GENETIC VARIABILITY OF TOTAL CAROTENOID CONTENT AND INDIVIDUAL COMPONENTS IN A TETRAPLOID WHEAT COLLECTION

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Introduction

Carotenoids are among the most important natural pigments for their wide distribution, different structure and several functions. The presence of these pigments generates the yellow colour, most important components of food quality. The nutritional importance of carotenoids comes principally from the provitamin A activity of β-carotene, β-cryptoxanthin, and others with at least one intact non-oxygenated β-ionone ring. In addition, carotenoids are antioxidants and have been recently involved in the prevention or protection against serious human disorders (Panfili *et al.*, 2004; Hildago *et al.*, 2006). The colour of semolina is also an important quality criterion with regard to pasta production (Hentschel *et al.*, 2002; Guarda *et al.*, 2004). Several analytical techniques have been developed to evaluate the carotenoids: chemical pigment extraction, light reflectance measurements and high-performance liquid chromatography (HPLC) (Fratianni *et al.*, 2005, Konopka *et al.*, 2006). Actually, there is a substantial interest into improve the content and composition of carotenoids in crops plants, including wheat, in order to advance the technological, commercial and nutritional values for human consumption. The objectives of the this study were: 1) phenotypic evaluation and 2) study of genetic variability of carotenoid content and individual components in a collection of cultivated and wild wheats.

Material and methods

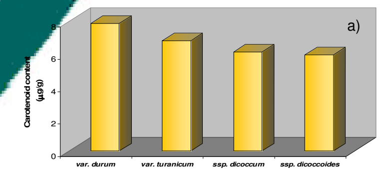
A collection of tetraploid wheat, consisting of durum wheat cultivars, *Triticum turgidum* ssp. *turgidum* var. *turanicum*, ssp. *dicoccoides* and spp. *dicoccum* accessions, was evaluated for carotenoid pigments content and yellow index. Field experiments were carried out at Valenzano (Bari, Italy) by a randomized complete block design with three replications for two years (2006 and 2007). Grain samples were milled and whole-meal flour obtained was used to evaluate carotenoid content and individual components using spectrophotometric technique, colorimetric system and HPLC analysis. The extraction of yellow pigments was carried out by incubation in water-saturated n-butyl alcohol and the absorbance of n-butyl alcohol extracts was measured at 435,8 nm (AACC 14-50). Brown and yellow indexes were estimated using the reflectance colorimetric Chroma Meter CR-300 (MINOLTA). The collection grown in 2006 season was subjected to HPLC analysis for the determination of main components of carotenoids. Each sample was extracted with hexane/acetone (80:20 v/v) and butyled hydroxytoluene (BHT) (0,1% w/v) as anti-oxidant were added. Afterwards, it was stored in the dark for 16h and then extracted another one. The organic layers were collected and evaporated to dryness under nitrogen. Next, the dry residues were dissolved in methanol: dichloromethane (45:55 v/v) and a sample volume of 20 µl was injected to the HPLC (Agilent 1100). Separation was done on a YMC C30 column (250 x 4.6 mm; 5 µm particle size). The mobile phase was MeOH:MTBE (89:11 v/v) at a constant flow of 1 ml / min. The statistical analysis was carried out using MSTAT-C software.

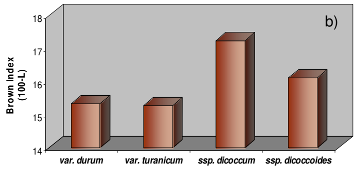
Results and discussion

The carotenoid content, brown and yellow indexes of the *Triticum turgidum* ssp. collection are reported in **Table 1**. The carotenoid pigments content ranged from 3.84 µg/g to 10.64 µg/g with an average of 6.77 µg/g. The var. *durum* cultivars showed the highest mean value (7.20 µg/g). The ssp. *dicoccum* and ssp. *turgidum* var. *turanicum* accessions exhibited similar carotenoid pigments content: 5.83 µg/g and 5.6 µg/g respectively. Ultimately, the ssp. *dicoccoides* accessions showed a range of variation rather small (range: 4.35 - 6.65 µg/g), with the exception for the accession MG 5510 (data not showed). In the collection, the yellow index exhibited a wide range of variation (12.62 – 19.13). Among the *Triticum* ssp., the var. *durum* cultivars showed the highest mean value, while the var. *turanicum* accessions presented a yellow index similar to var. *durum* cultivars, although slightly lower. Finally, the ssp. *dicoccoides* and *dicoccum* accessions presented the lowest values. These results reflected the carotenoid pigments content obtained by AACC method. As showed in **Table 1**, the performance of the brown index seems to be opposite to that of the yellow: the highest and the lowest mean values were detected in ssp. *dicoccum* accessions (19.05) and var. *durum* cultivars (16.11).

