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RESEARCH ARTICLE

Genetic variation of carotenoids in Chinese bread wheat cultivars and the effect of the 1BL.1RS translocation

Wenshuang LI[1], Shengnan ZHAI[1], Hui JIN[1], Weie WEN[1,2], Jindong LIU[1], Xianchun XIA[1], Zhonghu HE (✉)[1,3]

1 Institute of Crop Science/National Wheat Improvement Center, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100081, China
2 College of Agronomy, Xinjiang Agricultural University, Urumqi 830052, China
3 International Maize and Wheat Improvement Center (CIMMYT) China Office, c/o CAAS, Beijing 100081, China

Abstract Carotenoid content of wheat is an important criterion for prediction of the commercial and nutritional value of products made from bread wheat (*Triticum aestivum*) cultivars. The objective of this study was to determine the major components of carotenoids in Chinese wheat using ultra performance liquid chromatography (UPLC) including lutein, zeaxanthin, α-carotene and β-carotene. Grain carotenoid content was investigated in 217 cultivars from three major Chinese wheat regions and from seven other countries grown in two environments. Genotype contributed to the majority of variation in carotenoid components. Lutein, zeaxanthin and β-carotene concentrations varied from 18.3 to 100.1, 4.9 to 12.0 and 0.9 to 48.7 μg per 100 g in wheat flour with an average of 40.2, 7.2 and 18.2 μg per 100 g, respectively. Lutein (61.3%) was the main carotenoid component, followed by β-carotene (27.7%) and zeaxanthin (11.0%). No α-carotene was detected. Total carotenoids, lutein, zeaxanthin and β-carotene were all higher in cultivars with the 1BL.1RS translocation compared to those without the translocation. This is the first report on assay of lutein, zeaxanthin and β-carotene concentrations for a large number of wheat cultivars. These data will be useful for genetic improvement of wheat carotenoid content and for understanding of the carotenoid biosynthetic pathway in wheat.

Keywords *Triticum aestivum*, carotenoids, nutritional quality, UPLC

1 Introduction

Bread wheat (*Triticum aestivum*) is one of the most important crops in the world with China being the largest wheat producer and consumer, with about 24 million hm^2 sown and about 110 Mt harvested. Given its importance in human diets, it would be of great benefit to increase carotenoid concentrations of wheat grain.

Carotenoids are a diverse group of lipid-soluble organic pigments synthesized in fruits, vegetables and cereal grains that impart yellow/orange color to agricultural products[1]. More than 700 carotenoids have been described and divided into two classes, carotenes and xanthophylls, which are hydrocarbons and oxygenated derivatives, respectively[2]. Carotenoids perform important functions in plants, such as harvesting light for photosynthesis and protecting chlorophyll against oxidative damage[3]. Animals and humans are unable t1o synthesize carotenoids and must obtain them from their diet[4]. Carotenoids have several functions in improving human health and nutrition. Epidemiological studies have indicated associations between carotenoid-rich diets and reduced risk of chronic diseases such as cancer[5], cardiovascular disease[6] and amyotrophic lateral sclerosis[7]. Carotenoids, mainly lutein and zeaxanthin, are also crucial for prevention of frequently-occurring eye diseases, such as age-related macular degeneration, cataracts, and retinitis pigmentosa[8]. β-carotene is vitamin A precursor, which can reduce childhood mortality or growth retardation. In addition to their nutritional and health values, carotenoids are important components of yellow pigment content (YPC) in bread wheat grain and flour. YPC influences the color of the end products, which is an important quality criterion for the noodle and steamed bread industry. Several studies of carotenoids content in wheat or wheat relatives have been reported[1,9,10]. Adom et al.[11] found that lutein was the most abundant carotenoid in 11 wheat cultivars. Carotenoids had a wide range of concentrations and differed significantly between wheat cultivars. Okarter et al.[12] concluded that lutein and zeaxanthin concentrations were higher in eight diverse wheat cultivars than

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Correspondence: zhhecaas@163.com

reported for previous studies. Einkorn wheat was reported to exhibit the highest concentration of lutein, followed by durum, while bread wheat had the lowest concentration[1]. Studies of carotenoid content have mainly included durum and einkorn wheat[13], with much less data being reported for bread wheat cultivars. Knowledge of carotenoid content of wheat is important for the food industry and therefore breeding programs to develop new cultivars and products with enhanced nutritional and quality properties[14].

