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Original Paper

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Carotenoid and SSR marker-based diversity assessment among short duration maize (Zea mays L) genotypes

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Abstract

Based on analysis of variance using a CRD model, significant variation for kernel carotenoid content was found to be present in 25 maize (*Zea mays*) genotypes. Total carotenoid content was found to be at a minimum (0.94 µg/g) in the white kernel line Sikkim primitive-1, whereas as much as 38.25 µg/g was observed in a dark yellow colored kernel line (1490). TLC profiling of total carotenoids showed that out of 25, 11 lines also had high provitamin-A content, in addition to high kernel carotenoids. Kernel color did not resolve any strong correlation with either total carotenoid content or provitamin-A. Thereby, selection of genotypes for high carotenoid and provitamin-A based on kernel color may not be successful.
Jaccard's similarity coefficients, based on SSR data, were found to vary from 0.17 to 0.97. The highest value of genetic similarity (0.97) was found between Pop31B and Pop31C and therefore they seem to be most similar, whereas inbred lines Pop31D and POB-3, and 1586 and Tarun-1 were most divergent (0.17). The UPGMA dendrogram constructed using Jaccard similarity coefficients of SSR marker data divided the 25 lines into four groups (A, B, C, and D). Each broad group (Group A and B) was further divided into clusters, thus a total of seven clusters were formed. Cluster strength varied from a minimum of 1 member in cluster III of Group B to a maximum of 5 members in cluster II of Group B. Clustering patterns, in general, revealed that lines with high carotenoid content did not occupy the same cluster. A similar distribution was also observed for lines with a high provitamin-A content. The marker based clustering pattern therefore did not show strong correlation with quantitative data. Based on total carotenoids and relative provitamin-A content, 11 lines were identified to be a potential source for biofortification of carotene in maize.

Keywords: genetic diversity, maize, SSR marker, carotenoid

Introduction

Vitamin A deficiency (VAD) is an immunodeficiency disorder characterized by widespread alteration in immunity (Semba, 1994). VAD is a major cause of premature death in developing nations, particularly among children. Among the major cereals, yellow endosperm maize is the only crop having appreciable amount of carotenoids (Wurtzel, 2004) with a wide range of genetic variability. Several carotinoids have provitamin-A activity due to the presence of a vitamin structure that is a part of the overall carotinoid compound. Biofortification of maize for the improvement in provitamin-A content has received increased interest in recent years in an effort to overcome vitamin A deficiency resulting from the consumption of maize based diets in areas where the supply and availability of animal products, fruits and vegetables are limited.

Provitamin-A (carotenoids) and vitamin-E (tocopherols) are fat-soluble vitamins that occur in corn grain. Carotenoids are located in endosperm and tocopherols, a component of oil, in the germ (Weber, 1987). Carotenoids are a large class of yellow, red, and orange pigments derived from isoprenoids. In plants, carotenoids are both primary and secondary metabolites. As primary metabolites, carotenoids serve as regulators of plant growth and development, as accessory pigments in photosynthesis, as photoprotectors preventing photo-oxidative damage, and as precursors to the hormone abscisic acid (ABA). For humans, the presence of carotenoids in the endosperm and fruits of crop plants adds to their nutritional value. Dietary carotenoids are essential precursors to vitamin A and to retinoid compounds needed in animal morphogenesis (Bendich and Olson, 1989).

Two classes of carotenoid pigments are carotenes and xanthophylls, which are responsible for the yellow and orange color of maize endosperm. Yellow maize kernels contain several carotenoid isoforms, including two carotenes—alfa-carotene and beta-carotene and three xanthophylls—beta-cryptoxanthin, zeaxanthin, and lutein. Of these two carotenes, beta-carotene is present in the highest concentration, while either lutein or zeaxanthin is the most prevalent form of the xanthophylls.

