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Variation in yellow pigment content in bread (*Triticum aestivum*) and durum (*Triticum turgidum* var *durum*) wheat and synthetic hexaploids: genetic and environmental effects

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ABSTRACT

Four hundred and ten genotypes representing Indian wheat varieties, selected germplasm lines, synthetic hexaploids and durum wheats were used in the present investigation to explore variability in yellow pigment content (YPC) in both bread (*Triticum aestivum* L.) and durum (*Triticum turgidum* Lvar *durum*) wheats. Microlevel test utilizing 0.2 g of the flour was developed to measure YPC in this investigation. There was strong correlation (R^2=0.97) between values of YPC measured using microlevel test and AACC method. The data demonstrated large variation in YPC varying from 3.58 to 11.06 ppm with the average value of 7.08 ppm in durum wheats and 0.87 to 5.30 ppm with the average value of 3.19 ppm in bread wheat. Among bread wheat varieties, 1B/1R translocation lines showed statistically significant higher YPC as compared to non 1B/1R translocation lines (P<0.001). Sixteen advanced genotypes of bread wheat and 15 genotypes of durum wheat separately grown over four locations in North Western Plains Zones in India showed significant differences between means of YPC for genotypes, locations and also had interaction effect. The heritability of YPC was 0.79 in bread wheat and 0.72 in durum wheat. High heritability and large variations in YPC have great utility in improving end-product quality in wheat. Microlevel tests developed in the present investigation can assist in capturing larger numbers of QTLs indirectly during advancing the segregating lines in the breeding programme.

Key words: Genetic effect, Nutritional quality, Wheat, Yellow pigment

Yellow pigment concentration in wheat grains imparts commercial and nutritional value to different end-products such as bread, noodle, chapati and pasta products. The colour of the end-products is influenced by the concentration of the yellow pigments in grain, and also oxidative degradation by lipoxygenase during processing and storage (Pagnotta *et al.*2005). High yellow pigment content (YPC) is desirable for yellow alkaline noodles made from bread wheat (*Triticum aestivum* L.) and pasta products from durum wheat (*Triticum turgidum* L. var *durum*). In contrast, low yellow pigment content is desirable for white noodles, bread and chapati where bright white to creamy colour is preferred (He *et al.* 2004). Carotenoids are the main components of yellow pigments in wheat. There are two distinct classes of carotenoids in wheat as carotenes, which are tetraterpenoid hydrocarbons, and xanthophylls, which contain one or more oxygen groups (Van den Berg *et al.*2000). In addition to imparting colour to the end-products, carotenoids have nutritional value for human beings. Carotenoids are natural compounds that reduce the oxidative damage to biological membrane by scavenging peroxi-radicals such as those involved in certain human diseases and in the ageing process. Carotenoids are also associated with a reduced risk of cancer, decrease in cardiovascular diseases, protection of the macula region of the retina and prevention of cataracts, and increased levels of iron absorption (Garcia-Casal 2006).

Yellow pigments are usually analysed by using AACC method 14-50 (AACC, 2000), based on the extraction of pigments with water saturated n-butyl alcohol and subsequent spectrophotometrical determination. However, for screening large numbers of samples in the evaluation of germplasm and breeding lines there is a need of simple assay. The objective of this study was to develop simplified assay for the measurement of yellow pigment content and to explore the genetic variability in a diverse set of durum and bread wheat and synthetic hexaploids. This will provide information on the existing variability and genetic resources available to breeders to improve colour and nutritional value of different end-products.

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MATERIALS AND METHODS

Four hundred and ten genotypes representing Indian wheat varieties, selected germplasm lines, synthetic hexaploids and durum wheats were employed in the present study. A set of 96 elite synthetic hexaploids procured from CIMMYT, Mexico and grown at Directorate of Wheat Research, Karnal, India were used in this investigation. The pedigree of all these materials is available and can be provided if required. In addition, a set of 15 advanced lines of durum wheat and 16 of bread wheat grown at five locations (Ludhiana, Durgapura, Pantnagar, Delhi and Hisar) in North Western Plains Zone in India during 2009-2010, were used to identify genotype and environmental interaction on yellow pigment content (YPC). Harvested grains from two replicates in RBD from each location were used in this investigation.