The 1BL.1RS translocation is used in wheat breeding and is present in about 45% of Chinese wheat cultivars and advanced lines[15]. Studies of the effects of the 1BL.1RS translocation in bread wheat have concentrated on resistance to rust and powdery mildew, yield potential, and a detrimental effect on gluten strength, bread and noodle quality[16,17]. The effects of the 1BL.1RS translocation on flour color and YPC in bread wheat were reported in an earlier study[18]. However, the effects of the 1BL.1RS translocation on carotenoids components contents in wheat have not been assessed.

An ultra-performance liquid chromatography (UPLC) system offers higher sensitivity, faster analysis and better resolution than high performance liquid chromatography (HPLC), and was first developed by the Waters Corporation. Separation in UPLC is performed under very high pressure, and separation efficiency is significantly improved over HPLC[19]. Thus, the objective of the present study was to determine the variation in carotenoid content (including composition and concentrations) among 217 bread wheat cultivars using the UPLC system.

2 Materials and methods

2.1 Materials

2.1.1 Plant material

Grain samples from three trials grown during the 2012–2013 cropping season, including 217 cultivars, were used to determine the contents and components of carotenoids (Appendix A, Tables S1–S3). In Trial 1, 39 wheat cultivars, including 36 from the northern winter wheat region (Region 1) of China and three from the U.S.A, were planted in Beijing and Shijiazhuang, Hebei. In Trial 2, 119 Chinese cultivars from the Yellow and Huai River valleys facultative wheat region (Region 2) and 19 cultivars from five different countries were grown in Anyang, Henan, and Suixi, Anhui. In Trial 3, 40 Chinese cultivars from the middle and lower Yangtze River autumn-sown spring wheat region (Region 3) were grown at Suixi, Anhui, and Chengdu, Sichuan. All genotypes were planted in randomized complete blocks with two replicates at each location, and each accession was planted in a single 1.5 m row of 50 seeds with 25 cm between rows. All harvested samples were sound and showed no evidence of preharvest sprouting.

The 1BL.1RS translocation had a significant effect on yellow pigment content in a previous study; therefore the presence of the 1BL.1RS translocation was determined using molecular markers[18]. There were 9, 65 and 9 cultivars with the 1BL.1RS translocation in Region 1, Region 2, and Region 3 groups, respectively.

2.1.2 Chemicals used

Acetone and n-hexane were of analytical grade purchased from Beijing Chemical Factory. Butylated hydroxytoluene (BHT) and standards for lutein (90% purity), zeaxanthin (95% purity) and β-carotene (95% purity) were purchased from Sigma-Aldrich Co. LLC (St. Louis, Missouri, USA). α-Carotene standard was purchased from CaroteNature GmbH (Ostermundigen, Switzerland). Solvents used for UPLC, methanol, acetonitrile, ethyl acetate, dichloromethane, and triethylamine, were purchased from Sigma-Aldrich, and all solvents used were HPLC grade.

2.2 Methods

2.2.1 Sample preparation for carotenoids determination

After harvest, hard, medium hard and soft wheat samples were tempered to around 16.5%, 15.5% and 14.5% moisture contents, respectively, for 16 h and milled into flour using a Brabender Quandrumat Junior mill (Brabender GmbH & Co. KG, Duisburg, Germany). The milled samples were passed through a 60-mesh sieve using a rotary box for 5 min, and then reduced into flour by a gradual reduction system. To minimize the degradation of pigments by oxidative enzymes and light, flour samples were stored at 18°C in a room with dim light before analysis.

