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Genetic diversity of nutritionally important carotenoids in 94 pea and 121 chickpea accessions

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ABSTRACT

Biofortification of staple crops via breeding is an attractive strategy for reducing human micronutrient deficiencies. The objective of this research was to examine the concentration of carotenoids in diverse pea and chickpea accessions grown in Saskatchewan (Canada) using high performance liquid chromatography. In pea accessions mean concentration of lutein was highest (11.2 $\mu g\ g^{-1}$) followed by β-carotene (0.5 $\mu g\ g^{-1}$), zeaxanthin (0.3 $\mu g\ g^{-1}$), and violaxanthin (0.3 $\mu g\ g^{-1}$). Green cotyledon pea accessions were richer in β-carotene and total carotenoids compared to yellow cotyledon accessions. In chickpea accessions mean concentration of lutein (8.2 $\mu g\ g^{-1}$) was highest followed by zeaxanthin (6.2 $\mu g\ g^{-1}$), β-carotene (0.5 $\mu g\ g^{-1}$), β-cryptoxanthin (0.1 $\mu g\ g^{-1}$), and violaxanthin (0.1 $\mu g\ g^{-1}$). Desi chickpea accessions had higher carotenoid concentration than kabuli accessions. This research identified pea and chickpea accessions that can be utilized in breeding for the improvement of carotenoid concentration through biofortification.

1. Introduction

Pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.) are excellent sources of protein, slowly digestible starch, and dietary fibre (Duranti, 2006), and good sources of minerals including iron, calcium, zinc (Thavarajah and Thavarajah, 2012), folates (Rychlik et al., 2007; Hefni et al., 2010; Gupta et al., 2013), as well as carotenoids (Abbo et al., 2005, 2010; Marles et al., 2013). Carotenoids are natural pigments synthesized from plants. Humans and animals are incapable of carotenoid biosynthesis and therefore depend on dietary carotenoid sources (Fraser and Bramley, 2004). Plant-derived carotenes are metabolized to produce vitamin A from α- and β-carotene and β-cryptoxanthin (Ziegler, 1989; Olson, 1989), and the conversion of some carotenes to vitamin A occurs in animals (Williams et al., 2009).

Among the ~600 carotenoids, lutein and zeaxanthin do not have provitamin activity, but aid in prevention of age-related macular degeneration (Krinsky et al., 1990; Meydani et al., 1994; Olmedilla et al., 2001; Fraser and Bramley, 2004). Lutein also protects skin from UV-induced damage and reduces the risk of cataracts and cardiovascular diseases (Moeller et al., 2000; Alves-Rodrigues and Shao, 2004). Vitamin-A plays an important role in vision, reproduction, bone growth, cell differentiation and cell division in mammals (Stephens et al., 1996). β-Cryptoxanthin has a potential anabolic effect on bones due to stimulating osteoblastic bone formation and inhibiting osteoclastic bone resorption (Yamaguchi and Uchiyama, 2003, 2004; Yamaguchi, 2012). Carotenoids protect humans from skin disorders and several forms of cancer (Snodderly, 1995; Bramley, 2000; Bhosale et al., 2004; Story et al., 2010; Tanaka et al., 2012). Furthermore, carotenoids can reduce lipid peroxidation by efficiently scavenging free radicals and can protect cellular membranes and DNA from oxidative damage (Iannone et al., 1998; Sujak et al., 1999; Panasenko et al., 2000; Stahl et al., 2002).

The improvement of nutritional value of staple food crops through biofortification of pulses might bring significant impact given their high consumption globally. Chickpea and pea are among the most important pulse crops for human health in developing countries. Enriching food crops through plant breeding has been widely discussed in the fields of nutrition and public health at the international level (Bouis, 2002). The association of numerous physiological abnormalities with the intake of an imbalanced diet and malnutrition is a major health challenge in developed as well as developing countries. Biofortification of

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staple food crops is a new approach to control deficiencies of carotenoids, Fe, and Zn (Welch and Graham, 2005) and is seen as one of the key strategies for alleviating micronutrient malnutrition afflicting poor communities (White and Broadley, 2005).

Recent studies have been undertaken to evaluate carotenoid concentration in various crops (Abushita et al., 2000; Fraser et al., 2001; Hentschel et al., 2002; Menkir and Dixon, 2004; Ssemakula et al., 2007; Hidalgo and Brandolini, 2008; Amorim et al., 2009; Ramachandran et al., 2010; Haynes et al., 2010). Separation and quantification of putative carotenoids by high performance liquid chromatography (HPLC) equipped with diode array detector (DAD) is a sensitive, reliable and accurate method. To date, only limited research has been reported related to carotenoids in pea and chickpea seeds using HPLC analysis. Previously, lutein, zeaxanthin and β-cryptoxanthin were reported in chickpea seed (Abbo et al., 2005, Abbo et al., 2010), whereas violaxanthin, lutein, and β-carotene were reported in cotyledons and seed coats of field pea (McCallum et al., 1997; Holasová et al., 2009). Lutein was reported as the major carotenoid in chickpea (Abbo et al., 2005, 2010), pea (McCallum et al., 1997; Holasová et al., 2009; Marles et al., 2013) and wheat (Adom et al., 2003; Hidalgo et al., 2006; Ramachandran et al., 2010). Higher lutein concentration was reported in green cotyledon peas compared to yellow cotyledon (Holasová et al., 2009; Marles et al., 2013; Ashokkumar et al., 2014).

Thus far, the assessment of carotenoid profiles in pea and chickpea has been limited to small and relatively narrow sets of germplasm. The objective of this research was to assess the variability in concentration of nutritionally important carotenoids in seeds of large sets of genetically diverse pea and chickpea accessions. The derived information will be used in developing breeding strategies for enhancing carotenoid concentration in pulse crops.

2. Materials and methods

2.1. Plant material

Genetically diverse accessions derived from association mapping panels of pea (63 yellow and 31 green cotyledons) and chickpea (84 kabuli and 37 desi) were obtained from trials conducted in 2011 by the Crop Development Centre (CDC), University of Saskatchewan, Saskatoon, Canada. Trials were conducted using a randomized complete block design at Rosthern for pea and Elrose for chickpea in Saskatchewan, Canada. Seed subsamples were taken randomly from the entire harvested lot from 2 field plots for both crops, i.e. 2 biological replicates. Subsamples of 10 g of pea and chickpea seeds were air-dried to 14% moisture and stored at −20 °C until milled. Whole seed samples were ground in a Udy mill (Fort Collins, CO, USA) equipped with a 0.5 mm screen and ground samples were stored at −20 °C. One week prior to extraction, samples were kept at room temperature and 1 g sub-samples were weighed for extraction.

2.2. Carotenoid extraction, calibration and quantification

Carotenoid extraction was conducted using an optimized methanol–dichloromethane extraction method, which had low volatility and 100% recoveries for total carotenoids (Ashokkumar et al., 2014). All the standards and sample extractions were carried out under subdued light conditions at room temperature of 20 °C to protect the carotenoids from degradation. Sample extraction solvents were initially premixed with 0.1% butylated hydroxytoluene (BHT) in dichloromethane (DCM) and methanol (MeOH), v/v (1:1) and added at the rate of 5 mL/g tissue in Pyrex tubes capped with Teflon liners. Samples were immediately vortexed followed by shaking at 200 rpm for 1 h in a Bigger Bill shaker (Thermolyne Corporation, Dubuque, IA). After shaking the extracts were treated with an equal volume of 100% acetonitrile as the diluent to remove proteins and centrifuged at 11,000 × *g* for 5 min. The centrifuged supernatant was filtered (0.2 μm PVDF), placed in 250 μL glass inserts in 2 mL amber glass HPLC vials and then analyzed by HPLC.

The individual standards of lutein, zeaxanthin, β-cryptoxanthin, β-carotene (Sigma–Aldrich, Oakville, ON, Canada) and violaxanthin (ChromaDex, Irvine, CA, USA) were obtained. The purity of chemical standards was 97% for β-cryptoxanthin and 95% for the others. Carotenoids quantification was performed based on absolute calibration curves of lutein (447 nm), zeaxanthin (453 nm), β-carotene (455 nm) and others (450 nm) with a minimum of five concentration levels. The linear range for the carotenoid standard curves were as follows: 2–40 μg/mL violaxanthin and β-carotene, 2–20 μg/mL for zeaxanthin, 4–100 μg/mL for lutein and 2–400 μg/mL for β-cryptoxanthin. The above concentration ranges were chosen as the most appropriate for determination of individual carotenoid concentration and were based on our preliminary experiments. For calibration, regression coefficients achieved were as follows: violaxanthin ($y = 14.740x - 7.661$, $R^2 = 0.997$), lutein ($y = 12.45x + 18.821$, $R^2 = 0.998$), zeaxanthin ($y = 12.505x - 12.116$, $R^2 = 0.998$), β-carotene ($y = 10.401x + 34.651$, $R^2 = 0.999$), and β-cryptoxanthin ($y = 539.05x - 164.19$, $R^2 = 0.999$), where y denotes peak area and x concentration (μg/mL). The LOQs for violaxanthin, lutein, zeaxanthin, β-carotene and β-cryptoxanthin were 0.17, 0.44, 0.16, 0.20, and 2.00 μg/mL, respectively. The recovery percentages ($n = 3$) were as follows: violaxanthin (99.9%), lutein (100%), zeaxanthin (99.9%), β-carotene (99.7%) and β-cryptoxanthin (99.8%). Accuracy and repeatability was measured by relative standard deviation (RSD) of peak areas for three consecutive injections of the reference solution. The RSD for violaxanthin, lutein, zeaxanthin, β-carotene and β-cryptoxanthin were 0.75, 1.15, 1.40, 0.90, and 0.51 percentages, respectively. All standards had more than 97% purity, and standard extraction solvents were initially premixed with 0.1% BHT in DCM and MeOH, v/v (3:1) for β-carotene and v/v (1:1) for others.

