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Positive influence of *Lophopyrum ponticum* derived *Y* gene on yellow pigment content - A major durum wheat quality trait

Gagandeep Singh, Johar Singh Saini*, N. S. Bains and R. P. Singh

Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141 004

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Abstract

The *Lophopyrum ponticum* chromosome segment 7EL is known to carry rust resistance genes *Lr19* and *Sr25* besides a gene (*Y*) for yellow pigmentation of the flour. This chromosome segment, originally translocated to hexaploid wheat (chromosome 7D) and later retranslocated to durum wheat (Chromosome 7A) and was available for present study in form of durum wheat variety UC1113 (*Y*). Intenser yellow colour of durum wheat products is an important quality parameter on account of consumer preference and nutritional significance. The utility of *L. ponticum* chromosome segment was assessed for improvement of durum wheat quality, in view of availability of molecular marker tags for this segment and the need to raise yellow pigment content of Indian durum wheats. The molecular marker analysis of 188 F_3 progenies derived from a cross UC1113 (*Y*) X PDW291 was performed to identify progenies homozygous for presence and absence of *L. ponticum* segment carrying the *Y* gene. The two sets of progenies were then used to ascertain the influence of the *Y* gene on various quality parameters. Presence of yellow pigment gene resulted in an average increase in yellow pigment content of 24.03%, but lowered 1000 grain weight, test weight and grain hardness. Yellow pigment was positively correlated with sedimentation value. The presence of several *Y* gene positive F_3 progenies combining most of the desirable durum wheat quality traits showed that *Y* gene can be easily utilized to increase the yellow pigment content without unfavourably impacting other quality parameters.

Key words: *Triticum durum*, *Lophopyrum ponticum*, yellow pigment, grain quality

Introduction

Durum wheat (*Triticum durum* Desf.) represents the second most important wheat species after bread wheat and accounts for 5-6 % (~30 million metric tonnes) of total world wheat production (http://www.fas.usda.gov/). In India, durum wheat comprises a smaller portion (about 3 %) of the total wheat grown. Presently, in India durum wheat not only holds relevance for its semi-processed products, suitable for a fast urbanising society, but also for its export potential. Unlike common wheat in India which is mainly processed at home to make *chapatis*, durum wheat requires industrial processing for making specific products including pasta, macaroni, vermicelli, etc. The quality aspects, therefore, constitute a prime objective in durum wheat breeding. Grain vitreousness, protein content, gluten strength and yellow pigment content represent the most important durum wheat quality parameters. Yellow colour of durum wheat semolina is mainly due to yellow endosperm pigments *viz*., xanthophylls (leutin), carotenoids (carotene) and flavones (tricin) [1] which provide the bright yellow colour to pasta products. Besides consumer preferences yellow colour pigments are of nutritional value and are thus a major consideration in durum wheat quality [2]. A molecular marker tagged chromosome segment comprising the distal region of chromosome arm 7EL from *Lophopyrum ponticum* (Podp.) A Löve (Syn. *Thinopyrum ponticum* (Podp.) Barkworth & DR Dewey) carries a gene designated *Y* that increases yellow pigment in the endosperm. The 7EL segment initially available in hexaploid wheat was not preferred for breeding as *Y* gene gives yellowish tinge to flour, reducing its appeal in bread making. The 7EL segment initially translocated to 7D of bread wheat was retranslocated to 7A and thus became relevant for durum wheat improvement [3]. This alien segment also harbors useful rust resistance genes, *Lr19* and *Sr25* conferring leaf rust and stem rust resistance,

*Corresponding author's e-mail: joharsingh@pau.edu
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respectively. *Sr25* confers resistance to the original version of stem rust race *Ug99*. Incorporation of *Sr25* gene is of direct relevance as NWPZ falls in potential migration route of this race. *Lr19* is also effective against most of the leaf rust pathotypes of this region. The study assesses the role of yellow pigment enhancing gene (*Y*) from *L. ponticum* when introgressed in an elite durum wheat cultivar.