Carotenoids from the flour were extracted following the method of Ramachandran et al.[20], with the following modifications. Accurately-measured 8.0 g flour samples were transferred to 100 mL bottles with glass stoppers and extracted with 40 mL of solvent (n-hexane/acetone, 8:2, v/v, 0.1% BHT w/v). Samples were mixed on a vortex mixer for 1–2 min and shaken at 300 $r \cdot min^{-1}$ on a constant temperature gyratory shaker for 1 h at 35°C. Mixtures were moved to a 50 mL centrifuge tube and centrifuged at 13 500 $r \cdot min^{-1}$ for 15 min at 4°C. Supernatants were retained, transferred to 100 mL beakers and evaporated to dryness at 35°C under a nitrogen stream using a nitrogen evaporator system. Dry residues containing carotenoids were re-dissolved in 1.5 mL of methanol/ethyl acetate (68:32, v/v) with 0.1% BHT. The organic layers were transferred to a 2 mL micro-centrifuge tubes at –20°C before analysis. They were centrifuged at 12 000 $r \cdot min^{-1}$ for 20 min at 4°C. Supernatants were filtered through 0.22 μm polyvinylidene fluoride syringe filters into 2 mL

brown micro-centrifuge tubes and 200 μL samples were transferred into glass vials with screw caps for automatic sampling, using 8 μL for UPLC analysis. All procedures were carried out under dim light, and extracts were placed in brown tubes to avoid sample degradation by photo-oxidation.

2.2.2 Standards preparation

Pure standards of lutein, zeaxanthin, α-carotene, and β-carotene were first dissolved in dichloromethane and then diluted in methanol, to obtain 0.1 $mg \cdot mL^{-1}$ of lutein, zeaxanthin, α-carotene, and 0.05 $mg \cdot mL^{-1}$ of β-carotene standard solutions, then stored in darkness at –20°C. Each standard solution was diluted in methanol/ethyl acetate (68∶32, v/v) and injected alone, for detection of their retention times at 450 nm. Then, five-point calibration curves were built from a mix of all standard solutions.

2.2.3 UPLC analysis

UPLC was run on a Waters Acquity system (Waters Corporation, Milford, MA, USA), equipped with a photodiode array detector at 450 nm. Millennium 32 software from Waters was used for instrument control, data acquisition and data processing. A YMC Carotenoid Reverse Phase Column (4.6 mm × 100 mm i.d., 3 μm particle size) was used with gradients of acetonitrile/methanol/water/triethylamine (81∶14∶5∶0.05, v/v/v/v) (A) and methanol/ethyl acetate/triethylamine (68∶32∶0.05, v/v/v) (B). The mobile phase was first degassed by sonication for 20 min. The gradient was programmed as follows: (1) initial conditions of 100% solution A; (2) a 0.3 min linear gradient with 100% solution A; (3) a 11.7 min linear gradient with solution A from 100% to 0% and solution B from 0% to 100%; (4) a 8 min linear gradient with 100% solvent B; (5) a 2 min linear gradient with solution A from 0% to 100% and solution B from 100% to 0%; (6) a 3 min linear gradient with 100% solvent A. Flow rate was 0.4 $mL \cdot min^{-1}$. Column temperature was maintained at 35°C. Eight microliters of samples were injected for UPLC analysis.

Carotenoids were detected at 450 nm and identified by comparison of retention times and spectral analyses for pure standard solutions. The sample chamber was maintained at 15°C and samples were injected within 24 h after extraction.