Table 1. Mean values, range of variation, least significant difference ($LSD_{0.05}$), coefficient of variation (C.V.) and heritability (h^2_B) of carotenoid pigments content (AACC n° 14-50 method) and of colorimetric indexes (brown index and yellow index) in a *T. turgidum* ssp. collection grown at Valenzano (BA) during 2006.

Species	Accessions (n°)	Carotenoid content (µg/g)	Brown Index (100-L)	Yellow Index (b)
ssp. *turgidum* var. *durum*	52	**7.20** (3.84-10.64)	**16.11** (14.40-17.69)	**16.39** (12.96-19.13)
ssp. *turgidum* var. *turanicum*	16	**5.83** (4.75-8.52)	**16.14** (14.89-16.89)	**15.47** (14.15-18.74)
ssp. *dicoccum*	8	**5.57** (5.51-5.63)	**19.05** (18.53-19.07)	**14.10** (14.00-14.27)
ssp. *dicoccoides*	11	**5.41** (4.35-6.65)	**17.47** (16.24-18.89)	**14.39** (12.62-16.00)
Mean		**6.77**	**16.27**	**16.02**
Range		(3.84-10.64)	(14.40-19.57)	(12.62-19.13)
C.V. (%)		5.66	4.13	4.12
LSD (0.05)		0.62	1.08	1.07
h^2_B		0.94	0.59	0.81





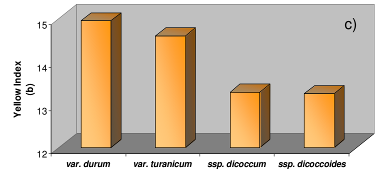
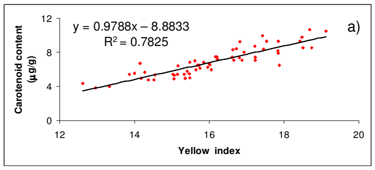
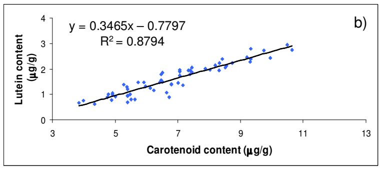


Fig. 1 Carotenoid content (AACC 14-50) (a), brown index (b) and yellow index (c) of the collection grown to Valenzano (BA) in 2007. Results were the mean of each accession.

In 2007, the carotenoid pigments content, the yellow and brown indexes confirmed the results obtained in 2006 season. The highest mean value of carotenoid content and yellow index was observed for var. *durum* cultivars (7.85 µg/g and 14.95, respectively), followed by var. *turanicum* accessions (6.80 µg/g and 14.80) (**Fig. 1a** and **1c**).

The results showed a performance of brown index opposite to yellow index: the ssp. *dicoccum* accessions exhibited the highest mean value (17.24), while the lowest was observed in var. *durum* cultivars (15.33) (**Fig. 1b**). The heritability (h^2_B) of carotenoid pigment content and yellow index was very high: 0.94 and 0.81 in 2006; 0.91 and 0.94 in 2007. The high heritability and the similar values in two years suggest that the carotenoid pigments content has high stability and little influence by environmental conditions. The correlation analysis of trial carried out at Valenzano during 2006 showed a strong positive correlation between the carotenoid pigments content and yellow index. These results confirmed the data reported by Fratianni *et al.* (2005) (**Fig. 2a**). Similar results were observed in 2007(data not showed).





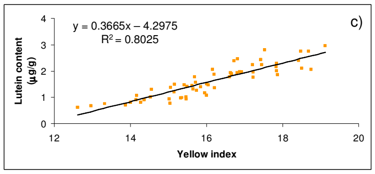


Fig. 2 Relationship between: a) carotenoid content (AACC n° 14-50) and yellow index; b) lutein concentration (HPLC) and carotenoid content (AACC n° 14-50); c) yellow index and lutein concentration (HPLC).

