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ORIGINAL PAPER

QTL analysis of pasta quality using a composite microsatellite and SNP map of durum wheat

W. Zhang · S. Chao · F. Manthey · O. Chicaiza · J. C. Brevis · V. Echenique · J. Dubcovsky

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Abstract Bright yellow color, firmness and low cooking loss are important factors for the production of good-quality pasta products. However, the genetic factors underlying those traits are still poorly understood. To fill this gap we developed a population of 93 recombinant inbred lines (RIL) from the cross between experimental line UC1113 (intermediate pasta quality) with the cultivar Kofa (excellent pasta quality). A total of 269 markers, including 23 SNP markers, were arranged on 14 linkage groups covering a total length of 2,140 cM. Samples from each RIL from five different environments were used for complete pasta quality testing and the results from each year were used for QTL analyses. The combined effect of different loci, environment and their interactions were analyzed using factorial ANOVAs for each trait. We identified major QTLs for pasta color on chromosomes 1B, 4B, 6A, 7A and 7B. The 4B QTL was linked to a polymorphic deletion in the *Lpx-B1.1* lipoxygenase locus, suggesting that it was associated with pigment degradation during pasta processing. The 7B QTL for pasta color was linked to the *Phytoene synthase 1* (*Psy-B1*) locus suggesting difference in pigment biosynthesis. QTLs affecting pasta firmness and cooking loss were detected on chromosomes 5A and 7B, and in both cases they were overlapping with QTL for grain protein content and wet gluten content. These last two parameters were highly correlated with pasta firmness ($R > 0.71$) and inversely correlated to cooking loss ($R < -0.37$). The location and effect of other QTLs affecting grain size and weight, gluten strength, mixing properties, and ash content are also discussed.

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W. Zhang · O. Chicaiza · J. C. Brevis · J. Dubcovsky (✉)
Department of Plant Sciences, One Shields Av.,
University of California, Davis, CA 95616, USA
e-mail: jdubcovsky@ucdavis.edu

S. Chao
USDA-ARS Biosciences Research Laboratory,
1605 Albrecht Blvd, Fargo, ND 58105, USA

F. Manthey
Department of Plant Sciences,
North Dakota State University, Fargo, ND 58105, USA

V. Echenique
Departamento de Agronomía, Universidad Nacional del Sur,
CERZOS (CONICET), San Andrés 800, 8000 Bahía Blanca,
Argentina

Introduction

Approximately 30 million tons of durum wheat (*Triticum turgidum* L. var. *durum*) is produced every year in different regions of the world (http://www.fas.usda.gov/pecad/highlights/2005/07/durum2005). Since durum wheat is mainly used for pasta, the varieties that meet the requirements of high-quality pasta products receive premium prices in the global market. These requirements include bright yellow color, high protein content and pasta firmness, and small cooking loss (for a review see Troccoli et al. 2000).

Pasta color is determined by grain carotenoid content and carotenoid degradation by lipoxygenases during pasta processing (Troccoli et al. 2000). The main carotenoid pigment in the durum grain is lutein, which contributes to both pasta quality and nutritional value (Hentschel et al. 2002).

Yellow pigment is mainly controlled by additive gene effects and has high heritability (Clarke et al. 2006; Clarke et al. 2000; Elouafi et al. 2001). Equally important parameters for pasta quality are pasta firmness and cooking loss, which are associated with grain protein content (GPC) and gluten strength (Sissons et al. 2005).

The construction of detailed durum wheat genetic maps using molecular markers has facilitated a more precise delimitation of the chromosome regions affecting some of these pasta quality traits. The first durum maps were constructed using restriction fragment length polymorphism (RFLP) markers (Blanco et al. 1998) and were then complemented with simple sequence repeat (SSR) markers (Korzun et al. 1999). Additional maps were published more recently integrating different types of molecular markers (Elouafi and Nachit 2004; Maccaferri et al. 2008; Nachit et al. 2001; Peleg et al. 2008; Pozniak et al. 2007). Several of these maps have been used to identify quantitative trait loci (QTL) for quality traits including grain yellow pigment content (Elouafi et al. 2001; Patil et al. 2008; Pozniak et al. 2007; Zhang and Dubcovsky 2008), protein content (Blanco et al. 2006; Joppa et al. 1997; Olmos et al. 2003; Uauy et al. 2006), test weight and kernel weight (Elouafi and Nachit 2004), and gluten strength (Elouafi et al. 2000).