2.2.4 Molecular detection of the 1BL.1RS translocation

Genomic DNA was extracted from three kernels of each cultivar using a method modified from Lagudah et al.[21]. A marker H20 was employed to detect 1BL.1RS translocations[22]. PCR was performed in volumes of 20 μL, including 10 μL 2 × GC buffer I, 100 μmol of each dNTP, 2 pmol of each primer, 1 U of ExTaq, and 80 ng of template DNA. All reagents were from Takara Biotechnology Co., Ltd. (http://www.takara.com.cn). The PCR program was: denaturing at 94°C for 5 min, followed by 35 cycles of denaturing at 94°C for 1 min, annealing at 52–60°C for 1 min, 72°C for 2 min, and a final extension at 72°C for 5 min. PCR products were separated by electrophoresis in 1% agarose gels stained with ethidium bromide and visualized using UV light.

2.2.5 Statistical analysis

The data are reported as mean±SD (standard deviation) for UPLC assay. Analysis of variance (ANOVA) was performed with the SAS/STAT software version 9.1 (SAS Institute Inc., Cary, NC, USA). PROC GLM was used in ANOVA, where genotypes were considered as fixed effects, whereas environments and replications nested in environment were considered as random effects. The effects of genotype (G), environment (E), and their interaction (G × E) on carotenoid content were evaluated for the 217 wheat cultivars. *t*-tests between cultivars with and without 1BL.1RS translocation were analyzed using SAS 9.1.

3 Results and discussion

3.1 Effects of genotype, environment and their interactions

The concentrations of carotenoid components, viz. lutein, zeaxanthin and β-carotene, were determined in 217 bread wheat cultivars from Trials 1, 2, and 3. No α-carotene was found in any of the samples. ANOVA was conducted for lutein, zeaxanthin, and β-carotene data across two environments. The significant variation in concentrations of carotenoid components was attributed to G, E, G × E interaction in the three trials (Table 1). In Region 1, G contributed to the majority of variation in lutein, zeaxanthin and β-carotene ($P < 0.01$). E made a significant contribution to lutein and β-carotene ($P < 0.01$). G × E interaction made a significant contribution to zeaxanthin ($P < 0.05$) and β-carotene ($P < 0.01$). The ANOVA indicated significant G, E and G × E interaction differences ($P < 0.01$) among 138 Region 2 bread wheat samples for carotenoid components. In Region 3, the effects of G and E were significant for variation in lutein, zeaxanthin and β-carotene ($P < 0.01$). G × E interaction contributed to a big variation ($P < 0.01$) in lutein and β-carotene.

The large differences in carotenoid components reported previously may be attributed to different extraction methods, milling fractions, particle size and HPLC procedures[2,9,14]. Several studies have shown that genetic effects and environmental conditions may significantly

Table 1 Analysis of variance of the concentration of carotenoid components for three sets of bread wheat cultivars across two environments. Data are the *F* value

Source of variance	Region 1		Region 2		Region 3	
	df	*F* value	*df*	*F* value	*df*	*F* value
Lutein						
Genotypes	38	26.08**	137	24.60**	39	61.53**
Environments	1	41.44**	1	274.23**	1	1242.99**
Replications (Environments)	2	2.27	2	1.98	2	9.87**
Genotypes × Environments	38	1.42	137	2.20**	39	3.58**
Zeaxanthin						
Genotypes	38	3.27**	137	5.85**	39	15.69**
Environments	1	0.09	1	267.84**	1	527.21**
Replications (Environments)	2	0.45	2	19.88**	2	2.86
Genotypes × Environments	38	1.74*	137	1.64**	39	1.21
β-carotene						
Genotypes	38	33.15**	137	23.30**	39	103.73**
Environments	1	8.65**	1	330.97**	1	71.74**
Replications (Environments)	2	8.60**	2	5.82**	2	7.04**
Genotypes × Environments	38	2.54**	137	2.80**	39	3.10**

Note: *, $P < 0.05$; **, $P < 0.01$. Region 1, Northern winter wheat region; Region 2, Yellow and Huai River valleys facultative wheat region; Region 3, middle and lower Yangtze River autumn-sown spring wheat region.