Whole meal was produced using Cyclotec Mill with 0.5 mm sieve. Moisture content were determined using NIR as per the approved methods 46-30 (AACC 2000) in wheat grains at 14 % mb.

Total yellow pigment content was measured using whole meal flour as per AACC Method 14-50 (AACC 2000). Water-saturated butyl alcohol (40 mL) was added to 8 g of whole meal (13.5% moisture basis), shaken and extracted for 16 h in the dark. Extract was filtered through Whatman No. 1 filter paper, and absorbance measured at 435 nm using a Microplate Reader (Biotec). Three individual absorbance measurements per extracted sample were recorded and values were averaged and converted to yellow pigment concentration (mg/kg) using the extinction coefficient for b-carotene. For the development of microlevel test, yellow pigments were extracted in 1 ml of the water-saturated butyl alcohol using 0.2 g of the flour.

Fifteen durum wheat genotypes and 16 bread wheat genotypes grown at 5 locations in RBD were tested for yellow pigment content. Data were analyzed statistically and means were compared with ANOVA and grouped by Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Development of microlevel test for the measurement of YPC

Elevated yellow pigment (YP) concentration is a desirable end-use quality trait in durum wheat and is the target of durum breeding programs worldwide. Yellow pigments are usually analyzed by using AACC method 14-50 (AACC 2000) using 8 g of the flour. The method is dependent on extraction of pigments with water saturated n-butyl alcohol in the dark. In the present investigation, yellow pigments were extracted using 0.2 g of the flour in 1 ml of the water-saturated butyl alcohol over night in the dark. In this method though similar principal with AACC method; the amount of the sample was reduced to 0.2 g. The extract was centrifuged at 10 000 rpm for 10 min and the supernatant was taken for the measurement of yellow pigment content in Microplate Reader. 200 µl of the supernatant was taken in 96 well microplate for the measurement of absorption at 336 nm using BioTec (Power Wave 340) spectrophotometer.

There was strong positive correlation ($R^2 = 0.97$) between values of yellow pigment content measured using microlevel test and AACC method. The average values of yellow pigment content measured using microlevel test and AACC method were 5.87 ug/g and 5.83 ug/g respectively. In addition there was very strong correlation ($R^2 = 0.92$) between duplicate readings of all the samples using microlevel test. This further demonstrated the accuracy and repeatability of the microlevel test for the measurement of yellow pigment. Earlier study also reported a test with lower quantity of flour for the measurement of β-carotene content (Santra *et al.* 2003) using only two cultivars. However, they reported β-carotene content in place of yellow pigment wrongly, because entire yellow pigment content was extracted instead of β-carotene. In addition, they used micro-cuvettes for spectrophotometric analysis. Bleggia *et al*. (2010) also reported micro-method for measuring YP content in durum wheat by injecting the extract from 100 mg of the flour in HPLC system. However, the method is not useful in analyzing large numbers of samples in breeding programme. The assay developed in present investigation uses microplate assay to measure yellow pigment content, it has greater utility in germplasm evaluation for the identification of desirable lines with both high and low yellow pigments. In addition, it can be very useful in breeding where segregating lines can be identified with desirable levels of yellow pigments for their subsequent advancement into next generation.

Variability for YPC

The data demonstrated large variation in yellow pigment content ranging from 3.58 to 11.06 ppm with the average value of 7.08 ppm in durum wheats. The frequency histogram showed normal distribution of yellow pigment content in this set of durum genotypes indicating oligogenic trait controlled by many genes. Most of the varieties had yellow pigment content in lower to medium range with the highest value in PDW 233 (7.61 µg/g). However, there was higher yellow pigment content in genotypes selected from international nurseries with the highest value of 11.06 µg/g. Therefore, international germplasm lines with higher yellow pigment content can be utilized in breeding for improving durum quality.