Chromatography was performed using the Agilent technologies 1200 HPLC system equipped with a refrigerated autosampler, and a diode array detector (DAD). Separation was done on a C30 carotenoid column (3 μm, 4.6 × 250 mm) (Waters, YMC America, Newtown, PA), preceded by a 20 × 4 mm C30 guard column (Waters, YMC America, Newtown, PA) were used at 24 °C. Spectrophotometric detection was achieved by means of a diode array detector in the range 190–600 nm. Extracts were eluted with 40 min isocratic elution (58:22:20, CH_3CN:CH_3OH:CH_2Cl_2) at flow rate was 0.8 mL/min, to separate compounds in the extracts, and injection volume was 20 μL/sample. A consistent column wash (40 °C) with 50:49:1 (v/v/w) 2-propanol: water: acetic acid was performed after every 25 samples (Ramachandran et al., 2010) at 0.2 mL flow rate and for 30 min. All eluent solvents were of HPLC grade. Each biological sample was extracted in two technical repeats and analyzed by HPLC.

2.3. Statistical analysis

Results were converted to $\mu g\, g^{-1}$, and each carotenoid quantified, individual value was reported as a mean of 2 biological replicates for both pea and chickpea, in every genotype (technical replicates were averaged for each biological replicate). Significant differences among means were determined by Duncan's Multiple Range Test. Pearson correlation analysis was computed using the PROC CORR statement, and was carried out among the four carotenoids for pea and five carotenoids for chickpea. All statistical procedures were computed using Statistical Analysis Software (SAS) version 9.3 for windows (SAS Institute, 2011).

3. Results

3.1. Carotenoids isolation

Chromatographic peaks were identified by comparing retention times and absorbance spectra to the respective carotenoid standards. A peak was identified as an individual carotenoid if characteristic triple maxima were observed in the absorbance spectrum and retention time was 6.4, 10.6, 12.4, 21.5 and 35.2 min for violaxanthin, lutein, zeaxanthin, β-cryptoxanthin, and β-carotene, respectively. In the present study, violaxanthin, lutein, zeaxanthin, and β-carotene were identified in the mature pea and chickpea seeds and were quantified using HPLC-DAD. In addition, low concentration of β-cryptoxanthin was detected in chickpea accessions only. Several unidentifiable peaks were also detected, but were not quantified as they were minor carotenoids with unreported or lesser nutritional benefits. Total carotenoid concentration was calculated as the sum of mean values of the four individual carotenoids for pea and five individual carotenoids for chickpea.

3.2. Carotenoid concentration in genetically diverse accessions of pea

The mean carotenoid concentration of 94 pea accessions is presented in Table 1. The mean and range of carotenoid

Table 1
Carotenoid concentration in 94 genetically diverse pea accessions evaluated in 2011 at Rosthern, Saskatchewan, Canada.

Entry	Genotype	Type[a]	Country of origin	Status	Mean carotenoid concentration ($\mu g\,g^{-1}$)				
					Violaxanthin	Lutein	Zeaxanthin	β-Carotene	Total carotenoids[b]
1	CN 112439	G	Belarus	Land race	0.70	9.91	0.38	0.70	11.69
2	CN 112441	G	Lithuania	Breeding line	0.62	13.15	0.44	0.82	15.03
3	CDC Patrick	G	University of Saskatchewan, Canada	Cultivar	1.31	17.44	0.56	1.51	20.82
4	Sovershenstov-65	G	Russia	Cultivar	0.43	8.76	0.22	0.67	10.08
5	Denica	G	Bulgaria	Cultivar	0.36	9.55	0.19	1.16	11.25
6	Ramonsky 90	G	Russia	Cultivar	0.61	10.44	0.40	0.66	12.10
7	Oryol-328	G	Russia	Cultivar	0.54	10.56	0.41	0.41	11.93
8	MPG87	G	Canada	Breeding line	1.06	24.49	0.44	1.47	27.46
9	CDC Striker	G	University of Saskatchewan, Canada	Cultivar	0.57	15.59	0.63	1.03	17.82
10	Cooper	G	Netherlands	Cultivar	1.01	16.22	0.55	1.22	19.00
11	Nitouche	G	Denmark	Cultivar	1.17	12.45	0.94	1.29	15.85
12	SW Sergeant	G	Sweden	Cultivar	0.79	13.55	0.83	1.33	16.50
13	MFR043	G	Canada	Cultivar	0.84	12.75	0.73	1.07	15.38
14	Orb	G	UK	Cultivar	0.92	12.15	1.00	1.12	15.19
15	CDC Montero	G	University of Saskatchewan, Canada	Cultivar	0.65	12.36	0.50	0.65	14.16
16	CDC Sage	G	University of Saskatchewan, Canada	Cultivar	0.73	14.17	1.07	1.43	17.40
17	Espace	G	Netherlands	Cultivar	0.96	13.55	0.77	1.15	16.43
18	Radley	G	UK	Cultivar	0.49	17.49	0.33	1.22	19.52
19	CDC Vienna	G	University of Saskatchewan, Canada	Cultivar	1.15	15.58	0.25	1.58	18.56
20	MI3391	G	Agriculture and Agri-Food Canada, Canada	Breeding line	0.67	18.41	0.21	2.04	21.32
21	MI3360	G	Agriculture and Agri-Food Canada, Canada	Breeding line	0.58	15.31	0.28	1.58	17.75
22	Crackerjack	G	Denmark	Cultivar	0.59	12.74	0.40	1.33	15.06
23	Woody	G	France	Cultivar	0.45	9.12	0.35	0.67	10.59
24	Lucy	G	France	Cultivar	0.58	15.33	0.42	1.11	17.45
25	PS05100632	G	USDA, USA	Breeding line	1.01	19.22	0.90	2.80	23.93
26	PS05100840	G	USDA, USA	Breeding line	0.99	16.22	0.88	1.78	19.86
27	Mini	G	USA	Cultivar	0.87	24.21	0.77	2.34	28.19
28	Rally	G	USA	Cultivar	0.33	10.84	0.31	1.22	12.69
29	SGL 2024	G	Poland	Breeding line	0.60	14.15	0.90	1.32	16.97
30	Aragorn	G	USA	Cultivar	1.21	18.03	1.32	2.23	22.79
31	CN 112431	G	Czechoslovakia	Unknown	0.34	17.32	0.18	0.69	18.52
32	Rambo	Y	Netherlands	Cultivar	0.21	8.25	0.25	0.24	8.95
33	40-10	Y	Germany	Cultivar	0.19	8.53	0.08	0.20	8.99
34	Laxtons Superb	Y	USA	Cultivar	0.22	8.53	0.10	1.02	9.87
35	Green	Y	Ukraine	Cultivar	0.20	15.79	0.35	0.22	16.55
36	CDC Treasure	Y	University of Saskatchewan, Canada	Cultivar	0.18	12.35	0.28	0.00	12.81
37	Kalininsky	Y	Russia	Cultivar	0.09	7.63	0.23	0.00	7.95
38	Krasnoobskii	Y	Russia	Cultivar	0.14	10.52	0.33	0.21	11.20
39	Ekaterininskyj Usatyj	Y	Russia	Cultivar	0.07	7.63	0.22	0.00	7.92
40	Omskii-9	Y	Russia	Cultivar	0.19	12.75	0.27	0.06	13.27
41	Agat	Y	Belarus	Cultivar	0.14	8.58	0.30	0.00	9.02
42	Vector	Y	Russia	Cultivar	0.09	7.52	0.23	0.00	7.85
43	Cutlass	Y	Univ. Sask./Alberta Agriculture, Canada	Cultivar	0.12	6.19	0.21	0.00	6.52
44	CDC Bronco	Y	University of Saskatchewan, Canada	Cultivar	0.15	10.17	0.35	0.11	10.78
45	CDC Centennial	Y	University of Saskatchewan, Canada	Cultivar	0.06	7.12	0.13	0.00	7.32

Table 1 (*Continued*)