affect carotenoid content of wheat[1,23]. Three einkorn accessions selected for a high concentration of lutein and a bread wheat cultivar grown in four environments were used to investigate the influence of location on the lutein content[1]. The lutein concentration showed significant differences among wheat accessions and at four locations. The effects of G, E and G × E on carotenoids were also investigated using flour samples of 10 soft winter wheat cultivars grown at four different locations[23]. The largest variation in lutein, zeaxanthin and total carotenoids were attributed to E, followed by G × E interaction and then G. Limited data have been published on the effects of genetic and environmental conditions for a large number of bread wheat cultivars. ANOVA from three trials in the present study demonstrated that G contributed to the greatest variation in carotenoid content, which was probably a consequence of the large number of cultivars assessed.

3.2 Carotenoids components

A wide range of variation for concentrations of all carotenoid components was observed in Chinese wheat. Lutein and zeaxanthin were detected in all samples. The lutein concentration varied from 18.3 to 100 μg per 100 g, with a mean of 40.2 μg per 100 g, and was the primary carotenoid in extracts. This finding is in agreement with the previous reports for diploid, tetraploid and bread wheat[3,9,11,13] where lutein accounted for a mean of about 90% of the total carotenoids in einkorn wheat and 70%–80% for bread wheat[3,24].

Zeaxanthin concentration varied from 4.88 to 12.0 μg per 100 g, with a mean of 7.21 μg per 100 g. Lutein and zeaxanthin concentrations in the present study were a little lower than previously reported for 11 wheat cultivars[11]. The difference may be attributed to the extraction and analytical conditions, genetic effects, different growing environments, and/or their interactions.

The concentration of β-carotene varied from 0.89 to 48.7 μg per 100 g, with a mean of 18.2 μg per 100 g. This was higher than reported for durum wheat and einkorn[9,14,25]. Most samples had β-carotene, but it was absent from nine cultivars (Chuanmai 42, Chuanmai 43, Chuannong 23, Emai 11, En 5, Fan 6, Jingmai 103, Yangmai 158, and Yangmai 10) probably because their β-carotene concentrations were too low to be detected by the UPLC system.

In this work, lutein accounted for 61.3% of three carotenoids components followed by β-carotene and zeaxanthin, sharing 27.7% and 11.0%, respectively. Of the total carotenoids (lutein + zeaxanthin + β-carotene) concentrations, the average proportion of β-carotene in bread wheat measured in this study was higher than those of durum wheat and einkorn[9,14,25]. In some bread wheat samples, the lutein concentration was higher than the others, whereas their β-carotene concentration was lower. The high accumulation of lutein and the relatively low levels of β-carotene could indicate dominance of the carotenoids biosynthetic pathway in producing the lutein. This is consistent with the findings of Humphries et al.[26], who reported high lutein concentrations as well as relatively low β-carotene concentrations. In addition, the lutein concentration was higher in durum than wild

tetraploid wheat; however, β-carotene concentration was lower in durum[14]. Bread wheat had a higher percentage of β-carotene and lower lutein concentration than durum. No α-carotene was found in the bread wheat cultivars. Moore et al.[25] also reported that lutein was the primary carotenoid present in eight wheat cultivars, along with significant amounts of zeaxanthin and β-carotene.

3.3 Variation between cultivars with and without 1BL.1RS translocation

In Region 1, there were no significant differences in total carotenoids (lutein + zeaxanthin + β-carotene), lutein, and β-carotene concentration between cultivars with and without 1BL.1RS translocation ($P < 0.05$, Table 2). However, total carotenoids, lutein, zeaxanthin, and β-carotene concentrations in cultivars with 1BL.1RS translocation were significantly higher than those without the translocation in Region 2 ($P < 0.05$, Table 3). For Region 3, the contents of total carotenoids and β-carotene were also significantly higher in cultivars with 1BL.1RS translocation than those without the translocation ($P < 0.05$, Table 4). Notably, a major quantitative trait locus for YPC was identified on chromosome