In bread wheat, yellow pigment content varied from minimum of 1.87 ppm to maximum of 5.30 ppm with the average value of 3.19 ppm. Bread wheat showed lower YPC than durum wheat because of genetic selection for high yellow pigment concentration in durum wheat breeding programs (Hentschel *et al.* 2002, Hidalgo *et al.* 2006 and Leenhardt *et al.* 2006). Among bread wheat varieties, genotypes having 1B/1R translocation showed higher YPC as compared to non 1B/1R translocation genotypes ($P<0.001$).

This supports earlier reports of the effect of the 1B/1R translocation on flour colour and YPC in bread and durum wheat (Zarco-Hernandez *et al.* 2005 and Zhang *et al.* 2009). This information is useful for breeders for the development of cultivars suitable for different end-use products. High yellow pigment content is desirable for yellow alkaline noodles of bread wheat and pasta products from durum wheat. In contrast low yellow pigment content is desirable for white noodles, bread and chapati where bright white to creamy colour is preferred (He *et al.* 2004). In addition, yellow pigments have properties beneficial with respect to nutritional quality (Garcia-Casal 2006).

Genotypic and location effect on YPC

Fifteen advanced genotypes of durum and 16 of bread wheat were grown over five locations (Ludhiana, Durgapura, Delhi, Pantnagar and Hisar) in North Western Plains Zone of India and analyzed for yellow pigment content (Table 1-2). The significant differences were observed between means for genotypes, locations and had interaction effect. Further, means were grouped by Duncan Multiple Range Test where similar superscripts reflect genotypes in same group with non-significant statistical difference.

Significant differences in yellow pigment levels were observed among locations with CD value of 0.33 for durum wheat and 0.13 for bread wheat. There were significant differences in YP levels between different centres. The yellow pigment content of bread wheat was highest in Ludhiana with mean value of 3.48 ppm and lowest in Durgapura (2.57 ppm). In case of durum wheat also, the mean value of yellow pigment content was highest in Ludhiana (5.88 ppm) and lowest in Durgapura (4.95 ppm). There were also significant genetic differences in YPC among the genotypes with CD value 0.59 in durum and 0.24 in bread wheat. Genotypic variation was greater than environmental variation.

The heritability of YPC in bread wheat was 0.79 and in durum wheat 0.72. Earlier reports indicated complex nature of inheritance of YP being controlled largely by additive gene action and higher variation in heritability (H^2 = 0.34 to 0.95) (Clarke *et al.* 2006). Several QTLs representing different regions in the genome have been identified in both durum and hexaploid wheat on different chromosomes (Pozniak *et al.* 2007, He *et al.* 2008 and Patil *et al.* 2008) indicating oligogenic nature of YPC. Large variations in YPC along with high heritability exhibited in this investigation demonstrated that the YPC can be manipulated genetically. Microlevel tests developed in the present investigation can be very useful in capturing larger numbers of QTLs indirectly during advancing the segregating lines in the breeding programme.

In conclusion, the study demonstrated that micro-level test using 0.2 g of the whole wheat flour can be employed in assaying YPC. There was three, four and five folds variation in YPC in aestivum, durum and synthetic wheats, respectively.