However, some of the previous QTL have been identified in crosses including wild tetraploid parental lines (Elouafi and Nachit 2004; Gonzalez-Hernandez et al. 2004; Joppa et al. 1997) and therefore, have limited application to modern durum germplasm. In addition, no information is available for more complex parameters that require full pasta evaluations, such as pasta color, pasta firmness or cooking loss. Even for the traits for which there is some QTL information, additional mapping populations are needed to obtain a more complete picture of the different genetic factors affecting pasta quality traits in the modern durum germplasm. To fill this gap in our understanding of the genetic factors underlying important quality parameters we developed a genetic map between adapted durum wheat varieties of different quality and performed full pasta analyses for five different environments. The QTL analyses of these data provided valuable information and molecular markers that will be useful to accelerate the selection of durum varieties with improved pasta quality.

Materials and methods

Materials

The durum mapping population was produced from the cross between UC1113 and Kofa. Kofa is a Desert Durum® variety developed by Western Plant Breeders (now WestBred) that has excellent pasta quality with optimal semolina and pasta color, high protein content, and strong gluten. UC1113 is a breeding line from the UC Davis wheat breeding program selected from CIMMYT cross CD52600 (KIFS//RSS/BD1419/3/MEXIS-CP/4/WAHAS/5/YAV79). This line has excellent agronomic performance but intermediate pasta quality parameters. A total of 93 recombinant inbred lines (RIL) and the two parental lines were grown at UC Davis, California in 2003, 2004, 2006 (Sacramento Valley, CA) and at the Desert Research and Extension Center (Imperial Valley, CA) in 2005 and 2006. Fertilization included at least 220 kg/ha of nitrogen and optimum irrigation. The field trials were organized in a randomized complete block design (RCBD) with three replications (plots were 1.2-m wide by 2.5–3.6 m long). Seeds from the three replications were pooled for quality analyses, which were performed at the Durum Wheat Quality Laboratory at North Dakota State University, Fargo, ND.

High levels of stripe rust infection were observed at UC Davis during the 2003 season and the RILs from the UC1113 × Kofa population showed segregation for different levels of resistance (no completely susceptible lines were observed). The 2004 season at UC Davis showed low levels of stripe rust, and no infection was detected in the UC1113 × Kofa RILs. In 2006 the RILs were treated with fungicide to prevent damage from stripe rust. The two experiments at Imperial Valley had no stripe rust or other diseases. In summary, only the 2003 results from the experiment at UC Davis might have been affected by stripe rust.

Genetic map

Parental lines were screened using 1,235 wheat microsatellite markers previously mapped in the A and B genomes. Amplification fragments were separated in 6% non-denaturing acrylamide gel (29:1) and stained directly with ethidium bromide (http://maswheat.ucdavis.edu/PDF/SSR_Protocol.pdf). The same parental lines were screened also with 275 wheat SNP markers generated by the NSF-Wheat SNP project (http://wheat.pw.usda.gov/SNP) by template-directed dye-terminator incorporation assay with fluorescence polarization detection (FP-TDI) (Chao et al. 2008; Hsu et al. 2001).

Linkage analysis was carried out using MapMaker version 3.0b (Lander et al. 1987). Map distances were computed with the Kosambi mapping function. The map was initially constructed at a LOD of 2.0. Additional markers were added using the TRY command and their order was fine-tuned using the RIPPLE command. Regions for which the marker orders are supported by LOD score values lower than 2.0 were indicated by vertical lines on the left side of the map (Fig. 1). Centromere localizations were estimated based on previous determinations of the arm location of