The first step in the carotenoid biosynthetic pathway is the production of C40 carotenoid by the tail-

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to-tail condensation of two molecules of geranyl geranyl pyrophosphate (GGPP) to form the colorless compound phytoene. The enzyme that commits GGPP to the carotenoid biosynthetic pathway is phytoene synthase. Phytoene desaturase is the second enzyme in the carotenoid biosynthetic pathway and is responsible for a two-step desaturation, taking phytoene to zeta (z)-carotene. Phytoene desaturase (PDS) has been associated with the mutant *viviparous 5* (*vp5*), a white endosperm mutant deficient in both carotenoids and ABA. Zeta-carotene desaturase (ZDS) is the third enzyme in the carotenoid biosynthetic pathway and is responsible for two-step saturation from zeta-carotene to lycopene. It has been associated with *viviparous 9* (*vp9*), another white endosperm mutant of maize. Other important genes in the carotenoid biosynthetic pathway are beta-lcy and epsilon-lcy encoding the enzymes beta-cyclase and epsilon-cyclase respectively, which convert the straight-chain lycopene into beta- and alfa-carotene (Cunningham et al. 1996) by adding two beta-rings to beta carotene, and one each of an epsilon and beta-ring to alfa-carotene.

The first steps in the development of varieties high in beta carotene, for use in human diets, include an assessment of genotypic variation in the germplasm. Significant genotypic variation in carotenoid, including beta-carotene has been reported in yellow endosperm maize, adopted to temperate environment (Brunson et al, 1963; Weber, 1987). Furthermore, beta-carotene is known to be a heritable trait in temperate maize. However very limited information is available about the range of genetic variation of kernel carotenoids in tropical yellow endosperm maize germplasm.

In the present investigation twenty-five maize genotypes were selected with the major objectives:

1. to analyze the quantitative variation in carotenoids content in the maize kernel using spectrophotometry;
2. use thin layer chromatography (TLC) to profile the total maize kernel carotenoids into provitamin-A and nonprovitamin A;
3. to study the SSR polymorphism linked with total kernel carotenoids;
4. to identify the lines with high carotenoids content.

Materials and Methods

The experimental material for the present investigation consisted of 25 lines (Table 1). This material was obtained from NE Borlaug Crop Research Center, Pantnagar. A carotenoid extraction protocol, developed by Torbert Rochefords' Lab, was used for the extraction of carotenoids from matured maize kernels (Rocheford, 2004). Spectrophotometry was used to record optical density (OD) values of each sample at 450 nm and subsequently, using the Lambert Beer equation, carotenoid content of each sample was determined as follows:

$$E = \varepsilon c d$$

where

E = extinction (photometer reading)
ε = molar extinction coefficient
c = concentration
d = distance =1, to determine concentration
$c = E/\varepsilon$

Lutein, Zeaxanthin and beta-carotene are the major carotenoids in maize. So an average value for ε and for the molecular mass was used.

ε lutein = 122,688 l/mol cm; M =568 g/mol
ε zeaxanthin = 133,480 l/mol cm; M = 568 g/mol
ε β-carotene = 134,000 l/mol cm; M = 537 g/mol
ε average = 130,056 l / mol cm;
M average = 557.7 g / mol.

Table 1 - List of experimental materials.

No	Lines	Developed from/ source material	No	Lines	Developed from/ source material
1	Pop31A	Population-31	14	POB-2	Population-446
2	YHP-Alm	Yellow Heterotic Pool-A	15	YHP-3	Yellow Heterotic Pool-A
3	Pop31B	Population-31	16	POB-3	Population-446
4	Pop31C	Population-31	17	CM-400	Co-ordinated Maize Improvement Programme
5	Pop31D	Population-31	18	Tarun-2	Pantnagar
6	CM-300	Co-ordinated Maize Improvement Programme	19	CM-129	Co-ordinated Maize Improvement Programme
7	YHP-1	Yellow Heterotic Pool-B	20	1490	Pantnagar
8	CM-139	Co-ordinated Maize Improvement Programme	21	1586	Pantnagar
9	CM -137	Co-ordinated Maize Improvement Programme	22	Pob-3	Population-445
10	Pob 445-54	Population-445	23	PoP31D-1	Population-31
11	Tarun-1	Pantnagar	24	Pop31-E	Population-31
12	YHP-2	Yellow Heterotic Pool-A	25	POB-4	Population-446
13	SIKKIM Primitive-1	SIKKIM Primitive Intercross			

Hence, c = OD / 130,056 x 557.7 x 1,000 x 10 / w (µg / g)
where
c = concentration of total carotenoid content (µg/g) in a given sample on dry weight basis;
OD = optical density taken at 450 nm wavelength using a spectrophotometer;
W = weight of sample (0.5 g).

Each sample was analyzed in three replications and the data were subjected to statistical treatment in completely randomized design (CRD).