Entry	Genotype	Type[a]	Country of origin	Status	Mean carotenoid concentration ($\mu g\,g^{-1}$)				
					Violaxanthin	Lutein	Zeaxanthin	β-Carotene	Total carotenoids[b]
46	CDC Golden	Y	University of Saskatchewan, Canada	Cultivar	0.08	9.11	0.21	0.00	9.41
47	CDC Mozart	Y	University of Saskatchewan, Canada	Cultivar	0.13	8.53	0.18	0.08	8.92
48	CDC Meadow	Y	University of Saskatchewan, Canada	Cultivar	0.15	12.71	0.30	0.00	13.16
49	DS Admiral	Y	Denmark	Cultivar	0.12	11.20	0.24	0.00	11.56
50	Eclipse	Y	Netherlands	Cultivar	0.21	11.54	0.19	0.05	11.99
51	Reward	Y	Agriculture and Agri-Food Canada, Canada	Cultivar	0.13	11.34	0.18	0.08	11.73
52	CDC Dundurn	Y	University of Saskatchewan, Canada	Cultivar	0.33	15.80	0.38	0.38	16.88
53	Kaspa	Y	Australia	Cultivar	0.19	10.32	0.22	0.06	10.79
54	Carneval	Y	Sweden	Cultivar	0.13	11.75	0.16	0.06	12.10
55	MP1401	Y	Agriculture and Agri-Food Canada, Canada	Breeding line	0.11	9.09	0.13	0.00	9.34
56	Alfetta	Y	Netherlands	Cultivar	0.10	7.59	0.14	0.00	7.83
57	SW Marquee	Y	Sweden	Cultivar	0.14	7.85	0.15	0.00	8.14
58	Terese	Y	France	Cultivar	0.14	9.76	0.18	0.05	10.12
59	Torsdag	Y	France	Cultivar	0.05	5.26	0.10	0.00	5.41
60	Delta	Y	Netherlands	Cultivar	0.15	7.89	0.17	0.00	8.21
61	CDC Acer	Y	University of Saskatchewan, Canada	Cultivar	0.27	12.19	0.22	0.19	12.87
62	CDC Leroy	Y	University of Saskatchewan, Canada	Cultivar	0.15	8.51	0.20	0.00	8.86
63	Naparnyk	Y	Ukraine	Cultivar	0.06	9.34	0.14	0.00	9.54
64	Trapper	Y	Agriculture and Agri-Food Canada, Canada	Cultivar	0.23	13.22	0.27	0.00	13.73
65	Highlight	Y	Sweden	Cultivar	0.13	10.36	0.18	0.00	10.67
66	Carrera	Y	Netherlands	Cultivar	0.09	5.91	0.09	0.00	6.08
67	CDC 1-150-81	Y	University of Saskatchewan, Canada	Breeding line	0.20	14.12	0.29	0.11	14.71
68	CDC 1-2347-144	Y	University of Saskatchewan, Canada	Breeding line	0.19	11.21	0.28	0.07	11.75
69	Agassiz	Y	Agriculture and Agri-Food Canada, Canada	Cultivar	0.13	6.08	0.12	0.00	6.33
70	Polstead	Y	Netherlands	Cultivar	0.09	7.62	0.13	0.00	7.84
71	Bilboquet	Y	France	Cultivar	0.12	8.06	0.11	0.00	8.29
72	Belote	Y	France	Cultivar	0.11	7.26	0.11	0.00	7.48
73	Rose	Y	Germany	Cultivar	0.09	9.10	0.12	0.00	9.31
74	Rocket	Y	Germany	Cultivar	0.09	9.08	0.15	0.00	9.32
75	Hardy	Y	France	Cultivar	0.11	6.70	0.10	0.00	6.92
76	Prelude	Y	France	Cultivar	0.06	7.69	0.15	0.00	7.90
77	Cartouche	Y	France	Cultivar	0.15	14.40	0.34	0.04	14.92
78	Argus	Y	Agriculture and Agri-Food Canada, Canada	Cultivar	0.08	8.94	0.19	0.00	9.21
79	Hugo	Y	Agriculture and Agri-Food Canada, Canada	Cultivar	0.14	8.12	0.18	0.03	8.47
80	Stella	Y	Agriculture and Agri-Food Canada, Canada	Cultivar	0.08	8.52	0.17	0.00	8.77
81	02H016P-03HO2004-06TGVP004	Y	Australia	Breeding line	0.13	9.99	0.19	0.08	10.39
82	03H107P-04HO2026	Y	Australia	Breeding line	0.11	10.19	0.21	0.04	10.54
83	03H267-04HO2006	Y	Australia	Breeding line	0.11	8.41	0.18	0.09	8.80
84	PS05101142	Y	USDA, USA	Breeding line	0.22	15.30	0.22	0.24	15.98
85	Superscout	Y	USA	Cultivar	0.15	13.30	0.38	1.22	15.05
86	Lacy Lady	Y	USA	Cultivar	0.04	5.43	0.08	0.25	5.81
87	Fallon	Y	USDA, USA	Cultivar	0.20	10.52	0.27	0.15	11.14
88	FDP2010	Y	France	Breeding line	0.10	6.03	0.15	0.00	6.28
89	P0309-09	Y	Agriculture and Agri-Food Canada, Canada	Breeding line	0.13	7.25	0.09	0.00	7.46
90	P0321-08	Y	Agriculture and Agri-Food Canada, Canada	Breeding line	0.14	8.90	0.13	0.00	9.16
91	P0316-04	Y	Agriculture and Agri-Food Canada, Canada	Breeding line	0.12	8.22	0.21	0.00	8.55
92	P0322-01	Y	Agriculture and Agri-Food Canada, Canada	Breeding line	0.23	9.49	0.25	0.04	10.00
93	Lido	Y	Denmark	Cultivar	0.11	10.34	0.15	0.00	10.59
94	Rialto	Y	Denmark	Cultivar	0.14	11.25	0.14	0.00	11.54
	SD[c]				0.33	3.84	0.25	0.66	4.78
	SE[d]				0.02	0.27	0.01	0.05	0.35

[a] Cotyledon colour, G = Green, Y = Yellow.
[b] Total carotenoids was calculated as the sum of four individual carotenoids.
[c] SD, standard deviation.
[d] SE, standard error ($N = 188$).

Table 2
Mean and range of carotenoid concentration among 94 accessions of green and yellow cotyledon pea evaluated in 2011 at Rosthern, Saskatchewan, Canada.

Trait	Carotenoid concentration ($\mu g\,g^{-1}$) ± SD[a]		
	Entire collection	Green cotyledon[†]	Yellow cotyledon[†]
Mean			
Violaxanthin	0.3 ± 0.33	0.8 ± 0.27[a]	0.1 ± 0.06[b]
Lutein	11.2 ± 3.84	14.6 ± 3.90[a]	9.6 ± 2.53[b]
Zeaxanthin	0.3 ± 0.25	0.6 ± 0.30[a]	0.2 ± 0.08[b]
β-Carotene	0.5 ± 0.66	1.3 ± 0.54[a]	0.1 ± 0.21[b]
Total carotenoid[b]	12.4 ± 4.78	17.1 ± 4.53[a]	10.0 ± 2.69[b]
Range			
Violaxanthin	0.0–1.3	0.3–1.3	0.0–0.3
Lutein	5.6–24	8.8–24	5.3–15.8
Zeaxanthin	0.1–1.3	0.2–1.3	0.1–0.4
β-Carotene	0.0–2.6	0.4–2.6	0.0–1.2
Total carotenoid[b]	5.8–26.9	10.1–26.9	5.8–16.9

[a] Standard deviation.
[b] Total carotenoid concentration was calculated as the sum of four individual carotenoids.
[†] Means followed by different letters differed significantly according to Duncan's Multiple Range Test (DMRT) $P < 0.05$.

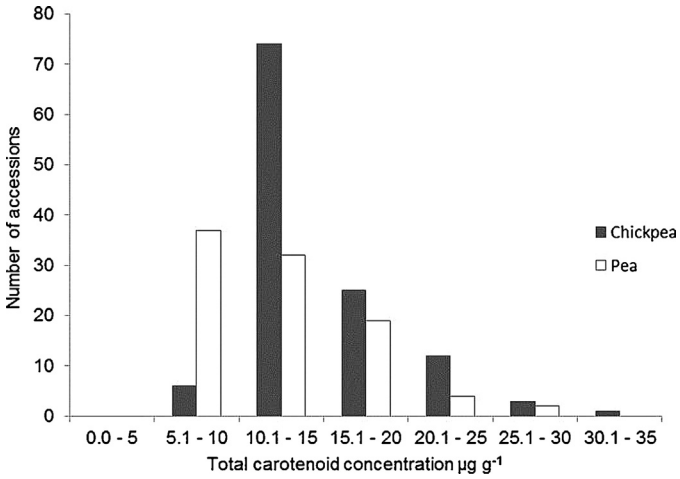

Fig. 2. Frequency distribution for total carotenoid concentration in 121 chickpea and 94 pea accessions.

concentration in the entire collection of pea, as well as by cotyledon colour is summarized in Table 2. As a whole, pea accessions were highest in lutein (11.2 $\mu g\,g^{-1}$), followed by β-carotene (0.5 $\mu g\,g^{-1}$), zeaxanthin (0.3 $\mu g\,g^{-1}$) and violaxanthin (0.3 $\mu g\,g^{-1}$). The mean lutein concentration of the pea collection ranged substantially from 5.6 $\mu g\,g^{-1}$ to 24.0 $\mu g\,g^{-1}$ and the zeaxanthin concentration ranged from 0.1 $\mu g\,g^{-1}$ to 1.3 $\mu g\,g^{-1}$ (Table 2). Frequency distribution for carotenoid concentration in pea accessions is presented in Fig. 1. Seventy-seven pea accessions had mean lutein concentration of 5–15 $\mu g\,g^{-1}$ and the two with the greatest concentration had 24 $\mu g\,g^{-1}$ (Fig. 1B). Twenty-two pea accessions had mean β-carotene concentration of 1.1–2.0 $\mu g\,g^{-1}$ and three had 2.0–3.0 $\mu g\,g^{-1}$ (Fig. 1D). MPG87 and Mini had the highest total carotenoid concentration (27.5 $\mu g\,g^{-1}$ and 28.2 $\mu g\,g^{-1}$, respectively). CDC Patrick, MI3391, PS05100632, and Aragorn had total carotenoid concentration in the range of 20.1–25 $\mu g\,g^{-1}$ (Fig. 2). Mean concentrations ($\mu g\,g^{-1}$) of lutein, β-carotene, violaxanthin, and zeaxanthin of green cotyledon pea

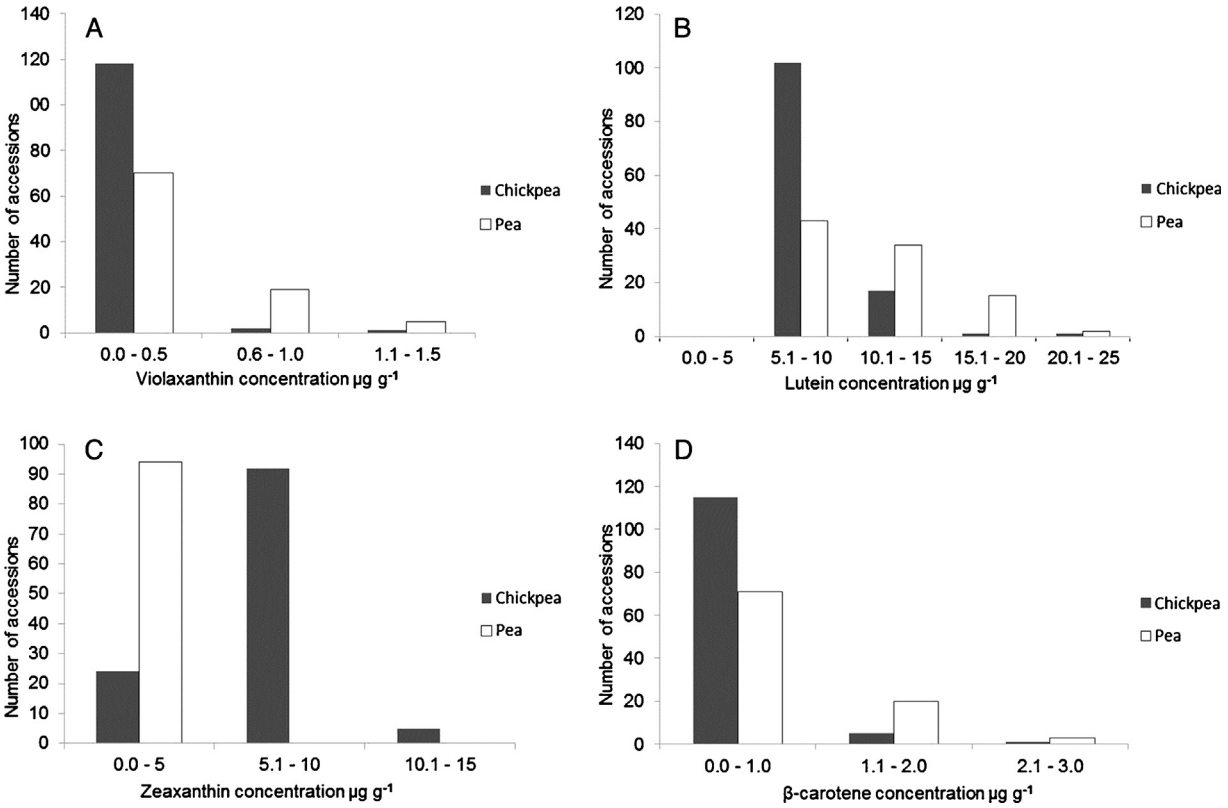

Fig. 1. Frequency distributions for carotenoid concentration in 121 chickpea and 94 pea accessions. (A) Violaxanthin; (B) Lutein; (C) Zeaxanthin; (D) β-carotene.