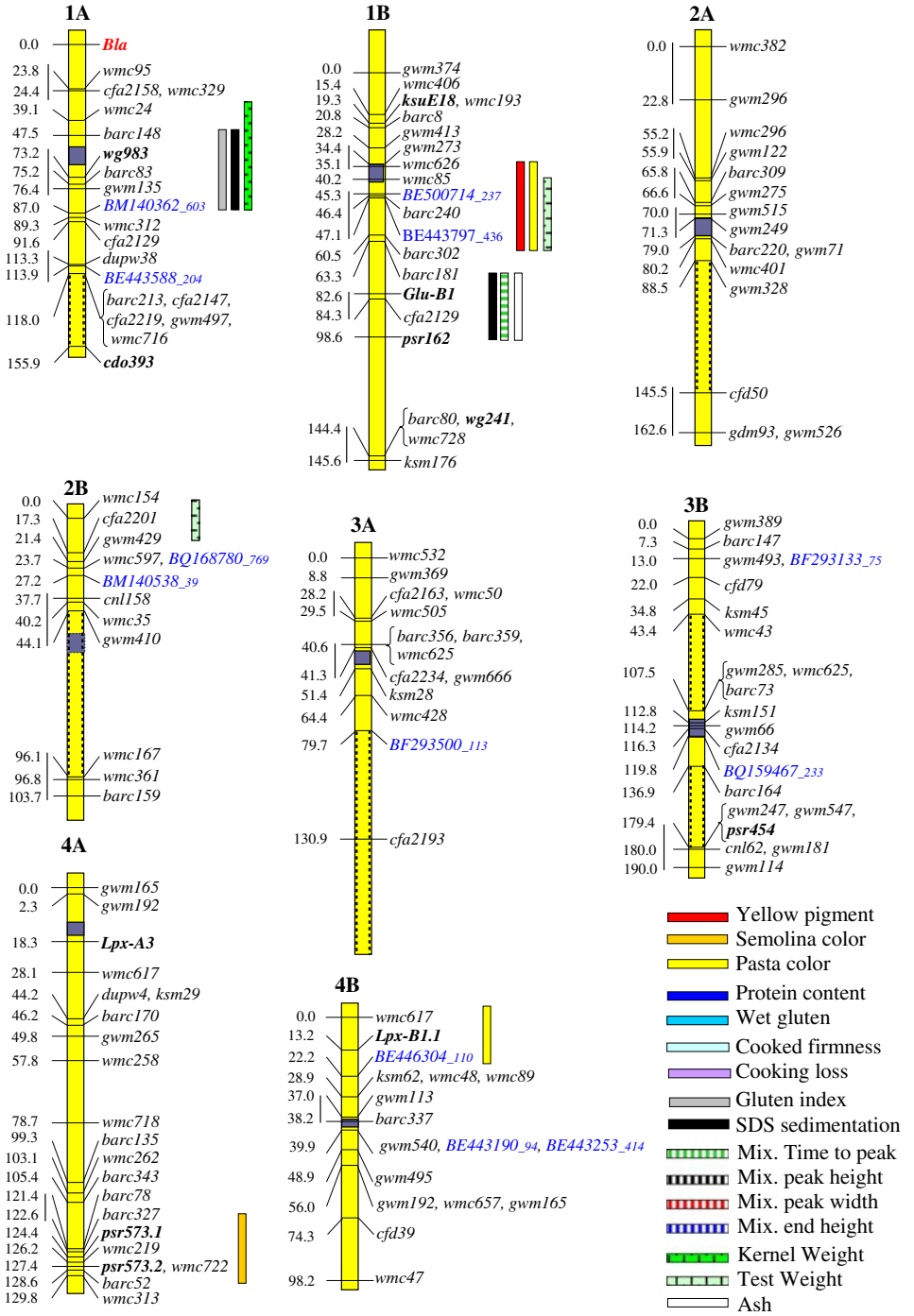


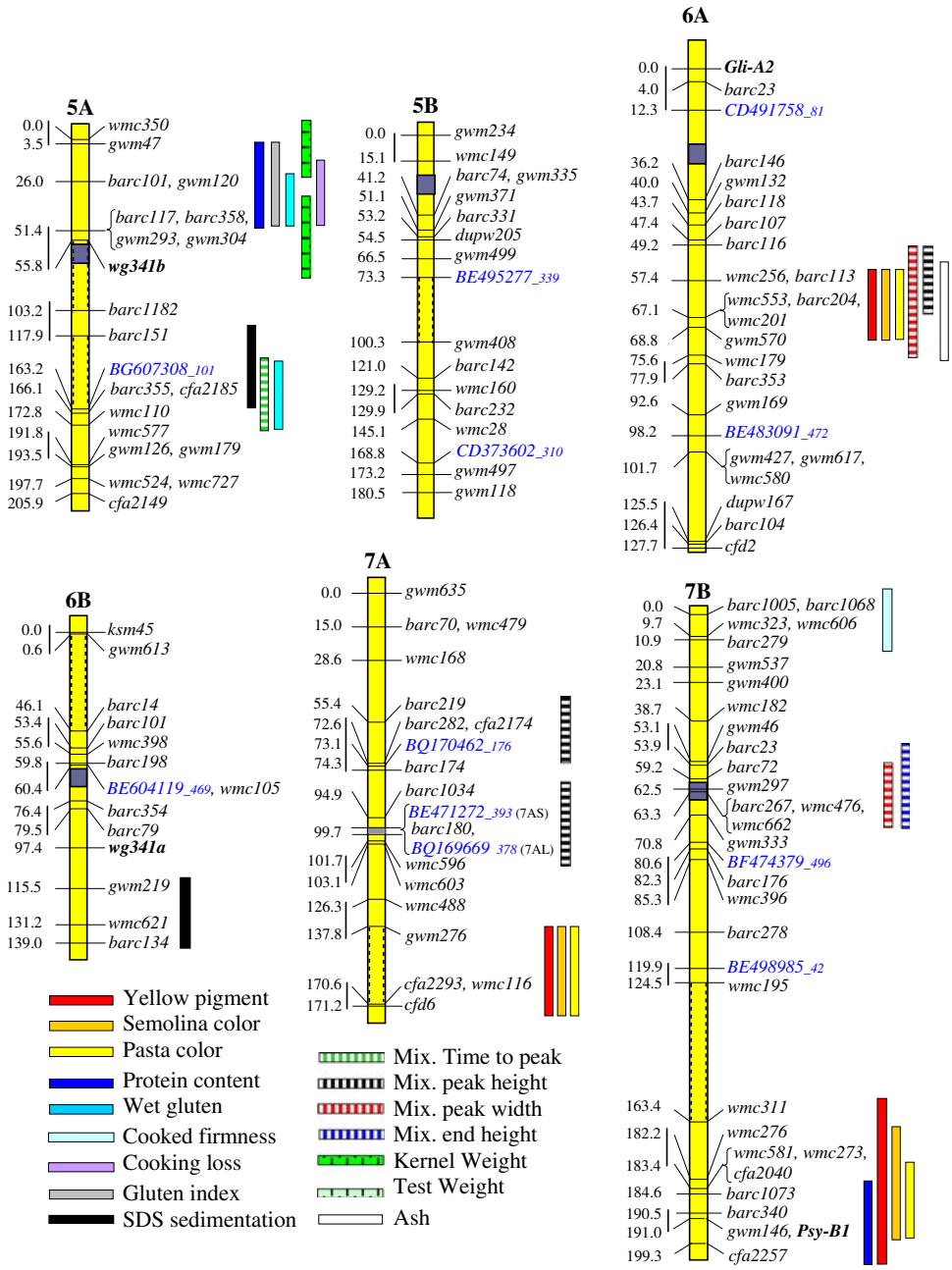
Fig. 1 Linkage map of the Kofa × UC1113 population. Map positions are given in cM. *Red* morphological marker (Black Glume); *Blue* SNP markers; *Bold* RFLP, STS and protein markers, rest SSR markers. Distances connected by a *vertical line* on the left side of the map indicate marker orders supported by LOD scores < 2.0. *Dotted lines* in the chromosome indicate distantly linked markers (LOD < 2.0), whose position is supported by independent information from nulli-tetrasomic analyses, physical mapping, or similar location in other maps (see ESM)

mapped markers. Unlinked markers known to belong to the same chromosome by nulli-tetrasomic analyses or by previous mapping studies were mapped at lower LOD scores and those connections were indicated in the maps by dotted lines and described in detail in the Electronic Supplementary Material (ESM). Since all markers (except *Bla* and *Glu-B1*) used in this study were DNA markers, the *X* preceding the marker name and used to indicate marker type (McIntosh et al. 2003) was omitted from text and figures.

