the HarvestPlus (HP) inbred lines analyzed in this study recorded moderate to high levels of total carotenoids, with an overall range of 14.50-30.10 µg/g (Table 1).

It is interesting to note the differences in the total carotenoid levels of DMRQPM 'sister' lines; DMRQPM-

03-102 recorded 15.30 μg/g for the target trait, while DMRQPM-03-121 and DMRQPM-03-118 showed 19.20 μg/g and 21.45 μg/g, respectively. Although all these three inbred lines were developed from the same source population, different gene/allele combinations could have segregated and got fixed in the genotypes, leading to varying levels of carotenoids (Table 1). Similar variation was also reported by Chander *et al*. [25] in a set of maize RIL populations developed by crossing By804 x B73.

The study also revealed similar trend of variation (high, medium and low) among the QPM inbred lines (12.20-28.40 μg/g) as compared to the non-QPM inbred lines (14.50-30.10 μg/g). This suggests that the QPM trait *per se* did not have any significant influence on total carotenoids in maize. This was further reinforced when one compares the total kernel carotenoid concentration of CM212 (non-QPM) versus VQL1 (QPM version of CM212) developed through molecular marker-assisted breeding at VPKAS, Almora [26]. The inbred lines revealed only minor difference in the total carotenoids (22.34 μg/g in CM212; 25.30 μg/g in VQL1) that could be attributed to some introgression of non-recurrent/donor parent during backcrossing.

Genotypes with deep orange kernel colour are usually expected to have higher levels of total carotenoids as compared to those with yellow or pale yellow kernel colour. The correlation between kernel colour with that of total carotenoid concentration was found to be significant (r = 0.49**). For example, majority (with a few exceptions) of the orange or deep orange kernel genotypes, such as DMRQPM-58, LM16, LM15, HP19-33, NAI125, VQL1, CM145, recorded high levels of total carotenoids (Table 1). In a situation where a very large number of maize genotypes/breeding materials/accessions need to be screened, preliminary selection based on the deep orange kernel colour may be useful, as is being practised in the CIMMYT-Maize HarvestPlus Program. However, a genotype with high total kernel carotenoid concentration may or may not reflect high β-carotene or provitamin A concentration.

In the present study, total carotenoids in maize inbred lines was estimated using slightly modified protocol of Kurilich and Juvik [19], which is a rapid and inexpensive method for screening of large number of genotypes for the target trait. Nevertheless, promising inbred lines with high amount of total carotenoids need to be further profiled for various components (α-carotene, β-carotene, zeaxanthin, lutein and β-cryptoxanthin) by using HPLC or UPLC technique.

The mean and range of carotenoids of various generations of the experimental cross are summarized in Table 2. The mean of P_1 (DMRQPM-58) was 29.32 μg/g and 31.04 μg/g at Delhi and Pantnagar, respectively. The same for the P_2 (DMRQPM-28-03) was 12.28 μg/g at Delhi, while it was 12.02 μg/g at Pantnagar. The mean values of $F_1$and F_2 generations were almost intermediate between the parents (F_1: 19.51 μg/g at Delhi; 22.66 μg/g at Pantnagar; F_2: 21.45 μg/g at Delhi; 16.93 μg/g at Pantnagar). In contrast, the ranges of P_1, P_2 and F_1 generations were quite narrow, but the range for F_2 generation was large at both Delhi (7.35-40.00 μg/g) and Pantnagar (8.20-33.50 μg/g). These observations clearly demonstrate that the total carotenoid concentration is under polygenic control, and the large variance in F_2 generation could be due to segregation and recombination among the favorable genes/alleles contributed by both the parental lines. Interestingly, the mean for BC_1P_1 generation was similar to P_1 parents, while the mean for BC_1P_2 was towards P_2 parent at both locations (Table 2). This suggested that the P_1 parent contributed more favorable genes/allelic combinations for the target trait, while it probably got diluted in BC_1P_2 generation due to unfavourable alleles contributed by the P_2 parent. The range for backcross generations was found to be much higher

Table 2. Estimates of total carotenoids in six generations of DMRQPM-58 x DMRQPM-28-03

Genotypes	Delhi			Pantnagar		
	Mean (μg/g)	±SE	Range(μg/g)	Mean (μg/g)	±SE	Range (μg/g)
DMRQPM-58 (P_1)	29.32	0.23	27.80-30.00	31.04	0.20	30.00-31.80
DMRQPM-28-03 (P_2)	12.28	0.30	11.00-14.30	12.02	0.38	10.35-13.70
DMRQPM-58 x DMRQPM-28-03 (F_1)	19.51	0.44	17.80-21.90	22.66	0.44	20.00-24.00
F_2	21.45	0.58	7.35-40.00	16.93	0.51	8.20-33.50
DMRQPM-58 x F_1 (BC_1P_1)	26.94	0.67	13.70-40.70	27.01	0.49	16.20-34.89
DMRQPM-28-03 x F_1 (BC_1P_2)	21.62	0.71	9.60-32.34	16.19	0.60	8.50-28.00

than the parents and F_1 but was little lesser than the F_2 generation.

High broad sense heritability for total carotenoids was estimated at both Delhi (96.9%) and Pantnagar (95.6%) locations. These estimates indicate that genetic factors play a major role in determining the total carotenoid concentration in maize. These observations are in congruence with report from another study [25] wherein 85% broad sense heritability for total carotenoids was estimated in a maize RIL population. Moderate to high heritability estimates for carotenoids have also been reported in the past for maize [22, 27]. High heritability of provitamin A could be due to the involvement of a few major genes in the carotenoid biosynthetic pathway [13, 28, 29].

The number of major gene blocks influencing the trait, as per the Burton's formula [20], was found to be at least three at Delhi, and four at Pantnagar. It is well established that *Yellow1* (*Y1* or *psy1*) gene codes for phytoene synthase which controls the first rate-limiting step in the carotenoid biosynthetic pathway. Mutation of the *Y1* allele to its recessive version *y1,* leads to no synthesis of phytoene synthase, and consequently no carotenoids in the maize kernel, leading to white kernel phenotype [30]. Besides phytoene synthase, other enzymes such as phytoene desaturase (*pds*), zeta carotene desaturase (*zds*), lycopene beta cyclase (*lcyB*), lycopene epsilon cyclase (*lcyE*) and beta carotene hydroxylase 1 (*crtRB1*) are also involved in important catalytic steps of the pathway [17, 18]. In addition to such major genes, there could be an array of other major/ minor genes that could together influence the levels of total carotenoids in maize.

Stability of carotenoids across locations is an important factor to decide whether location-specific or location-independent breeding programmes should be undertaken. The present study showed that the mean performances of the same set of genotypes at both Delhi and Pantnagar remained reasonably stable, and environment had lesser influence on the target trait (Table 2). This is also indicated by high heritability estimates of total carotenoids as detected at both the locations. Menkir et al. [31] also reported stable nature of carotenoids in maize across environments. Similar small effect of environments on carotenoids was reported in tropical open-pollinated varieties as well as temperate inbred lines and hybrids [19, 28, 29].

Thus, the present investigation revealed the presence of considerable genetic variation for total carotenoids in the selected Indian and exotic (HarvestPlus) tropical maize lines, signifying the scope for genetic improvement of this important trait. Specific genotypes with high levels of total carotenoids need to be further profiled for components of carotenoids, especially β-carotene. Inbred lines with high β-carotene can be further utilized as potential donors in breeding for nutritionally enriched maize adapted to the Indian context.

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