After estimation of total carotenoid content in different genotypes, thin layer chromatography (TLC) was performed for the separation of provitamin-A and non-provitamin-A components of total carotenoid. Provitamin-A is the precursor of vitamin A in maize kernel. For this purpose, Silica TLC plates were prepared by applying silica gel G (with binder) paste on glass plates with the help of an applicator, making a 1.5 mm thick layer. Before use, these TLC plates were activated at 110ºC for 2 hours and then 2 ml of sample aliquot was air dried and re-suspended into 50 µl of chloroform and loaded onto a silica TLC plate using an eppendorf pipette. TLC plates were run in PE+DE+acetone (40+10+10 v, v, v) for only 6-7 cm (about 15 minutes).

Isolation of high molecular weight genomic DNA was done from maize seeds using a CTAB method. For this, 25 genotypes were selected based on their average carotenoid content. The DNA was quantified by spectrophotometry and quality analysis was done on a 0.8% agarose gel.

Twelve pairs of SSR markers (Table 2) were used to screen the 25 selected maize inbred lines. For the resolution of amplified product 8 % of 19:1 acrylamide with 42 % urea, denaturing gels were used.

Table 2 - List of SSR primers.

Sl.No.	Primer	Sequence	T_m °C
1.	Phi308707forward	ATCTTGCTTCCATAAGATGCACTGCTCT	60.0
2.	Phi308707reverse	CTCAGCTTCGGTTCCTACACAGT	58.6
3.	Umc1403 forward	GTACAACGGAGGCATTCTCAAGTT	57.3
4.	Umc1403 reverse	TGTACATGGTGGTCTTGTTGAGGT	58.2
5.	Umc2047 forward	GACAGACATTCCTCGCTACCTGAT	58.2
6.	Umc2047 reverse	CTGCTAGCTACCAAACATTCCGAT	57.0
7.	Umc2115 forward	CTGTCTGTCTACCCAACCCAACAG	59.2
8.	Umc2115 reverse	GGGGATAGGCGTGTGTATGTACTG	58.8
9.	LcyE forward	TTTACGTGCAAATGCAGTCAA	57.1
10.	LcyE reverse	TGACTCTGAAGCTAGAGAAAG	58.4
11	Umc 2332 forward	GTCGGAGAAGGAGCTACTGAGCTA	59.3
12.	Umc2332 reverse	CACAGGTACGTCTGGATGCTGT	59.0
13.	Umc1595 forward	GCTGCTGGTCTACAACCTCTTGTT	59.2
14.	Umc1595 reverse	CGCTTGAAATGGAAAGGTAGAAAG	54.4
15.	Umc2373 reverse	TATGGTACAGGCACAGCAGCAGTA	59.9
16.	Umc2373 forward	ACCCAAGTGAGGTGAAGTGAAGC	59.3
17.	Bcm1028 forward	AGGAAACGAACACAGCAGCT	58.6
18.	Bcm1028 reverse	TGCATAGACAAAACCGACGT	55.3
19.	Umc1506 forward	AAAAGAAACATGTTCAGTCGAGCG	56.1
20.	Umc1506 reverse	ATAAAGGTTGGCAAAACGTAGCCT	57.2
21	Y1 forward	CAAGAAGAGGAGAGGCCGGA	58.7
22.	Y1 reverse	TTGAGCAGGGTGGAGCACTG	59.9
23	Umc1792 forward	CGGGAATGAATAAGCCAAGA	52.5
24	Umc1792 reverse	GCGCTCCTTCACCTTCTTTA	54.9

The gels were pre-run at a constant voltage of 400V for 20 min; 10 µl of PCR product was mixed with DNA sequencing stop solution and then electrophoresed in 1X-TBE buffer (pH 8.0) at constant voltage (100V) for 4 h. After electrophoresis silver staining of gel was done.

Mean kernel carotenoid content for each genotype was estimated. The analysis of variance of carotenoid content for each genotype was carried out in a complete randomized design using SPSS 16.0. The SSR data was recorded as present (1) or absent (0) of bands, each of which was treated as an independent character. The amplified products were scored separately for each primer. Genetic diversity analysis was conducted on the basis of scores. The dendrogram was constructed by the unweighted pair-group method with arithmetic mean (UPGMA) clustering with the software NTSYS-pc version 2.10 (Rohlf, 1998)

Results and Discussion

The data on kernel carotenoids were analyzed in a completely randomized design (CRD) to test the statistical validity of variance among different genotypes for maize kernel carotenoids content. Analysis of variance revealed that significant variation was present among the selected lines for kernel carotenoids, indicating the presence of wide genetic variability for total carotenoid concentration in the selected lines, which is the basic need to take up any breeding program for improvement of any trait.