QTL analysis

Windows QTLCartographer (Wang et al. 2004) was used to conduct composite interval mapping with a 1-cM walking speed and a 10-cM window size. QTLs were detected by Composite Interval Mapping (CIM) as implemented in QTLCartographer, Model 6, with five markers and a 10-cM window. We used a LOD threshold of 2.5 for individual traits, which is above the average LOD 2.4 calculated from

Fig. 1 continued



ten permutations using a significance level of 0.01. The average of the five locations for each trait was also used for a combined QTL analysis.

In addition to the CIM analyses, we performed separate factorial ANOVAs for each trait including environment, loci at the peak of significant QTL, and all possible two way interactions in the models. For highly correlated traits, loci significant for at least one of the traits were included in the ANOVA model. Epistatic interactions among QTLs and variance component of the different QTL and QTL interaction were analyzed using SAS version 9.1 PROC GLM and PROC VARCOMP (SAS Institute 2006).

Quality traits

Grain protein content was determined using an Infratec 1226 Grain Analyzer (Foss Analytical, Hoganas, Sweden). Test weight (TWT) was determined according to AACC Method 55-10 (AACC 2000). Thousand-Kernel weight (TKW) was determined by counting the number of kernels in 10 g of clean seed using an electronic seed counter. The weight of 1,000 kernels was calculated.

Milling and pasta processing procedures were described in detail before (Carrera et al. 2007). Briefly, durum was milled to semolina using a Bühler experimental mill fitted

with two Miag laboratory scale purifiers (Bühler-Miag, Minneapolis, MN, USA). Hydrated semolina was extruded under vacuum as spaghetti using a DeMaCo semi-commercial laboratory extruder (DeFrancisci Machine Corp, Melbourne, FL, USA). Spaghetti was dried in a laboratory pasta drier (Standard Industries, Fargo, ND, USA) using a low temperature (40°C) drying cycle.

Grain yellow pigment content (GYPC) was determined by extracting carotenoid pigments from 0.5 g of integral flour using 1.5 ml of water-saturated *n*-butanol for 1 h. After centrifugation (13,000 rpm, 5 m) the supernatant was collected and light transmission at 448-nm was measured using a spectrophotometer (Du6400). Water-saturated *n*-butanol was used as a control. Semolina (SC) and dry spaghetti (PC) color (yellowness) was quantified as CIE *b*-values using a colorimeter (Minolta chromameter model CR310, Minolta Corp., Ramsey, NJ) and are the average of three separate measurements. To minimize the effect of semolina particle size on CIE *b*-values, all grain samples were milled on the same mill by the same miller.

Semolina ash content (ASH) and wet gluten content (WG) were determined by AACC Methods 08-01 and 38-12, respectively. Gluten strength, was evaluated by the SDS micro-sedimentation (SDSS) test (Dick and Quick 1983), by the Gluten Index method (GI, AACC Method 38-12), and by using mixing curves from mixograph tests (AACC Method 54-40A). Mixogram results were reported as time to peak (TTP), peak height (PkHT), peak width (PkWd), and end height (EdHt). TTP was reported in minutes while PkHt, PkWd, and EdHt were reported in mm.

Spaghetti (10 g, 5 cm long) was cooked in 300 ml boiling distilled water for 12 m. The water was drained and the spaghetti was allowed to cool for 3 m. Cooked firmness (CFN) was determined as described by Walsh and Gilles (1971). Cooked firmness was measured as the work required to shear five cooked strands of spaghetti at a right angle using a specially designed plexiglass tooth fitted to TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Cooking loss (CL) was the percent weight of solids lost after evaporating the cooking water to dryness at 110°C in a forced air oven.