Materials and methods

The plant material for the study was a set of 188 F_3 progenies randomly derived from a cross between high yielding durum wheat cultivar PDW291 and a *Y* gene donor line UC1113. A single row of 10-15 plants of each of the F_3 progenies was sown in randomized complete block design with two replications in crop season 2010-11 in the experimental area of Department of Plant Breeding and Genetics, PAU, Ludhiana. The pooled DNA from each progeny was used for *Y gene* identification. For marker analysis of parents and checks as well as F_3 lines, DNA was isolated from young leaves using CTAB (Cetyl trimethyl ammonium bromide) method [4]. Quantity and quality of DNA was estimated using 0.8% agarose gel prepared in 0.5X TBE buffer (Tris base-45mM, Boric acid 45mM and EDTA 1mM).

The molecular markers BF145935 [5] and Gb [6] were used for monitoring *Y gene* presence. The amplification protocol followed was: 4 min at 94°C, 30 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 30 s and a final extension for 5 min at 72°C for marker Gb, and 4 min at 94°C, 35 cycles of 45 s at 94°C, 30 s at 60°C, 45 s at 72°C and a final extension for 7 min at 72°C for marker BF145935. The PCR products were resolved on 2.5% agarose gel.

Progenies homozygous for *Y* gene identified using molecular marker data were subjected to estimation of yellow pigment content and other grain quality parameters *viz*., grain protein content (GPC), thousand grain weight (TGW), test weight (TW), grain hardness (GH) and sedimentation value (SV). Grain protein content (%) was determined using “Infratec1241” grain analyser supplied by M/S Foss Analytical AB, Sweden. Yellow pigment was extracted from whole meal sample (4g) with n-butanol and optical density was measured spectrophotometrically using standard procedure [7]. Grain hardness was determined by crushing 10 randomly taken grains using the grain hardness tester supplied by M/S Ago Seiki Co. Ltd., Japan. Sedimentation value of the durum wheat whole meal samples was determined following method [8]. The data on thousand Grain weight, test weight and days to flowering was recorded following standard procedures. The analysis of variance was performed using mean observations of all the trials under study. The general Pearson correlations were used to find the relationship between yellow pigment content and other quality parameters.

Results and discussion

The 7EL *L. ponticum* segment offers an effective way of enhancing yellow pigment content with help of completely linked molecular. The *Y* gene harboured by this alien segment is likely to be different from the durum wheat resident pigment genes. It was important to assess the phenotypic effect of *Y* gene in durum background and evaluating its potential for improving yellow pigment content over and above that of recipient durum wheat line. Once assured of the positive effect of the gene, systematic introgression schemes (based on marker assisted backcrossing) can be taken up. To rapidly achieve this objective two random set of F_3 progenies, with and without Y gene were studied. Besides pigment content, the effect of alien segment was also studied on other quality traits to ascertain its overall utility for durum wheat quality improvement. This segment was first translocated to 7D of bread wheat was retranslocated to 7A [3] and thus became relevant for durum wheat improvement. UC1113, a durum wheat line carrying this retranslocated chromosome was used as a donor. The cultivar, PDW291, is a high yielding durum wheat cultivar recommended for North Western Plains zone of India. It has good pasta quality but falls short of optimum levels of yellow pigment. Two markers BF145935 (co-dominant) and Gb (dominant) linked to Sr25 were used to to confirm the presence of this gene in parental lines (Fig. 1).

A set of 188 F_3 progenies derived from the above cross were classified into homozygous carrier, heterozygous carrier and non carrier lines using these markers. The segregation ratio however deviated from the expected ratio of 1:2:1 for the gene in homozygous condition, heterozygous condition and absent respectively. The observed frequencies of these classes and the *Chi* square test are given in Table 1. Deviation from expected 1:2:1 segregation probably owes to differential transmission of *Lophopyrum ponticum* segment as reported in literature [3, 9].