HPLC analysis identified five main components of carotenoids: lutein (main component of carotenoids in wheat), zeaxanthin, β-cryptoxanthin (minor component), α-carotene and β-carotene. The collection showed a wide range of variation for all components identified (**Table 2**). The highest mean value of carotenoid pigments content was found in the cultivars var. *durum* (270.3 µg/100g), while the lowest in the accessions of ssp. *dicoccum* (139.2 µg/100g). The accessions of ssp. *turgidum* var. *turanicum* exhibited a yellow pigments content and its components similar to var. *durum*. The ssp. *dicoccoides* and *dicoccum* accessions, spite have also presented as the main component lutein, showed proportionately more zeaxanthin content; in addition, β-cryptoxanthin was present only in traces. A predominant genetic effect ($h^2_B \geq 0.70$) was estimated for lutein, zeaxanthin, β-carotene and carotenoid pigments content. These results confirmed the high stability of trait. The β-cryptoxanthin and α-carotene exhibited an heritability less than 0.50 (**Table 2**). The correlations analysis indicated a highly significant correlation (P ≤0001) among all the components analysed by HPLC and carotenoid pigments content estimated by the method official AACC n° 14-50. The higher correlations were observed with the lutein (r = 0.54) and carotenoid pigment content (r = 0.2), as reported by Fratianni *et al.* (2005). The **fig. 2b** and **2c** show the relationship between lutein concentration and carotenoid pigments content and yellow index of the *Triticum turgidum* ssp. collection grown in 2006.

Table 2. Mean values, range of variation, least significant difference ($LSD_{0,05}$), coefficient of variation (C.V.) and heritability (h^2_B) of carotenoid pigments content and its components in a *T. turgidum* ssp. collection grown at Valenzano (BA) during 2006.

Species	Accessions (n°)	Lutein (µg/100g)	Zeaxanthin (µg/100g)	β-criptoxanthin (µg/100g)	α-carotene (µg/100g)	β-carotene (µg/100g)	Other components[a] (µg/100g)	Carotenoid content (µg/100g)
ssp. *turgidum* var. *durum*	52	**175.6** (66.9-294.8)	**11.3** (7.5-17.2)	**0.58** (0.28-1.22)	**1.19** (0.43-3.12)	**1.38** (0.61-3.01)	**70.6** (16.8-127.4)	**260.3** (114.2-439.0)
ssp. *turgidum* var. *turanicum*	16	**128.1** (77.2-239.5)	**13.3** (10.7-18.6)	**0.53** (0.29-0.92)	**1.32** (0.38-2.95)	**1.16** (0.50-1.86)	**66.6** (39.7-193.8)	**210.5** (137.6-420.6)
ssp. *dicoccum*	5	**79.2** (61.5-87.7)	**15.3** (11.9-16.5)	traces	**0.50** (0.20-0.74)	**1.14** (0.78-1.43)	**43.2** (35.2-55.4)	**139.2** (114.1-154.6)
ssp. *dicoccoides*	8	**99.4** (62.6-148.6)	**13.7** (8.1-21.9)	traces	**1.04** (0.76-1.34)	**1.29** (0.78-1.61)	**55.1** (34.1-77.3)	**170.2** (115.3-236.6)
Mean		**152.7**	**12.18**	**0.56**	**1.16**	**1.32**	**66.6**	**234.1**
Range		(61.5-294.8)	(7.5-21.9)	(0.28-1.22)	(0.20-3.12)	(0.50-3.01)	(16.8-193.8)	(114.1-439.0)
C.V. (%)		11.07	11.00	87.83	42.60	35.82	15.59	8.68
$LSD_{0.05}$		33.65	2.67	0.73	0.99	0.94	20.65	40.44
h^2_B		0.89	0.70	0.23	0.44	0.33	0.78	0.91

a = Obtained as difference between total carotenoids and the sum of the components identified.

Conclusions

The evaluation of the carotenoid pigments content carried out on the collection of tetraploid wheat allowed to highlight a good performance for cultivars latest, sign of a renewed interest in this issue. This may be due to a selection of genotypes with a high pigment concentration by the breeders The heritability value was more than 90% in all experiments, confirming the strong genotypic component of carotenoid content. The results obtained in this study, showed the correspondence between the reflectance measurement and carotenoid pigments content and established that carotenoids, and lutein in particular, are the most important pigments responsible for the yellow colour of durum wheat. HPLC technique, although expensive, resulted in a sensitive, selective and reliable method for the determination of the qualitative and quantitative distribution of carotenoid compounds in cereals.

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