Table 2 Comparison of carotenoid content of bread wheat cultivars with and without 1BL.1RS translocation in the northern winter wheat region

Component	Genotype	Number of accessions	Mean (μg per 100 g)	*SD*	Range (μg per 100 g)
Total carotenoids (Lutein + zeaxanthin + β-carotene)	1BL.1RS	9	78.5[a]	12.34	63.3–97.7
	Non-1BL.1RS	30	69.5[a]	13.90	46.2–106.6
Lutein	1BL.1RS	9	48.4[a]	7.01	39.3–56.8
	Non-1BL.1RS	30	41.7[a]	9.42	24.6–62.1
Zeaxanthin	1BL.1RS	9	7.0[a]	0.29	6.5–7.6
	Non-1BL.1RS	30	6.7[b]	0.61	5.3–7.6
β-carotene	1BL.1RS	9	23.0[a]	12.12	2.0–37.9
	Non-1BL.1RS	30	21.1[a]	7.47	3.4–36.9

Note: Values with different letters in each column are significantly different at $P < 0.05$.

Table 3 Comparison of carotenoid content of bread wheat cultivars with and without 1BL.1RS translocation in the Yellow and Huai River valleys facultative wheat region

Component	Genotype	Number of accessions	Mean (μg per 100 g)	*SD*	Range (μg per 100 g)
Total carotenoids (Lutein + zeaxanthin + β-carotene)	1BL.1RS	65	76.1[a]	17.85	42.2–121.7
	Non-1BL.1RS	73	61.3[b]	13.90	39.6–115.9
Lutein	1BL.1RS	65	46.2[a]	14.96	26.2–100.1
	Non-1BL.1RS	73	39.7[b]	10.73	23.5–93.9
Zeaxanthin	1BL.1RS	65	8.1[a]	1.25	5.8–11.9
	Non-1BL.1RS	73	7.4[b]	0.94	5.4–12.0
β-carotene	1BL.1RS	65	21.9[a]	11.74	4.0–48.7
	Non-1BL.1RS	73	14.3[b]	6.45	3.3–32.4

Note: Values with different letters in each column are significantly different at $P < 0.05$.

Table 4 Comparison of carotenoid content of bread wheat cultivars with and without 1BL.1RS translocation in the middle and lower Yangtze River autumn-sown spring wheat region

Component	Genotype	Number of accessions	Mean (μg per 100 g)	*SD*	Range (μg per 100 g)
Total carotenoids (Lutein + zeaxanthin + β-carotene)	1BL.1RS	9	59.4[a]	13.20	46.0–82.5
	Non-1BL.1RS	31	48.2[b]	9.34	34.9–73.8
Lutein	1BL.1RS	9	30.1[a]	13.92	20.3–64.5
	Non-1BL.1RS	31	28.2[a]	7.14	18.3–51.8
Zeaxanthin	1BL.1RS	9	6.2[a]	1.40	5.1–9.1
	Non-1BL.1RS	31	5.9[a]	1.04	4.9–8.1
β-carotene	1BL.1RS	9	23.1[a]	10.80	0.0–39.4
	Non-1BL.1RS	31	14.1[b]	11.35	0.0–36.6

Note: Values with different letters in each column are significantly different at $P < 0.05$.

1BL.1RS[18], which provides further evidence that 1RS has favorable allele(s) for carotenoids and therefore yellow pigment content.

4 Conclusions

Significant variation in carotenoid content among 217 bread wheat cultivars was detected by UPLC. Cultivars with the 1BL.1RS translocation usually had higher carotenoid content than those without the translocation. It is possible to modify the contents of carotenoids through genetic improvement and study the related genes involved in the of carotenoids biosynthetic pathway in bread wheat.

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Compliance with ethics guidelines Wenshuang Li, Shengnan Zhai, Hui Jin, Weie Wen, Jindong Liu, Xianchun Xia, and Zhonghu He declare that they have no conflict of interest or financial conflicts to disclose.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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