accessions were 14.6, 1.3, 0.8, and 0.6, respectively, and 9.6, 0.1, 0.1, and 0.2 for yellow cotyledon accessions, respectively. Mean total carotenoid concentration was 1.7 fold higher in green cotyledon (17.1 $\mu g\ g^{-1}$) than in yellow cotyledon pea accessions (10.1 $\mu g\ g^{-1}$).

3.3. Carotenoid concentration in genetically diverse accessions of chickpea

The mean carotenoid concentration of 121 chickpea accessions is presented in Table 3. The mean and range of carotenoid

Table 3
Carotenoid concentration in 121 genetically diverse chickpea accessions evaluated in 2011 at Elrose, Saskatchewan, Canada.

Entry	Genotype	Type[b]	Origin	Status	Mean carotenoid concentration ($\mu g\ g^{-1}$)					
					Violaxanthin	Lutein	Zeaxanthin	β-Cryptoxanthin	β-Carotene	Total carotenoids[a]
1	BS1-D-15	D	Unknown	Germplasm	0.04	9.93	10.49	0.12	0.76	21.34
2	DH45-1	D	Unknown	Germplasm	0.08	14.14	14.79	0.13	0.87	30.00
3	ICC12004	D	Unknown	Landrace	0.07	6.99	6.52	0.05	0.63	14.27
4	ICRISAT-121D	D	ICRISAT, India	Germplasm	0.08	12.64	12.09	0.19	1.18	26.18
5	Sel85363	D	ICARDA, formerly at Syria	Breeding line	0.03	9.79	7.94	0.07	0.47	18.30
6	Sel85454	D	ICARDA, formerly at Syria	Breeding line	0.03	8.46	6.44	0.04	0.47	15.43
7	Myles	D	USDA/ARS	Cultivar	0.17	11.50	9.41	0.06	0.73	21.88
8	ICCV96029	D	ICRISAT, India	Cultivar	0.06	8.34	7.20	0.07	0.61	16.27
9	92040-52D	D	University of Saskatchewan, Canada	Breeding line	0.05	7.11	5.95	0.03	0.44	13.60
10	92056-8	D	University of Saskatchewan, Canada	Breeding line	0.13	10.96	8.63	0.09	0.86	20.67
11	CDC_ChiChi	D	University of Saskatchewan, Canada	Cultivar	0.11	9.73	6.23	0.07	0.97	17.11
12	CDC_Anna	D	University of Saskatchewan, Canada	Cultivar	0.11	10.52	8.56	0.08	0.76	20.03
13	CDC_Cabri	D	University of Saskatchewan, Canada	Cultivar	0.10	9.62	5.65	0.05	0.53	15.96
14	CDC_Desiray	D	University of Saskatchewan, Canada	Cultivar	0.05	6.37	6.22	0.07	0.36	13.08
15	CDC_Nika	D	University of Saskatchewan, Canada	Cultivar	0.02	6.01	5.38	0.03	0.28	11.73
16	FLIP94-510C	D	ICARDA, formerly at Syria	Breeding line	0.08	7.64	6.27	0.03	0.52	14.54
17	CDC_Ebony	D	University of Saskatchewan, Canada	Cultivar	0.70	15.70	7.33	0.07	1.02	24.84
18	CDC_Jade	D	University of Saskatchewan, Canada	Cultivar	1.28	21.54	5.85	0.05	2.57	31.29
19	303T-24	D	University of Saskatchewan, Canada	Breeding line	0.11	9.44	8.08	0.13	0.61	18.37
20	304-40	D	University of Saskatchewan, Canada	Breeding line	0.11	10.62	9.05	0.05	0.60	20.42
21	CDC_Vanguard	D	University of Saskatchewan, Canada	Cultivar	0.05	9.03	7.20	0.06	0.56	16.90
22	316B-42	D	University of Saskatchewan, Canada	Breeding line	0.03	5.31	4.19	0.00	0.29	9.82
23	381T-4	D	University of Saskatchewan, Canada	Breeding line	0.05	7.72	6.27	0.07	0.51	14.63
24	418-59	D	University of Saskatchewan, Canada	Breeding line	0.10	12.76	8.31	0.05	0.63	21.86
25	425-14	D	University of Saskatchewan, Canada	Breeding line	0.05	7.85	6.77	0.09	0.52	15.27
26	463-2	D	University of Saskatchewan, Canada	Breeding line	0.11	11.16	8.00	0.04	0.44	19.74
27	512-51	D	University of Saskatchewan, Canada	Breeding line	0.10	10.07	8.69	0.09	0.57	19.52
28	548aS-20	D	University of Saskatchewan, Canada	Breeding line	0.20	9.72	5.95	0.08	1.07	17.02
29	551-1	D	University of Saskatchewan, Canada	Breeding line	0.05	9.39	8.14	0.07	0.77	18.42
30	553-1	D	University of Saskatchewan, Canada	Breeding line	0.10	12.80	10.85	0.13	1.13	25.00
31	603-3	D	University of Saskatchewan, Canada	Breeding line	0.06	9.21	7.30	0.06	0.56	17.20
32	612-4	D	University of Saskatchewan, Canada	Breeding line	0.10	10.76	7.41	0.09	1.24	19.61
33	CDC_Corinne	D	University of Saskatchewan, Canada	Cultivar	0.07	12.67	11.12	0.05	0.76	24.67
34	GPE094	D	University of Saskatchewan, Canada	Breeding line	0.05	8.54	6.53	0.05	0.52	15.69
35	Y9563-028	D	University of Saskatchewan, Canada	Germplasm	0.10	12.20	10.25	0.04	0.71	23.30
36	CDC_Cory	D	University of Saskatchewan, Canada	Cultivar	0.10	15.16	12.97	0.13	0.73	29.09

Table 3 (*Continued*)

Entry	Genotype	Type[b]	Origin	Status	Mean carotenoid concentration ($\mu g\,g^{-1}$)					
					Violaxanthin	Lutein	Zeaxanthin	β-Cryptoxanthin	β-Carotene	Total carotenoids[a]
37	BC_73_(CP73)	D	University of Saskatchewan, Canada	Breeding line	0.19	11.20	9.41	0.09	1.06	21.94
38	BlancoLechoso	K	Spain	Cultivar	0.02	7.13	3.72	0.03	0.27	11.17
39	GI	K	USDA/ARS	Germplasm	0.02	9.96	6.03	0.12	0.64	16.77
40	Kolorit	K	Ukrainian	Germplasm	0.10	12.33	10.11	0.12	0.83	23.50
41	Dwelley	K	USDA/ARS	Cultivar	0.00	6.99	5.16	0.03	0.32	12.50
42	Evans	K	USDA/ARS	Cultivar	0.00	7.12	5.30	0.03	0.36	12.81
43	Sanford	K	USDA/ARS	Cultivar	0.00	6.81	5.03	0.00	0.33	12.17
44	CDC_Marengo	K	University of Saskatchewan, Canada	Cultivar	0.07	10.40	10.42	0.10	0.67	21.67
45	92073-60D	K	University of Saskatchewan, Canada	Breeding line	0.13	8.71	7.14	0.07	0.79	16.84
46	CDC_Chico	K	University of Saskatchewan, Canada	Cultivar	0.02	7.16	5.32	0.02	0.24	12.77
47	CDC_Diva	K	University of Saskatchewan, Canada	Cultivar	0.00	6.95	4.72	0.02	0.24	11.93
48	CDC_Verano	K	University of Saskatchewan, Canada	Cultivar	0.60	12.29	3.94	0.05	1.86	18.74
49	CDC_Xena	K	University of Saskatchewan, Canada	Cultivar	0.03	7.10	4.67	0.03	0.41	12.24
50	CDC_Yuma	K	University of Saskatchewan, Canada	Cultivar	0.02	7.31	5.54	0.04	0.38	13.29
51	93-120-63K	K	University of Saskatchewan, Canada	Breeding line	0.02	8.03	5.55	0.04	0.44	14.10
52	CDC_Luna	K	University of Saskatchewan, Canada	Cultivar	0.02	6.34	4.67	0.05	0.40	11.47
53	95168-64	K	University of Saskatchewan, Canada	Breeding line	0.02	8.43	5.66	0.04	0.38	14.52
54	95177-47	K	University of Saskatchewan, Canada	Breeding line	0.02	8.75	5.41	0.05	0.09	14.31
55	95NN-12	K	University of Saskatchewan, Canada	Breeding line	0.01	6.89	5.55	0.04	0.40	12.90
56	CDC_Frontier	K	University of Saskatchewan, Canada	Cultivar	0.00	6.51	5.20	0.00	0.30	12.01
57	FLIP95-48C	K	ICARDA, formerly at Syria	Breeding line	0.01	5.39	4.06	0.00	0.35	9.81
58	S95420	K	ICARDA, formerly at Syria	Breeding line	0.01	7.38	6.04	0.03	0.35	13.80
59	Amit	K	Israel	Cultivar	0.01	7.51	6.63	0.03	0.24	14.42
60	S96123	K	ICARDA, formerly at Syria	Breeding line	0.06	9.37	8.85	0.08	0.57	18.93
61	242-2	K	University of Saskatchewan, Canada	Breeding line	0.02	7.63	5.91	0.06	0.41	14.02
62	97-Indian2-112	K	India	Germplasm	0.01	7.26	5.82	0.03	0.35	13.48
63	FLIP97-101C	K	ICARDA, formerly at Syria	Breeding line	0.02	8.97	6.57	0.06	0.53	16.15
64	FLIP97-45C	K	ICARDA, formerly at Syria	Breeding line	0.02	7.45	4.69	0.04	0.27	12.46
65	328S-8	K	University of Saskatchewan, Canada	Breeding line	0.02	7.72	5.77	0.07	0.41	14.00
66	CA9890234W	K	USDA/ARS	Breeding line	0.01	7.27	4.36	0.03	0.36	12.03
67	FLIP98-135C	K	ICARDA, formerly at Syria	Breeding line	0.01	5.97	4.42	0.02	0.14	10.56
68	ICARDA-GP-LTRCIYT-SP-98ENTRY21	K	ICARDA	Germplasm	0.03	7.76	5.53	0.05	0.38	13.74
69	438-29	K	University of Saskatchewan, Canada	Breeding line	0.03	7.59	6.09	0.04	0.40	14.15
70	439as-22	K	University of Saskatchewan, Canada	Breeding line	0.02	7.69	5.14	0.03	0.44	13.32
71	441-34	K	University of Saskatchewan, Canada	Breeding line	0.01	7.32	5.01	0.05	0.55	12.95
72	CA2969	K	Spain	Germplasm	0.02	7.65	6.62	0.10	0.48	14.87
73	CIABN-99PL27119	K	ICARDA, formerly at Syria	Germplasm	0.01	6.68	5.21	0.04	0.38	12.33
74	CDC_Orion	K	University of Saskatchewan, Canada	Cultivar	0.00	6.46	5.11	0.06	0.35	11.97
75	492-3	K	University of Saskatchewan, Canada	Breeding line	0.00	7.01	5.93	0.02	0.30	13.26
76	CDC_Leader	K	University of Saskatchewan, Canada	Cultivar	0.00	6.86	7.52	0.04	0.30	14.71
77	494-4	K	University of Saskatchewan, Canada	Breeding line	0.00	6.94	5.03	0.04	0.40	12.41
78	561aS-18	K	University of Saskatchewan, Canada	Breeding line	0.03	10.35	8.15	0.05	0.42	19.01