The mean value of kernel carotenoid content varied from a minimum of 0.94 µg/g dry weight in Sikkim primitive-1 (white) to the maximum of 38.25 µg/g dry weight in line 1490 with a population mean of 24.48 µg/g. Wong et al (2003) and Hulsof et al (2007) reported a range of 5.69-33.21 µg/g and 9.90-39.96 µg/g, respectively for kernel carotenoids in maize. So, the range of total carotenoids observed in the present investigation was comparable to earlier findings reported for the target trait. However, Weber et al (1987) and Harjes et al (2008) reported much broader ranges of 30-77 µg/g and 5.5-66 µg/g respectively. The differences with regard to kernel carotenoids could be due to the effect of modifiers in addition to presence/absence of favorable gene combination.

When only colored endosperm were taken in consideration, the range of kernel carotenoids content was found to vary from a minimum of 16.55 µg/g in CM-129 to a maximum of 38.25 µg/g dry weight in line 1490 with a population mean of 27.65 µg/g. Three white lines (CM-300, CM-400, Sikkim primitive-1) showed a mean value of 1.08 µg/g, ranging from 0.94-1.28 µg/g. Carotenoid biosynthesis occurs during seed development (Li et al, 2008) and the accumulation of carotenoids imparts a yellow–orange color to the endosperm, an easily scored phenotype. So the results showing colored endosperm with a higher mean value for carotenoid are in accordance

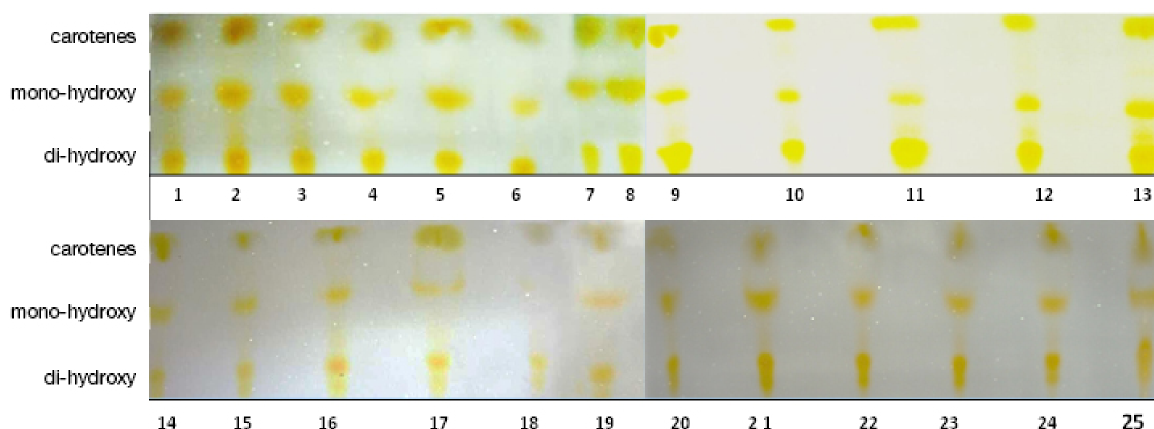

Figure 1 - Chromatogram showing separation of carotenoids for provitamin-A and non-provitamin-A (genotypes: 1 - CM-400, 2 - POB-3, 3 - Pob445-54, 4 - YHP-2, 5 - POB-2, 6 - PoP31D-1, 7 - Pob-3, 8 - CM-137, 9 - Pop31A, 10 -YHP-1, 11 - Pop31B, 12 - CM-139, 13 - Tarun-1, 14 - YHP-3, 15 - CM-129, 16 - Tarun-2, 17 - 1490, 18 - SIKKIM Primitive-1, 19 - CM-300, 20 - Pop31C, 21 - YHP-Alm, 22 - POB-4, 23 - 1586, 24 - Pop31D, 25 - Pop31-E).

with the expectation. The kernel carotenoids content approaching to a minimum in Sikkim primitive-1 is obvious because of its white kernel which contains a very small amount of colored carotenoid. The white kernel is due to a mutation in the *y1* and *vp9* genes, which are involved in biosynthetic pathways of carotenoids. Allelic variations and dosage effects may be responsible for the wide range of variability for carotenoids in yellow maize (Chander et al, 2008).