Table 1 Influence of genotype and locations on yellow pigment content (ppm) in bread wheat. Means were compared with ANOVA and further grouped by Duncan Multiple Range Test. Genotypes with similar superscript were in same group having non significant statistical difference

Variety	Locations					
	Ludhiana	Durgapura	Delhi	Pantnagar	Hisar	Average
PBW 621	2.96	2.04	2.79	2.37	2.50	2.53 ij
DBW 50	2.92	2.60	3.06	2.46	2.99	2.81 gh
PBW 343	4.09	2.97	3.81	3.76	3.69	3.66 ab
DBW 17	3.71	3.47	3.96	3.54	3.23	3.58 abc
PBW 550	3.31	2.00	2.74	2.42	2.81	2.66 hi
HD 2967	3.31	2.99	3.59	2.84	3.36	3.22 def
PBW 631	2.62	1.92	2.71	2.20	2.61	2.41 ij
PBW 634	3.46	2.89	3.58	3.09	3.69	3.34 cde
PBW 635	2.81	2.01	3.12	2.62	2.39	2.59 hij
PBW 636	3.79	3.12	4.02	3.59	2.61	3.43 bcd
DBW 55	4.09	3.19	3.86	3.94	3.46	3.71 a
DBW 58	3.54	2.54	3.47	2.82	4.34	3.34 cde
HD3024	3.29	2.86	3.48	3.16	2.73	3.10 ef
HD 3027	3.16	2.86	3.07	2.71	3.19	2.99 fg
HUW 635	3.09	1.95	2.62	2.32	2.77	2.55 hij
HUW 636	2.42	1.66	2.80	2.22	2.55	2.33 j
Average	3.28 a	2.57 d	3.29 a	2.88 c	3.06 b	

CD at 5% for location (L) = 0.1327; CD at 5% for variety (V) = 0.2375; CD at 5% for V × L = 0.3697; Genotypic variance (G) = 0.248; Environmental variance (E) = 0.064; Phenotypic variance (P) = 0.318; Heritability (H^2) = 78.8

Table 2 Influence of genotype and locations on yellow pigment content (ppm) in durum wheat. Means were compared with ANOVA and further grouped by Duncan Multiple Range Test. Genotypes with similar superscript were in same group having non-significant statistical difference

Variety	Locations					
	Ludhiana	Durgapura	Delhi	Pantnagar	Hisar	Average
PDW 315	5.24	3.67	5.31	4.05	4.54	4.56 gh
PDW 317	5.09	4.36	6.53	4.66	6.06	5.34 def
WHD 943	7.15	6.05	8.22	5.95	6.84	6.84 b
PDW 233	7.96	7.04	8.04	7.88	7.12	7.61 a
PDW 291	5.13	4.40	6.62	4.81	4.30	5.05 efg
WH 896	6.88	5.46	7.39	5.34	5.62	6.14 c
PDW 314	5.00	4.15	5.43	4.58	4.29	4.69 fgh
PDW 322	4.26	3.07	5.57	6.29	4.43	4.72 fgh
WHD 946	7.35	6.08	8.08	6.54	6.78	6.97 b
HD 4722	4.70	4.69	5.93	4.89	4.93	5.03 efg
GW 1255	5.50	4.45	7.00	5.22	4.83	5.40 de
UAS 429	6.13	5.75	6.61	5.27	5.24	5.80 cd
HI 8703	5.38	5.08	5.69	4.87	5.58	5.32 def
NIDW 577	6.03	6.00	7.89	5.30	5.62	6.17 c
DDW 16	8.02	6.11	8.04	8.58	7.34	7.62 a
Average	5.99 b	5.09 d	6.82 a	5.62 c	5.57 c	5.82

CD at 5% for location (L) = 0.3302; CD at 5% for variety (V) = 0.5906; CD at 5% for V X L = 0.9196; Genotypic variance (G) = 0.9656; Environmental variance (E) = 0.3948; Phenotypic variance (P) = 1.3608; Heritability (H^2) = 71.7

*PBW 343 is bread wheat

Among bread wheat varieties, 1B/1R translocation lines showed statistically significant higher YPC as compared to non 1B/1R translocation lines (P<0.001). The heritability of YPC was 0.79 in bread wheat and 0.72 in durum wheat. Higher variability in YPC along with high heritability demonstrated greater scope in improving nutritional and industrial quality of wheat. Microlevel tests developed in the present investigation can assist in capturing larger numbers of QTLs indirectly during advancing the segregating lines in the breeding programme.

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