Table 3 (*Continued*)

Entry	Genotype	Type[b]	Origin	Status	Mean carotenoid concentration ($\mu g\,g^{-1}$)					
					Violaxanthin	Lutein	Zeaxanthin	β-Cryptoxanthin	β-Carotene	Total carotenoids[a]
79	701-6	K	University of Saskatchewan, Canada	Breeding line	0.02	8.00	7.53	0.08	0.40	16.03
80	889-8	K	University of Saskatchewan, Canada	Breeding line	0.00	7.30	5.28	0.03	0.26	12.87
81	1041-3	K	University of Saskatchewan, Canada	Breeding line	0.02	8.00	6.22	0.06	0.42	14.73
82	1044-6	K	University of Saskatchewan, Canada	Breeding line	0.02	7.93	5.62	0.04	0.29	13.90
83	1045-1	K	University of Saskatchewan, Canada	Breeding line	0.01	8.70	6.89	0.07	0.45	16.12
84	CDC_Alma	K	University of Saskatchewan, Canada	Cultivar	0.01	7.39	6.44	0.10	0.51	14.45
85	CA05-73-6	K	University of Saskatchewan, Canada	Breeding line	0.02	7.31	5.96	0.02	0.30	13.61
86	CA05-75-16	K	University of Saskatchewan, Canada	Breeding line	0.02	7.43	6.00	0.03	0.49	13.97
87	FLIP81-71C	K	ICARDA, formerly at Syria	Breeding line	0.01	7.61	6.32	0.01	0.38	14.33
88	FLIP81-293C	K	ICARDA, formerly at Syria	Breeding line	0.02	8.39	7.01	0.04	0.32	15.78
89	FLIP82-150C	K	ICARDA, formerly at Syria	Breeding line	0.02	8.07	6.39	0.01	0.29	14.78
90	FLIP83-7C	K	ICARDA, formerly at Syria	Breeding line	0.03	8.08	4.79	0.02	0.54	13.45
91	FLIP84-92C	K	ICARDA, formerly at Syria	Breeding line	0.02	7.09	6.03	0.02	0.36	13.51
92	FLIP84-48C	K	ICARDA, formerly at Syria	Breeding line	0.02	9.14	7.90	0.02	0.44	17.52
93	FLIP84-188C	K	ICARDA, formerly at Syria	Breeding line	0.02	8.33	7.08	0.07	0.32	15.82
94	FLIP86-5C	K	ICARDA, formerly at Syria	Breeding line	0.02	7.30	5.82	0.06	0.47	13.67
95	FLIP86-6C	K	ICARDA, formerly at Syria	Breeding line	0.05	7.12	4.80	0.06	0.53	12.56
96	FLIP85-1C	K	ICARDA, formerly at Syria	Breeding line	0.01	5.69	2.91	0.01	0.24	8.86
97	FLIP85-17C	K	ICARDA, formerly at Syria	Breeding line	0.03	6.81	4.90	0.02	0.32	12.08
98	FLIP87-45C	K	ICARDA, formerly at Syria	Breeding line	0.04	8.07	6.80	0.05	0.37	15.33
99	FLIP87-8C	K	ICARDA, formerly at Syria	Breeding line	0.00	6.30	3.57	0.02	0.22	10.11
100	FLIP88-85C	K	ICARDA, formerly at Syria	Breeding line	0.01	6.36	3.28	0.01	0.24	9.89
101	FLIP90-96C	K	ICARDA, formerly at Syria	Breeding line	0.02	7.90	5.24	0.05	0.37	13.57
102	FLIP91-77C	K	ICARDA, formerly at Syria	Breeding line	0.02	6.85	5.36	0.00	0.27	12.50
103	FLIP93-93	K	ICARDA, formerly at Syria	Breeding line	0.02	7.34	3.99	0.00	0.29	11.64
104	FLIP93-146C	K	ICARDA, formerly at Syria	Breeding line	0.01	7.29	6.06	0.03	0.27	13.67
105	FLIP93-58C	K	ICARDA, formerly at Syria	Breeding line	0.01	6.85	5.74	0.02	0.34	12.96
106	FLIP97-137C	K	ICARDA, formerly at Syria	Breeding line	0.04	8.84	7.31	0.06	0.45	16.69
107	FLIP97-263C	K	ICARDA, formerly at Syria	Breeding line	0.02	8.17	5.27	0.07	0.42	13.97
108	FLIP97-281C	K	ICARDA, formerly at Syria	Breeding line	0.04	8.28	5.41	0.02	0.50	14.26
109	FLIP97-503C	K	ICARDA, formerly at Syria	Breeding line	0.02	7.75	7.43	0.08	0.78	16.06
110	FLIP97-530C	K	ICARDA, formerly at Syria	Breeding line	0.01	6.00	4.37	0.02	0.27	10.67
111	FLIP97-677C	K	ICARDA, formerly at Syria	Breeding line	0.00	7.59	5.84	0.04	0.24	13.71
112	FLIP97-706C	K	ICARDA, formerly at Syria	Breeding line	0.00	7.41	6.15	0.02	0.28	13.86
113	FLIP98-121C	K	ICARDA, formerly at Syria	Breeding line	0.00	7.75	5.30	0.04	0.26	13.35
114	ILC588(RIL_Parent)	K	ICARDA, formerly at Syria	Landrace	0.03	7.90	5.94	0.06	0.36	14.28
115	ILC_72	K	ICARDA, formerly at Syria	Landrace	0.03	8.79	5.42	0.05	0.30	14.59

Table 3 (*Continued*)

Entry	Genotype	Type[b]	Origin	Status	Mean carotenoid concentration ($\mu g\,g^{-1}$)					
					Violaxanthin	Lutein	Zeaxanthin	β-Cryptoxanthin	β-Carotene	Total carotenoids[a]
116	ILC_195	K	ICARDA, formerly at Syria	Breeding line	0.03	7.75	5.84	0.04	0.31	13.96
117	ILC_484	K	ICARDA, formerly at Syria	Landrace	0.03	8.29	6.13	0.03	0.31	14.79
118	ILC_2555	K	ICARDA, formerly at Syria	Landrace	0.03	10.12	7.04	0.03	0.32	17.54
119	ILC_3279(RII_Parent)	K	Former USSR	Landrace	0.02	7.80	7.36	0.04	0.27	15.48
120	Elixir	K	ICARDA, formerly at Syria	Cultivar	0.04	9.29	7.46	0.07	0.40	17.26
121	ILC482	K	ICARDA, formerly at Syria	Breeding line	0.03	6.54	5.36	0.04	0.20	12.17
	SD[c]				0.15	2.37	2.02	0.03	0.31	4.34
	SE[d]				0.01	0.21	0.18	0.00	0.02	0.42

[a] Total carotenoids was calculated as the sum of five individual carotenoids.
[b] Market class type, D = Desi, K = Kabuli.
[c] SD, standard deviation.
[d] SE, standard error ($N = 242$).

Table 4
Mean and range of carotenoid concentration ($\mu g\,g^{-1}$) among 121 accessions of desi and kabuli chickpea evaluated in 2011 at Elrose, Saskatchewan, Canada.