Thin layer chromatography (TLC) was performed for all 25 genotypes to separate the provitamin- A carotenoids from non-provitamin-A carotenoids in total carotenoids of maize kernel. Vitamin-A is a C20 enzymatic cleavage product made in humans from plant carotenoids containing an unmodified β-ring. Due to their single unmodified β-ring, α-carotene and β-cryptoxanthin have provitamin-A potential but β-carotene is the most efficient source, as two retinol molecules may be derived from each β-carotene molecule. Three different bands were obtained after running the TLC plates in a suitable solvent. The upper two bands (Figure 1) represent provitamin-A carotenoids while the lowest band represents non-provitamin-A carotenoids (Rocheford, 2004) since 1) β-carotene have no hydroxyl group attached and migrate with the solvent front; 2) monohydroxylated compounds like cryptoxanthin, migrate at an intermediate distance; 3) dihyroxylated compounds (lutein and zeaxanthin) remain close to the origin.

Observations from thin layer chromatogram revealed that 15 lines (Pop31A, YHP-Alm, Pop31B, Pop31D, Pop31-E, POB-4, POB-2, YHP-3, POB-3, Tarun-2, 1490, 1586, Pob-3, Pob445-54, Tarun-1) possessed relatively higher amounts of provitamin-A compared to the remaining 10 genotypes (Pop31C, CM–300SPC, YHP-1, CM-139, CM-137, YHP-2, Sikkim primitive-1, CM-400, CM–129, Pop31D-1). These lines are, therefore, said to be a potential source for vitamin-A precursor. Genotypes Pop31A, Pop31B, YHP-3, 1490 (Figure 1 - 9, 11, 14, 17) showed more carotene activity whereas Pob445-54, POB-2, Pob-3, Tarun-2, YHP-Alm, POB-4, 1586, Pop31D, Pop31-E (Figure1 - 3, 5, 7,16, 21, 22, 23, 24, 25) showed more monohydroxy activity. Only the two Tarun-1 and POB-3 lines (Figure 1 - 13, 2) exhibited both carotene and monohydroxy activity together.

From TLC analysis, we can see that most of the genotypes showed darker bands for dihydoy non-provitanin-A compound. The reason behind this is that provitamin-A compounds are biosynthetic

Table 3 - Comparison of kernel color with total carotenoid and Provitamin-A.

kernel colour	genotypes	total carotenoids content (μg/g) dryweight	relative provitaminA content
Orange	Pop31A	37.64	High
	Pop 31B	31.81	High
	1586	28.18	High
	Tarun-2	37.56	High
Light Orange	YHP-Alm	27.18	High
	Pob 445-54	32.50	High
	CM-139	30.36	Low
Light Yellow	PoP31D-1	26.32	Low
	CM-129	16.55	Low
	YHP-1	23.58	Low
	CM-137	30.78	Low
	YHP-2	18.01	Low
	POB-3	31.15	High
	POB-2	23.98	High
Dull Yellow	Pop31C	22.81	Low
	Pop31D	24.87	High
	Tarun-1	20.32	High
	Pop31-E	29.58	High
	Pob-3	26.4	High
	POB-4	21.61	High
Dark Yellow	1490	38.25	High
	YHP-3	29.42	High
White	CM-300	1.03	Low
	CM-400	1.28	Low
	Sikkim Primitive-1	0.94	Low