Sixty three F_3 lines, 27 for presence and 36 for absence of *Y* gene were used for analysis of quality traits *viz*., yellow pigment content, 1000 grain weight,

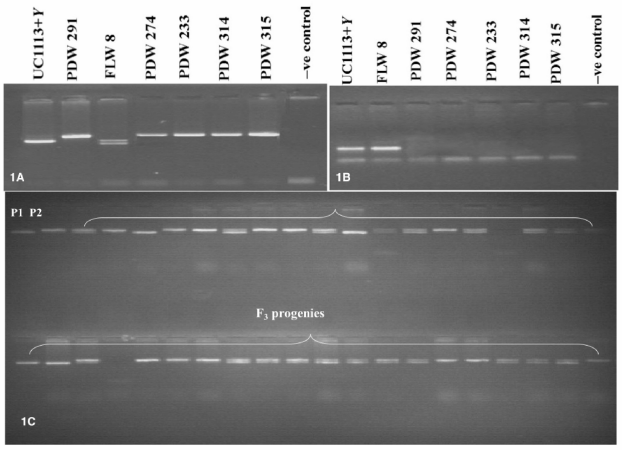

Fig. 1A and 1B: Amplification pattern of marker BF145935 (1A) and Gb (1B) on parental lines; 1C: Marker *BF145935* based genotyping of F_3 progenies, P_1 is UC1113 (*Y*) P_2 is PDW 291

Table 1. Segregation frequency of F_3 lines for yellow pigment (*Y*) gene

Class	Observed	Expected	$(O-E)^2/E$
Homozygous (Y+ve)	27	47	8.51
Heterozygous	125	94	9.57
Homozygous (Y–ve)	36	47	2.57
	$\chi^2 = (O-E)^2/E$		20.65
	Probability (P)		0.00003

test weight, grain hardness, grain protein content, sedimentation value and days to 50% flowering. The analysis of variance (Table 2) showed significant differences between F_3 lines for yellow pigment content, 1000 grain weight, test weight, grain hardness, grain protein content and sedimentation value. Days to flowering showed non-significant difference between F_3 lines. Variance component based on comparison of *Y* gene positive and negative progenies revealed significant differences for yellow pigment content, 1000 grain weight, test weight, grain hardness, sedimentation value and days to flowering between two progeny groups. Grain protein content showed non-significant difference between two progeny groups.

The results of mean and range of performance of Y Gene positive and negative lines for yellow pigment content and other quality traits is presented in Table 3. Progenies carrying Y gene showed mean yellow pigment content of 7.33 ppm ranging from 5.28-9.92 ppm and progenies not carrying this gene showed mean yellow pigment content 5.91 ppm with range from 4.31-8.13 ppm. Progenies carrying Y gene showed decrease in mean 1000 grain weight, test weight and grain hardness, but on the contrary sedimentation value showed increase. This may be the result of decrease in grain size and grain hardness in the Y gene positive lines. Progenies carrying Y gene showed marginal delay in flowering but slight increase in the protein content. Several F_3 progenies combining high yellow pigment content and other quality parameters were identified.

Correlation coefficients between yellow pigment and other quality traits under study were estimated and are presented. These results showed that yellow pigment content was negatively and significantly correlated with thousand grain weight, test weight and grain hardness. However, the correlation with grain protein content was non-significant. These correlations support the results obtained from analysis of variance discussed in the previous section. Protein content showed no correlation with yellow pigment content and days to 50% flowering. Protein content showed positive correlation with sedimentation value which is as expected. Yellow pigment content showed significant positive correlation with sedimentation value (0.43) and days to 50 % flowering. This correlation may be on account of lower 1000 grain weight and test weight in high yellow pigment lines. Sedimentation value showed significant positive correlation (0.33) with days to 50 %

Table 2. ANOVA for yellow pigment colour and other quality traits of F_3 lines with and without the *Y* gene