Trait	Carotenoid concentration ($\mu g\,g^{-1}$) ± SD[a]		
	Entire collection	Desi[†]	Kabuli[†]
Mean			
Violaxanthin	0.1 ± 0.15	0.1 ± 0.20[a]	0.0 ± 0.13[b]
Lutein	8.2 ± 2.37	10.1 ± 3.02[a]	7.5 ± 2.31[b]
Zeaxanthin	6.2 ± 2.02	7.9 ± 2.05[a]	5.5 ± 1.87[b]
β-Cryptoxanthin	0.1 ± 0.03	0.1 ± 0.03[a]	0.0 ± 0.03[b]
β-Carotene	0.5 ± 0.31	0.7 ± 0.39[a]	0.4 ± 0.31[b]
Total carotenoid[b]	15.1 ± 4.34	18.9 ± 4.78[a]	13.4 ± 4.18[b]
Range			
Violaxanthin	0.0–1.2	0.0–1.2	0.0–0.6
Lutein	5.3–21.5	5.3–21.5	5.4–12.3
Zeaxanthin	2.9–14.8	4.2–14.8	2.9–10.0
β-Cryptoxanthin	0.0–0.2	0.00–0.2	0.0–0.1
β-Carotene	0.1–2.6	0.3–2.6	0.1–1.9
Total carotenoid[b]	9.2–31.3	9.8–31.3	9.2–23.1

[a] Standard deviation.
[b] Total carotenoid concentration was calculated as the sum of five individual carotenoids.
[†] Means followed by different letters differed significantly according to Duncan's Multiple Range Test (DMRT) $P < 0.05$.

concentration in the entire collection of chickpea accessions is summarized in Table 4. Chickpea accessions were highest in lutein (8.2 $\mu g\,g^{-1}$) followed by zeaxanthin (6.2 $\mu g\,g^{-1}$), β-carotene (0.5 $\mu g\,g^{-1}$), β-cryptoxanthin (0.1 $\mu g\,g^{-1}$), and violaxanthin (0.1 $\mu g\,g^{-1}$). Mean zeaxanthin concentration ranged from 2.9 $\mu g\,g^{-1}$ to 14.8 $\mu g\,g^{-1}$, and β-carotene concentration ranged from 0.1 $\mu g\,g^{-1}$ to 2.6 $\mu g\,g^{-1}$ (Table 4). One hundred nineteen chickpea accessions had lutein concentration of 5.1–15 $\mu g\,g^{-1}$, with two accessions having greater than 15 $\mu g\,g^{-1}$ (Fig. 1B). Ninety two accessions had mean zeaxanthin concentrations of 5.1–10 $\mu g\,g^{-1}$ and five had greater than 10 $\mu g\,g^{-1}$ (Fig. 1C). Five chickpea accessions had greater than 1.1 $\mu g\,g^{-1}$ of β-carotene (Fig. 1D). Among the 121 chickpea accessions ICRISAT-121D, CDC Cory, DH45-1, and CDC Jade had the highest total carotenoid concentration (25.1–35 $\mu g\,g^{-1}$).

The desi chickpea accessions had higher mean total carotenoid concentration (18.9 $\mu g\,g^{-1}$) than the kabuli accessions (13.4 $\mu g\,g^{-1}$; Table 4). Mean concentration of the five individual carotenoids was higher in desi chickpea accessions than found in kabuli chickpea accessions (Table 4). Chickpea seeds had a greater average zeaxanthin concentration (6.2 $\mu g\,g^{-1}$) than that found in pea seeds (0.3 $\mu g\,g^{-1}$). A typical chromatogram of the carotenoid profile of desi chickpea accession 551-1 and green pea accession CDC Patrick are presented in Fig. 3A and B, respectively.

3.4. Correlation between carotenoids

Correlation coefficients were calculated for individual carotenoids (Table 5). Positive correlation ($P = <0.0001$) between all four carotenoids and total carotenoids were observed in pea. Similarly in chickpea, positive correlations ($P = <0.0001$) were observed

Table 5
Pearson correlation coefficients among carotenoids in pea ($n = 188$) and chickpea ($n = 242$) accessions.

	Violaxanthin	Lutein	Zeaxanthin	β-Cryptoxanthin[a]	β-Carotene	Total carotenoids
Pea						
Violaxanthin	1.0000	0.6753***	0.8503***	–	0.8704***	0.7762***
Lutein		1.0000	0.6322***	–	0.7257***	0.9866***
Zeaxanthin			1.0000	–	0.7573***	0.7217***
β-Carotene				–	1.0000	0.8214***
Total carotenoids						1.0000
Chickpea						
Violaxanthin	1.000	0.724***	0.109ns	0.153ns	0.751***	0.546***
Lutein		1.000	0.650***	0.518***	0.779***	0.944***
Zeaxanthin			1.000	0.682***	0.404***	0.862***
β-Cryptoxanthin				1.000	0.494***	0.653***
β-Carotene					1.000	0.720***
Total carotenoids						1.000

*** Significance at 0.0001% level; ns, not significant.
[a] β-Cryptoxanthin was not detected in pea accessions.

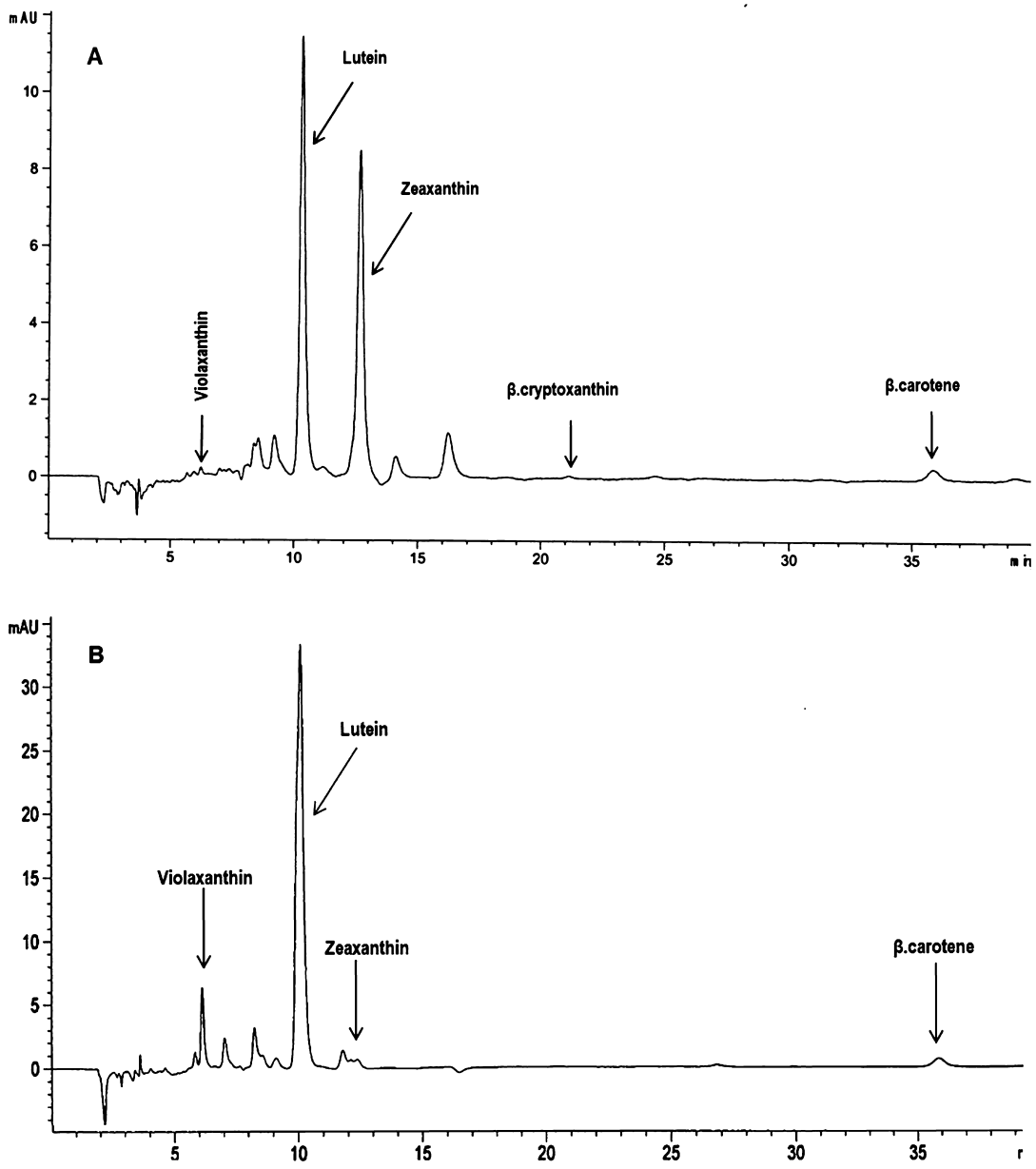

Fig. 3. Typical chromatogram of nutritionally important carotenoids of desi chickpea accession 551-1 (A) and pea accession CDC Patrick (B).

between the five carotenoids and total carotenoids except between violaxanthin and zeaxanthin and violaxanthin and β-cryptoxanthin.

4. Discussion

Genetic resources are crucial for developing new varieties and the greatest challenge is to identify useful genetic variation from existing germplasm collections (Upadhyaya et al., 2007). Carotenoid concentration could be influenced by several factors including seed maturation stage, soil type, storage conditions, climatic conditions, geographic location and particularly, genotype (Setiawan et al., 2001; Souza et al., 2004). In the current research, the most important factor determining the carotenoid concentration in seeds was the accession, since other factors were relatively similar for all pea and chickpea accessions, for example, all accessions were analyzed at the same stage of seed maturation and the seeds were stored under the same conditions.

Previously, only limited reports were available on the variability in the levels of carotenoids in pea and chickpea (Abbo et al., 2005; Marles et al., 2013; Ashokkumar et al., 2014). These studies used only a small number of genotypes. These initial observations suggested that evaluation of the carotenoid profile in a wider genotypic range of pea and chickpea is required to identify material with even greater potential for biofortification.