$LSD_{0.05}$ between the means of genotype: 1.48 μg/g

Table 4 - Jaccard's similarity coefficient between pair of lines in maize (Serial numbers are according to Table1).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	1																								
2	0.62	1																							
3	0.86	0.64	1																						
4	0.88	0.62	0.97	1																					
5	0.43	0.3	0.46	0.47	1																				
6	0.52	0.57	0.58	0.56	0.47	1																			
7	0.5	0.47	0.53	0.5	0.4	0.7	1																		
8	0.3	0.34	0.36	0.33	0.36	0.59	0.58	1																	
9	0.37	0.41	0.43	0.4	0.33	0.72	0.67	0.76	1																
10	0.3	0.34	0.36	0.33	0.36	0.59	0.58	0.85	0.76	1															
11	0.41	0.36	0.44	0.41	0.34	0.53	0.46	0.39	0.56	0.43	1														
12	0.46	0.36	0.45	0.46	0.34	0.5	0.39	0.31	0.48	0.35	0.78	1													
13	0.45	0.38	0.4	0.41	0.38	0.49	0.46	0.34	0.43	0.34	0.53	0.61	1												
14	0.35	0.42	0.4	0.38	0.27	0.56	0.46	0.43	0.56	0.39	0.37	0.37	0.44	1											
15	0.36	0.51	0.45	0.43	0.29	0.54	0.51	0.37	0.49	0.37	0.35	0.35	0.42	0.74	1										
16	0.28	0.29	0.27	0.28	0.17	0.32	0.31	0.36	0.41	0.41	0.29	0.33	0.32	0.38	0.45	1									
17	0.42	0.46	0.44	0.45	0.52	0.78	0.54	0.56	0.65	0.56	0.49	0.46	0.49	0.49	0.46	0.34	1								
18	0.38	0.42	0.44	0.41	0.27	0.56	0.59	0.61	0.61	0.56	0.41	0.33	0.36	0.49	0.54	0.43	0.49	1							
19	0.38	0.42	0.44	0.41	0.34	0.61	0.64	0.79	0.77	0.79	0.44	0.37	0.44	0.41	0.42	0.38	0.53	0.68	1						
20	0.35	0.32	0.42	0.39	0.34	0.43	0.48	0.62	0.5	0.56	0.29	0.21	0.28	0.33	0.28	0.23	0.42	0.57	0.57	1					
21	0.44	0.44	0.43	0.44	0.28	0.38	0.37	0.22	0.3	0.22	0.17	0.23	0.23	0.27	0.4	0.3	0.29	0.27	0.31	0.18	1				
22	0.49	0.5	0.51	0.49	0.27	0.39	0.42	0.25	0.33	0.25	0.24	0.2	0.26	0.3	0.42	0.29	0.31	0.3	0.33	0.22	0.84	1			
23	0.47	0.48	0.49	0.47	0.27	0.47	0.47	0.31	0.41	0.31	0.3	0.26	0.32	0.39	0.51	0.32	0.4	0.42	0.39	0.23	0.7	0.84	1		
24	0.53	0.46	0.48	0.45	0.25	0.42	0.5	0.29	0.4	0.29	0.31	0.28	0.33	0.38	0.42	0.34	0.35	0.38	0.38	0.21	0.69	0.83	0.88	1	
25	0.42	0.43	0.48	0.45	0.22	0.39	0.42	0.33	0.4	0.33	0.31	0.28	0.3	0.41	0.5	0.43	0.32	0.41	0.41	0.24	0.61	0.67	0.71	0.71	1

pathway intermediates, while the end products are nonprovitamin-A compounds, therefore usually provitamin-A compounds are not the predominant carotenoids in endosperm (Wurtzel, 2012). Genotypes Sikkim primitive-1, CM-400 and CM-300 (Figure 1 - 18, 1, 19), due to their white kernel, showed bands in TLC plate much less intense compared to other that indicates less amount of both total carotenoid as well as provitamin-A. A total of 11 (1490, Pop31A, Tarun-2, Pob445-54, Pop31B, POB-3, Pop31E, YHP-3, 1586, YHP-Alm, Pob-3) genotypes were identified for high total carotenoid and high provitamin-A.

Maize kernel color for all the genotypes was recorded and grouped into six groups i.e. orange, light orange, light yellow, dull yellow, dark yellow, and white to assess the relation between kernel color and total carotenoid and provitamin-A (Table 3). In the orange kernel group, carotenoid content varied from 28.18 µg/g to 37.64 µg/g whereas it varied from 27.18 to 32.50 µg/g in the light orange group. In the light yellow group, kernel carotenoids varied from 16.55 to 31.15 µg/g and from 20.32 to 29.58 µg/g in the dull yellow group, whereas in the dark yellow group, which consist of two genotypes only, it varies from 29.42 µg/g to 38.25 µg/g. In the white kernel group, carotenoids content varies from 0.94 to 1.28 µg/g. Different kernel colors recorded on 25 genotypes were also analyzed in relation to relative provitamin-A content. Lines within each group of orange, light orange, dull yellow, light yellow, dark yellow colored kernel exhibited both relatively high and relatively low provitamin-A except for the genotypes belonging to the orange (high provitamine-A) and white (low provitamin-A) kernel carotenoid.