Source	Mean squares						
	TGW (g)	TW (kg/hl)	GH (kg)	GPC (%)	YPC (ppm)	SV (ml)	DTF (days)
Treatments (F_3 lines)	38.429**	6.744**	3.328**	0.944**	3.068**	26.702**	4.837
Gene +ve lines	21.848	3.955	0.764	1.130	2.926	16.859	1.972
Gene -ve lines	37.295	4.387	2.318	0.796	1.491	11.771	5.884
Gene +ve vs -ve	509.221**	161.726**	105.318**	1.266	61.917**	805.219**	42.669**
Error	8.640	1.133	0.962	0.475	0.445	7.381	3.924

**Significant at 1% level of significance

Table 3. Mean performance of *Y*- gene positive and negative F_3 lines for different traits

Trait	Mean		Range	
	Y Gene +ve	*Y* Gene -ve	*Y* Gene +ve	*Y* Gene -ve
Yellow pigment (ppm)	7.33	5.91	5.28-9.92	4.31-8.13
1000 grain weight (g)	38.31	42.37	32.72-44.64	33.01-51.49
Test weight (kg/hl)	73.8	76.1	70.8-76.5	72.0-78.3
Hardness (kg)	8.74	10.58	7.40-9.74	8.85-13.35
Protein content (%)	11.64	11.44	10.15-13.15	10.27-12.76
Sedimentation value (ml)	37	32	31-44	27-36
DTF (days)	103	102	102-105	98-107

Table 4. Correlation matrix between different quality traits

Trait	TW(kg/hl)	GH (kg)	GPC (%)	YP (ppm)	SV (ml)	DTF (days)
TGW (g)	0.66**	0.47**	-0.06	-0.65**	-0.23	-0.30*
TW (kg/hl)		0.58**	-0.37**	-0.48**	-0.39**	-0.35**
GH (kg)			0.01	-0.50**	-0.43**	-0.34**
GPC (%)				0.06	0.25*	-0.18
YP (ppm)					0.43**	0.31*
SV (ml)						0.33**

*Significant at 5% level of significance **Significant at 1% level of significance

Table 5. *Y* gene positive lines carrying desirable trait combinations

S.No.	Genotypes	TGW (g)	TW (kg/hl)	GH (kg)	GPC (%)	YP (ppm)	SV(ml)	DTF (days)
1	D11 60	39.13	74.8	8.52	11.05	7.60	36	105
2	D11 63	33.58	74.0	8.81	11.29	9.92	36	104
3	D11 85	39.50	75.8	9.26	12.64	8.94	43	104
4	D11 123	39.01	75.5	8.81	12.25	7.48	37	102
5	D11 145	34.26	75.5	8.68	10.30	7.31	37	104
6	D11 166	39.62	76.5	9.74	10.15	8.06	38	103
7	PDW 291	47.74	76.9	9.83	10.88	5.04	28	98

flowering which may be in part mediated by high yellow pigment content, low 1000 grain weight and low test weight of late maturing genotypes. The negative associations of yellow pigment as are evident may be because of the smaller and softer grains and late maturing traits possessed by the donor line UC1113 (data not given). The donor traits tend to be transmitted in the progenies due to limited recombination opportunities available till F_3 generation. The list of F_3 lines carrying *Y* gene combining good of all the traits is presented in Table 5. On an average, the group of F_3 lines positive for *Y* gene showed lower values for test weight and thousand grain weight as discussed presently is undesirable, but lines possessing adequate quality across several traits could be identified.

Translocation of 7EL segment from 7D to 7A represents a significant basic advance [5] which made this segment relevant for durum wheat improvement. The results showed that *Y* gene is relevant for durum wheat improvement. Considering excellent physical grain characteristics of Indian durum cultivars and generally inadequate levels of yellow pigment content, the *Y* gene seems to fit the durum wheat improvement

needs very well. Y gene has clear positive effect on yellow pigment content and can easily be tracked with molecular markers. Several durum wheat quality traits in this study were impacted negatively by Y gene, particularly physical grain attributes. This is most likely due to linkage drag from the unadapted donor parent UC1113. Backcross approach to nullify negative donor genotype influence and large population size to ensure recombination opportunities are likely to ensure reconstitution of elite durum parent traits along with high yellow pigment.

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