Of the carotenoids observed in the current research more is known about the health benefits of lutein than the other carotenoids. Lutein was the predominant carotenoid in both pea and chickpea. Similar results were observed in previous studies in pea (McCallum et al., 1997; Holasová et al., 2009; Marles et al., 2013), chickpea (Abbo et al., 2005, 2010), and wheat (Adom et al., 2003; Hidalgo et al., 2006; Ramachandran et al., 2010). Mean lutein concentration of the pea accessions ranged from 5.6 $\mu g\, g^{-1}$ to 24 $\mu g\, g^{-1}$ which is greater than the previously reported of 7 $\mu g\, g^{-1}$ in yellow cotyledon pea, and 8 $\mu g\, g^{-1}$ to 14 $\mu g\, g^{-1}$ in green cotyledon pea (Holasová et al., 2009). Currently, there is no

recommended daily allowance (RDA) for lutein. However, the average consumption of lutein in the United States was estimated at 1.3 mg d^{-1}, and total carotenoids were approximately 6 mg d^{-1} (Chug-Ahuja et al., 1993). The effects of cooking on carotenoid concentration of pea and chickpeas were not assessed in research, however, other studies reported that lutein and the carotenes are stable under the normal cooking conditions, whereas violaxanthin is may be destroyed by heat (Khachik et al., 1992).

The mean β-carotene concentration observed in this research ranged from 0.0 to 2.6 μg g^{-1} and was similar to the range of 1–2 μg g^{-1} of β-carotene previously reported (Holasová et al., 2009). The average total carotenoid concentration of 94 pea accessions was 12.4 μg g^{-1}. Englberger et al. (2003) reported average total carotenoid concentration of 11.1 μg g^{-1} in 21 banana accessions. The range of total carotenoid concentration in pea accessions (5.8–26.9 μg g^{-1}) was greater than the range in diploid potato accessions (1.4–14.3 μg g^{-1}) (Lu et al., 2001).

Green cotyledon pea accessions were 13 fold richer in mean β-carotene concentration (1.3 μg g^{-1}) than yellow cotyledon accessions (0.1 μg g^{-1}). Similarly, Holasová et al., 2009 reported that green cotyledon pea had 10 fold higher β-carotene concentration than yellow and orange cotyledon pea. The highest concentration of β-carotene (2.6 μg g^{-1}) in green cotyledon pea was greater than the β-carotene concentration (1.6 μg g^{-1}) observed in Golden Rice endosperm (Beyer et al., 2002). Green cotyledon pea accessions (10–27 μg g^{-1}) had approximately 1.7 fold greater total carotenoids than found in yellow cotyledon pea accessions (5–17 μg g^{-1}). This could be due to greater expression of the lycopene cyclase gene in green cotyledon accessions (Demmig-Adams and Adams, 2002). Lutein and β-carotene levels are controlled by the transcriptional activity of the lycopene beta-cyclase gene in kiwifruit (*Actinidia chinensis* Planch.; Khattak et al., 2008) and microalga *Dunaliella salina* (Zhu et al., 2008).

Mean lutein concentration of chickpea in this research ranged from 5.3 μg g^{-1} to 21.5 μg g^{-1} was higher than the concentrations (2.8–6.2 μg g^{-1}) previously reported (Abbo et al., 2010). Average β-carotene concentration of the chickpea accessions was 0.5 μg g^{-1} which was equal to that reported previously (Khattak et al., 2008). Desi chickpea accessions had greater total carotenoid concentration than kabuli accessions and this could be due to the rich pigmentation intensity in extracts, as the seed coat of desi chickpea seeds had greater pigmentation than kabuli. Yellow flesh diploid potato clones had 4–22 times more total carotenoids than white fleshed potato cultivars (Lu et al., 2001). Yellow colour is the result of the carotenoid pigments present in wheat seeds and it is a selection criterion for durum wheat breeding worldwide (Troccoli et al., 2000). Pigmentation intensity corresponded with carotenoid accumulation in transgenic *Arabidopsis thaliana* (L.) Heynh seeds and seed specific expression of phytoene synthase increased the concentrations of carotenoids, xanthophylls, and abscisic acid (Lindgren et al., 2003).

In our previous research we observed that desi chickpea seed coat extracts had higher yellow pigment intensity than kabuli and it was positively associated with carotenoid concentration (Ashokkumar et al., 2014). Extraction and analysis of carotenoid concentration in chickpea is time consuming and expensive. In chickpea breeding programmes, it is not easy to analyze the carotenoid concentration of large segregating populations to select genotypes with higher concentration. Hence, the intensity of yellow pigment in seed coats could be used as an inexpensive indirect selection criterion for carotenoid concentration.

Chickpea seeds were richer in zeaxanthin than pea seeds. In the carotenoid biosynthetic pathway, lycopene β-cyclase produces β-carotene which is hydroxylated in a two-step reaction to produce zeaxanthin (Demmig-Adams and Adams, 2002), and accordingly, greater expression of the lycopene β-cyclase gene may increase the levels of carotenoids in chickpea seeds. The average total carotenoids concentration of 121 chickpea accessions (15.0 μg g^{-1}) were 3 fold higher than found in previously reported 42 banana accessions (4.7 μg g^{-1}) and 37 potato accessions (4.4 μg g^{-1}). The range of total carotenoids concentration of chickpea accession (9.2–31.3 μg g^{-1}) was greater than found in banana accessions (1.1–19.2 μg g^{-1}) and potato accessions (0.5–15.5 μg g^{-1}) reported Amorim et al. (2009) and Fernandez-Orozco et al. (2013), respectively.

Individual as well as total carotenoids were generally positively correlated each other in pea and chickpea (Table 5). Positive correlations were previously reported between lutein and chlorophyll concentration in pea ($r = 0.77$, $P < 0.01$) (Holasová et al., 2009) and sweet basil ($r = 0.68$, $P = <0.0001$), between lutein and zeaxanthin concentration in sweet basil ($r = 0.76$, $P = <0.0001$) (Kopsell et al., 2005) and chickpea ($r = 0.66$, $P = <0.05$) (Abbo et al., 2005). High lutein concentration in chickpea seed was recessive to low lutein concentration. High lutein concentration was associated with lower grain weight and it could be due to chromosomal linkage and pleiotropy (Abbo et al., 2005). In this research, desi accessions had generally higher lutein concentration than kabuli accessions (Table 3). Since kabuli accessions generally have greater seed weight than desi accessions, breeding large seeded chickpea with improved lutein concentration may be difficult due to the association of lutein promoting alleles with low grain weight alleles. Breaking the linkage between low grain weight and high lutein concentration may pave the way for breeding large seeded kabuli with improved lutein concentration (Abbo et al., 2005).

5. Conclusions

Functional and nutritional (biofortification) breeding for improved carotenoid profiles, allied with improved yield and disease resistance, should result in better nutritional value in peas and chickpeas. This research revealed new sources of variation for nutritionally important traits like β-carotene, lutein and zeaxanthin that can be exploited to enhance the genetic potential of pea and chickpea. These results indicated that diverse pea and chickpea accessions are rich in carotenoids, greater than previously reported in rice, wheat, potato, banana, and cassava. Consumption of peas and chickpeas with enhanced carotenoid concentration could address the problem of vitamin A deficiencies and age-related macular degeneration.

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References

Abbo, S., Bonfil, D.J., Berkovitch, Z., Reifen, R., 2010. Towards enhancing lutein concentration in chickpea, cultivar and management effects. Plant Breed. 129, 407–411.

Abbo, S., Molina, C., Jungmann, R., Grusak, M.A., Berkovitch, Z., Reifen, R., Kahl, G., Winter, P., Reifen, R., 2005. QTL governing carotenoid concentration and weight in seeds of chickpea (*Cicer arietinum* L.). Theor. Appl. Genet. 111, 185–195.

Abushita, A.A., Daood, H.G., Biacs, P.A., 2000. Changes in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. J. Agric. Food Chem. 48, 2075–2081.

Adom, K.K., Sorrells, M.E., Liu, R.H., 2003. Phytochemical profiles and antioxidant activity of wheat varieties. J. Agric. Food Chem. 51, 7825–7834.

Alves-Rodrigues, A., Shao, A., 2004. The science behind lutein. Toxicol. Lett. 150, 57–83.

Amorim, E.P., Vilarinhos, A.D., Cohen, K.O., Amorim, V.B.O., Santos-Serejo, J.A.D., Silva, S.O., Pestana, K.N., Santos, V.J.D., Paes, N.S., Monte, D.C., Reis, R.V.D., 2009. Genetic diversity of carotenoid-rich bananas evaluated by Diversity Arrays Technology (DArT). Genet. Mol. Biol. 32, 96–103.

Ashokkumar, K., Tar'an, B., Diapari, M., Arganosa, G., Warkentin, T.D., 2014. Effect of cultivar and environment on carotenoid profile of pea and chickpea. Crop Sci. 54, 2225–2235.

Beyer, P., Al-Babili, S., Ye, X., Lucca, P., Schaub, P., Welsch, R., Potrykus, I., 2002. Golden rice: introducing the β-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. J. Nutr. 132, 506S–510S.

Bhosale, P., Ermakov, I.V., Ermakov, M.R., Gellermann, W., Bernstein, P.S., 2004. Resonance Raman quantification of nutritionally important carotenoids in fruits, vegetables, and their juices in comparison to high-pressure liquid chromatography analysis. J. Agric. Food Chem. 52, 3281–3285.

Bouis, H.E., 2002. Plant breeding: a new tool for fighting micronutrient malnutrition. J. Nutr. 132, 491S–494S.

Bramley, P.M., 2000. Is lycopene beneficial to human health? Phytochemistry 54, 233–236.

Chug-Ahuja, J.K., Holden, J.M., Forman, M.R., Mangels, A.R., Beecher, G.R., Lanza, E., 1993. The development and application of a carotenoid database for fruits, vegetables, and selected multicomponent foods. J. Am. Diet. Assoc. 93, 318–323.

Demmig-Adams, B., Adams, W.W., 2002. Antioxidants in photosynthesis and human nutrition. Science 298, 2149–2153.

Duranti, M., 2006. Grain legume proteins and nutraceutical properties. Fitoterapia 77, 67–82.

Englberger, L., Darnton-Hill, I., Coyne, T., Fitzgerald, M.H., Marks, G.C., 2003. Carotenoid-rich bananas: a potential food source for alleviating vitamin A deficiency. Food Nutr. Bull. 24, 303–318.

Fernandez-Orozco, R., Gallardo-Guerrero, L., Hornero-Méndez, D., 2013. Carotenoid profiling in tubers of different potato (Solanum sp) cultivars: accumulation of carotenoids mediated by xanthophyll esterification. Food Chem. 141, 2864–2872.