Though the majority of orange kernel colored lines (except 1586) recorded high levels of total carotenoids and all the white maize exhibited the least amount of total kernel carotenoid, kernel carotenoid did not show significant relationship with other kernel colored groups. Again, the TLC profile of total kernel carotenoids indicated that all the orange and dark yellow genotypes are a potential source for vitamin-A precursor whereas all the white maize genotypes were poor in their provitamin-A content of total carotenoid. In a situation where a large number of genotypes are to be tested, kernel color (orange) may serve as a good physical marker for preliminary selection of high carotenoid lines. However, no strong affinity of kernel color with relative provitamin-A was observed. So, the presence of high carotenoid in the lines may not be reflecting the presence of high provitamin-A (Harjes et al, 2008).

The 25 lines were taken for characterization and diversity analysis using 12 pairs of simple sequence repeat (SSR) primers, including Y1 SSR markers specific to total carotenoids content and representing quantitative trait loci (QTLs) located on different chromosomes.

Based on the SSR marker data Jaccard's similarity coefficients (Table 4) were calculated between pairs of lines. The similarity coefficients were found to vary from 0 .17 to 0.97. The highest value for genetic similarity (0.97) was found between Pop31B and Pop31C and the lowest between Pop31D and POB-3 (0.17). Tarun-1 and 1586 were also having the same genetic distance that is found between Pop31D and POB-3.

A dendrogram (Figure 2) of selected maize genotypes revealed by UPGMA ordered the populations of 25 inbred lines into four groups (A, B, C, and D). Two

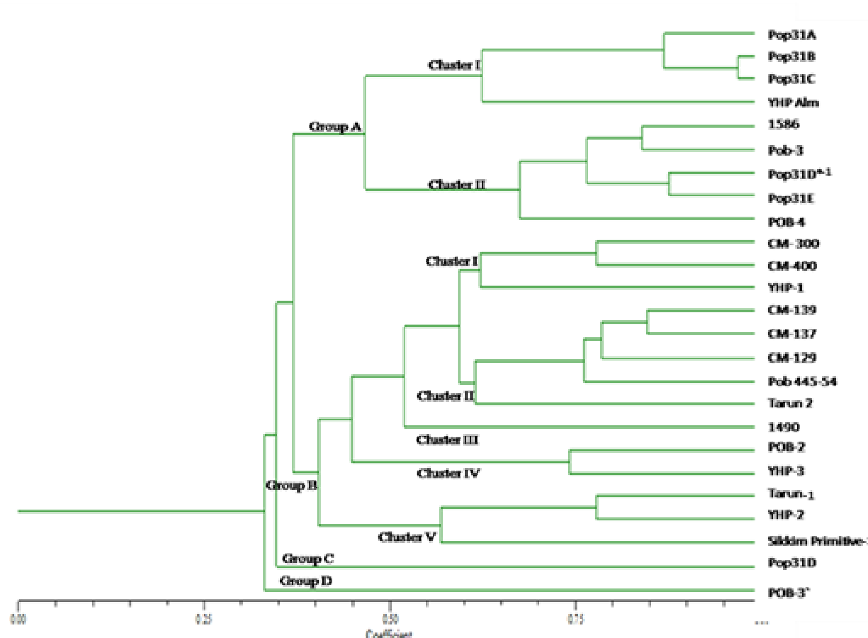

Figure 2 - Dendrogram of selected maize genotypes revealed by UPGMA cluster analysis based on SSR data.

broad groups were Group-A and Group-B and two individual inbred lines (Pop31D and POB-3) occupied two separate groups at a similarity coefficient 0.34. Group-A consisted of nine genotypes and further sub-divided into two clusters. Cluster I contained four genotypes (Pop31A, YHP-Alm, Pop31C, Pop31B) whereas cluster II contained five genotypes namely (1586, Pob-3, POB-4, Pop31-E and Pop31D-1). Both clusters have a similarity coefficient of 0.46. Group B consisted of 14 genotypes, which were further sub-divided into five clusters. Cluster I has three genotype (CM–300, YHP-1 and CM- 400) and cluster-II five genotypes (CM-139, CM -137, CM–129, Tarun-2, Pob445-54). Both these clusters share a similarity coefficient of 0.59. Cluster-III consisted of only one genotype i.e 1490. Within Cluster-IV and Cluster-V there are two (POB-2, YHP-3) and three (Tarun-1, YHP-2, Sikkim primitive-1) genotypes, respectively. As already mentioned, the least genetic similarity was shown by genotypes Pop31D and POB-3 and also by Tarun-1 and 1586; accordingly they are also placed into different clusters. Genotype Pop31D occupied Group C, whereas POB-3 was placed in Group D. Similarly, Tarun-1 came under cluster V of Group B but 1586 occupied cluster ll of Group A.

The experimental data displayed the presence of substantial variation between and within groups at the molecular level. Out of the 11 identified lines as a rich source of high carotenoid and provitamin-A, six belonged to cluster I (Pop31A, Pop31B, YHP-Alm) and cluster II (1586, Pob-3, Pop31E) of Group A, four genotypes belonged to cluster II (Tarun-2, Pob445-54), cluster III (1490), cluster IV (YHP-3) of Group B and genotype POB-3 alone occupied Group C. Though 11 lines were identified, for hybridization breeding, lines should be chosen from different cluster because to get maximum heterosis lines should be of different genetic background.

Analysis of cluster mean (Table 5) revealed that in Group-A both the cluster having high mean (>25 µg/g) carotenoid content. But in each cluster carotenoid value of individual line varies from low (<25 µg/g) to high. In Group-B inbred line 1490 belonging to cluster-III exhibited maximum carotenoid value. All the inbred lines belonging to Cluster-I and cluster-V of Group-B showed low kernel carotenoid with low mean value. Except CM-129 all the lines consisting of Cluster-II displayed high carotenoid with high mean value. Cluster-IV made up of two genotypes YHP-3 (high carotenoid) and POB-2 (low carotenoid) exhibited high mean value. Group-C and Group-D consisted of only one genotype each with low and high mean carotenoid respectively. Thus, except Cluster-I and Cluster-V (both exhibited low kernel carotenoid) of Group-B, the pattern of marker based clustering in the present investigation does not reveal strong correlation with quantitative data.

Conclusion

In the present investigation, 25 lines of maize, which constitute a fraction of the germplasm, were assessed for total carotenoids content and relative provitamin-A in the maize kernel. Considering both the parameters together, 11 lines were identified with high carotenoids and relatively more provitamin-A content in their kernel. Line 1490 was identified as one of the potential sources of carotenoids and provitamin-A as it exhibited the highest carotenoid content and also a relatively high provitamin-A content among all the 25 inbred lines. These lines, identified based on spectrophotometric and TLC data, are expected

Table 5 - Cluster wise mean carotenoids values of genotypes.

Group	Cluster	Genotypes	Carotenoids content (µg/g)	Cluster mean (µg/g)	Relative pro-vitamin A content
Group A	Cluster I	Pop31A	37.64	29.86	High
		Pop31B	31.81		High
		YHP-Alm	27.18		High
		Pop31C	22.81		Low
	Cluster II	1586	28.18	26.41	High
		Pob-3	26.40		High
		POB-4	21.61		High
		Pop31E	29.58		High
		PoP31D-1	26.32		Low
Group B	Cluster I	CM-300	1.03	8.63	Low
		CM-400	1.28		Low
		YHP-1	23.58		Low
	Cluster II	CM-137	30.78	29.55	Low
		Tarun-2	37.56		High
		Pob 445-54	32.50		High
		CM-129	16.55		Low
		CM-139	30.36		Low
	Cluster III	1490	38.25	38.25	High
	Cluster IV	POB-2	23.98	26.70	High
		YHP-3	29.42		High
	Cluster V	Tarun-1	20.32	13.09	High
		YHP-2	18.01		Low
		Sikkim primitive-1	0.94		Low
Group C	-	Pop31D	24.87	24.87	High
Group D	-	POB-3	31.15	31.15	High

to be potential donor parents in biofortification programmes aimed to improve both kernel carotenoid and provitamin-A. At the same time, genotypes can be taken from different clusters of the dendrogram for the development of high carotenoid hybrids.

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