Fraser, P.D., Bramley, P., Seymour, G.B., 2001. Effect of Cnr mutation on carotenoid formation during tomato fruit ripening. Phytochemistry 58, 75–79.

Fraser, P.D., Bramley, P.M., 2004. The biosynthesis and nutritional uses of carotenoids. Prog. Lipid Res. 43, 228–265.

Gupta, D.S., Dil, T., Knutson, P., Thavarajah, P., McGee, R.J., Coyne, C.J., Kumar, S., 2013. Lentils (*Lens culinaris* L.), a rich source of folates. J. Agric. Food Chem. 61, 7794–7799.

Haynes, K.G., Clevidence, B.A., Rao, D.D., Vinyard, B.T., White, J.M., 2010. Genotype × environment interactions for potato tuber carotenoid content. J. Am. Soc. Hortic. Sci. 135, 250–258.

Hefni, M., Öhrvik, V., Tabekha, M., Witthöft, C., 2010. Folate content in foods commonly consumed in Egypt. Food Chem. 121, 540–545.

Hentschel, V., Kranl, K., Hollmann, J., Lindhauer, M.G., Bohm, V., Bitsch, R., 2002. Spectrophotometric determination of yellow pigment content and evaluation of carotenoids by high-performance liquid chromatography in durum wheat grain. J. Agric. Food Chem. 50, 6663–6668.

Hidalgo, A., Brandolini, A., 2008. Kinetics of carotenoids degradation during the storage of einkorn (*Triticum monococcum* L. ssp. *monococcum*) and bread wheat (*Triticum aestivum* L. ssp. *aestivum*) flours. J. Agric. Food Chem. 56, 11300–11305.

Hidalgo, A., Brandolini, A., Pompei, C., Piscozzi, R., 2006. Carotenoids and tocols of einkorn wheat (*Triticum monococcum* ssp. Monococcum L.). J. Cereal Sci. 44, 182–193.

Holasová, M., Dostálova, R., Fieldlerová, V., Horáček, J., 2009. Variability of lutein content in peas (*Pisum sativum* L.) in relation to the variety, season and chlorophyll content. Czech J. Food Sci. 27, S188–S191.

Iannone, A., Rota, C., Bergamini, S., Tomasi, A., Canfield, L.M., 1998. Antioxidant activity of carotenoids: an electron-spin resonance study on beta-carotene and lutein interaction with free radicals generated in a chemical system. J. Biochem. Mol. Toxicol. 12, 299–304.

Khachik, F., Goli, M.B., Beecher, G.R., Holden, J., Lusby, W.R., Tenorio, M.D., Barrera, M.R., 1992. Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. J. Agric. Food Chem. 40, 390–398.

Khattak, A.B., Zeb, A., Bibi, N., 2008. Impact of germination time and type of illumination on carotenoid content, protein solubility and in vitro protein digestibility of chickpea (*Cicer arietinum* L.) sprouts. Food Chem. 109, 797–801.

Kopsell, D.A., Kopsell, D.E., Celentano, J.C., 2005. Carotenoid and chlorophyll pigments in sweet basil grown in the field and green house. Hortscience 40, 1230–1233.

Krinsky, N.I., Russett, M.D., Handelman, G.J., Snodderly, D.M., 1990. Structural and geometric isomers of carotenoids in human plasma. J. Nutr. 120, 1654–1662.

Lindgren, O.L., Stålberg, K.G., Höglund, A.S., 2003. Seed specific overexpression of an endogenous arabidopsis phytoene synthase gene results in delayed germination and increased levels of carotenoids, chlorophyll, and abscisic acid. Plant Physiol. 132, 779–785.

Lu, W., Haynes, K., Wiley, E., Clevidence, B., 2001. Carotenoid content and colour in diploid potatoes. J. Am. Soc. Hortic. Sci. 126, 722–726.

Marles, M.A.S., Warkentin, T.D., Bett, K.E., 2013. Genetic abundance of carotenoids and polyphenolics in the hull of field pea (*Pisum sativum* L.). J. Sci. Food Agric. 93, 463–470.

McCallum, J., Timmerman-Vaughan, G., Frew, T., Russel, A., 1997. Biochemical and genetic linkage analysis of green seed colour in field pea. J. Am. Soc. Hortic. Sci. 122, 218–225.

Menkir, A., Dixon, M.B., 2004. Influence of genotype and environment on β. Carotene content in tropical yellow-endosperm maize genotypes. Maydica 49, 313–318.

Meydani, M., Martin, A., Ribaya Mercado, J.D., Gong, J., Blumberrg, J.B., Russel, R.M., 1994. Beta-carotene supplementation increases antioxidant capacity of plasma in older women. J. Nutr. 124, 2397–2403.

Moeller, S.M., Jacques, P.F., Blumberg, J.B., 2000. The potential role of dietary xanthophylls in cataract and age-related macular degeneration. J. Am. Coll. Nutr. 19, 522S–527S.

Olmedilla, B., Granado, F., Blanco, I., Vaquero, M., Cajigal, C., 2001. Lutein in patients with cataracts and age-related macular degeneration: a long-term supplementation study. J. Sci. Food Agric. 81, 904–909.

Olson, J.A., 1989. Biological actions of carotenoids. J. Nutr. 119, 94–95.

Panasenko, O.M., Sharov, V.S., Briviba, K., Sies, H., 2000. Interaction of peroxynitrite with carotenoids in human low density lipoproteins. Arch. Biochem. Biophys. 373, 302–305.

Ramachandran, A., Pozniak, C.J., Clarke, J.M., Singh, A.K., 2010. Carotenoid accumulation during grain development in durum wheat. J. Cereal Sci. 52, 30–38.

Rychlik, M., Englert, K., Kapfer, S., Kirchhoff, E., 2007. Folate contents of legumes determined by optimized enzyme treatment and stable isotope dilution assays. J. Food Compos. Anal. 20, 411–419.

SAS Institute Inc., 2011. SAS/STAT® 9.3 User's Guide, 2nd ed. Cary, NC.

Setiawan, B., Sulaeman, A., Giraud, D.W., Driskell, J.A., 2001. Carotenoid content of selected Indonesian fruits. J. Food Compos. Anal. 14, 169–176.

Snodderly, D.M., 1995. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. Am. J. Clin. Nutr. 62, 1448S–1461S.

Souza, S.L., Moreira, A.P.B., Santana, H.M., Alencar, E.R., 2004. Carotenes and provitamin A contents of fruits commercialized in Viçosa, MG. Acta Sci. Agron. 26, 453–459.

Ssemakula, G., Dixon, A.G.O., Maziya-Dixon, B., 2007. Stability of total carotenoid concentration and fresh yield of selected yellow-fleshed cassava (*Manihot esculenta* Crantz). J. Trop. Agric. 45, 14–20.

Stahl, W., Ale-Agha, N., Polidori, M.C., 2002. Non-antioxidant properties of carotenoids. Biol. Chem. 383, 553–558.

Stephens, D., Jackson, P.L., Gutierrez, Y., 1996. Subclinical vitamin A deficiency: a potentially unrecognized problem in the United States. Pediatr. Nurs. 22, 377–389.

Story, E.N., Kopec, R.E., Schwartz, S.J., Harris, G.K., 2010. An update on the health effects of tomato lycopene. Annu. Rev. Food Sci. Technol. 1, 189–210.

Sujak, A., Gabrielska, J., Grudzinski, W., Borc, R., Mazurek, P., Gruszecki, W.I., 1999. Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects. Arch. Biochem. Biophys. 371, 301–307.

Tanaka, T., Shnimizu, M., Moriwaki, H., 2012. Cancer chemoprevention by carotenoids. Molecules 17, 3202–3242.

Thavarajah, D., Thavarajah, P., 2012. Evaluation of chickpea (*Cicer arietinum* L.) micronutrient composition: biofortification opportunities to combat global micronutrient malnutrition. Food Res. Int. 49, 99–104.

Troccoli, A., Borrelli, G.M., De Vita, P., Fares, C., Di Fonzo, N., 2000. Durum wheat quality: a multidisciplinary concept. J. Cereal Sci. 32, 99–113.

Upadhyaya, H.D., Dwivedi, S.L., Gowda, C.L.L., Singh, S., 2007. Identification of diverse germplasm lines for agronomic traits in a chickpea (*Cicer arietinum* L.) core collection for use in crop improvement. Field Crops Res. 100, 320–326.

Welch, R.M., Graham, R.D., 2005. Agriculture: the real nexus for enhancing bioavailable micronutrients in food crops. J. Trace Elem. Med. Biol. 18, 299–307.

White, P.J., Broadley, M.R., 2005. Biofortifying crops with essential mineral elements. Trends Plant Sci. 10, 586–593.

Williams, J.A., Kondo, N., Okabe, T., Takeshita, N., Pilchak, D.M., Koyama, E., Ochiai, T., Jensen, D., Chu, M.-L., Kane, M.A., Napoli, J.L., Enomoto-Iwamoto, M., Ghyselinck, N., Chambon, P., Pacifici, M., Iwamoto, M., 2009. Retinoic acid receptors are required for skeletal growth, matrix homeostasis and growth plate function in postnatal mouse. Dev. Biol. 328, 315–327.

Yamaguchi, M., 2012. Role of carotenoid b-cryptoxanthin in bone homeostasis. J. Biomed. Sci. 19, 1–13.

Yamaguchi, M., Uchiyama, S., 2003. Effect of carotenoid on calcium content and alkaline phosphatase activity in rat femoral tissues in vitro: the unique anabolic effect of β-cryptoxanthin. Biol. Pharm. Bull. 26, 1188–1191.

Yamaguchi, M., Uchiyama, S., 2004. β-Cryptoxanthin stimulates bone formation and inhibits bone resorption in tissue culture in vitro. Mol. Cell. Biochem. 258, 137–144.

Zhu, Y.H., Jiang, J.G., Chen, Q., 2008. Characterization of cDNA of lycopene β-cyclase responsible for a high level of β-Carotene accumulation in *Dunaliella salina*. Biochem. Cell Biol. 86, 285–292.

Ziegler, R.G., 1989. A review of epidemiologic evidence that carotenoids reduce the risk of cancer. J. Nutr. 119, 116–122.