Results

Genetic map

A total of 269 markers were mapped including 230 SSR, 23 SNP, 10 RFLP, 3 STS, 2 proteins, and 1 morphological marker (Fig. 1). The number of markers per chromosome varied from 12 to 32 with an average of 19 markers per chromosome. As expected, microsatellite markers were more polymorphic (18.6%) than SNP markers (8.4%). In addition to the new SNP markers, 22 microsatellite markers not mapped before were added to the maps (Table S1, table and figure numbers preceded by an S belong to the ESM). Most SSR markers mapped to the same chromosome locations where they have been mapped before, but 15 were mapped on different chromosome (Table S1). The relative order of markers in our map was very similar to that found in previously published maps, and the exceptions are discussed in the ESM.

The total length of the map was 2,140 cM (Fig. 1) with an average chromosome length of 153 cM. In this map chromosome 4B was the shortest (98 cM) and 5A the longest (206 cM). In spite of our efforts to select markers evenly distributed across the chromosomes, some regions showed no polymorphisms suggesting the presence of chromosome segments that were identical by descent. The number of non-polymorphic SSR markers tested in each of these regions was described in the ESM. Markers on both sides of a gap that were mapped to the same chromosome arm by nulli-tetrasomic analysis or that were previously mapped at similar distances were assigned to the same linkage group and connected by dotted lines (Fig. 1).

QTL analyses

Yellow pigment, semolina and pasta color

Yellow pigment (GYPC), semolina color (SC) and pasta color (PC) traits were highly correlated. Across the five environments GYPC showed highly significant correlations ($P < 0.0001$) with SC ($R = 0.74$) and PC ($R = 0.66$). The correlations between SC and PC were relatively greater ($P < 000.1$, $R = 0.82$). All three color parameters showed a significant ($P < 0.001$) negative correlation with TKW (GYPC $R = -0.28$, SC $R = -0.22$, PC $R = -0.17$) and TWT (GYPC $R = -0.24$, SC $R = -0.28$, PC $R = -0.25$) suggesting that differences in grain size and shape might have affected grain pigment concentration in this segregating population.

We identified four major overlapping QTL for GYPC, SC, and PC on chromosomes 1B, 6AL, 7AL and 7BL and a smaller one with a larger effect on pasta color on chromosomes 4B (Fig. 1, Table 1 and Fig. S1). The QTL peaks for GYPC, SC and PC on chromosome 6A were all mapped between *barc113* and *gwm570* (Figs. 1, 2). This QTL showed LOD scores above the 2.5 threshold for all five environments (Table 1). The overlapping QTL for GYPC, SC and PC on chromosomes 7A and 7B were both located on the telomeric region of the long arm (Figs. 1, 2) and were significant for most of the environments (Table 1). The *Psy-B1* locus was mapped at the peak of the 7BL QTL. A more variable QTL was detected on chromosome 1B between markers *wmc626* and *barc302*. This QTL was in

Table 1 QTL with significant effects in at least two locations (*values in italics*)

Chr	% of explained variation						Peak (cM)	Interval (cM)	Flanking markers	Allele high
	03D	04D	05I	06D	06I	Average				
GYPC										
1B	*0.08*	*0.20*	0.01	*0.13*	0.02	0.09	46	38–60	*wmc626-barc302*	Kofa
6A	*0.11*	*0.12*	*0.15*	*0.11*	*0.21*	0.14	66	60–68	*barc113-gwm570*	Kofa
7A	*0.30*	*0.19*	*0.20*	*0.14*	*0.27*	0.22	171	163–171	*gwm276-cfd6*	UC1113
7B	0.07	0.03	*0.08*	*0.06*	*0.09*	0.07	191	174–197	*wmc311-cfa2257*	Kofa
SC										
4A	0.02	*0.05*	0.04	*0.12*	0.01	0.05	127	123–128	*barc327-barc52*	Kofa
6A	0.08	*0.08*	*0.17*	*0.07*	*0.16*	0.11	65	59–68	*barc113-gwm570*	Kofa
7A	*0.16*	*0.31*	*0.21*	*0.35*	*0.23*	0.25	171	166–171	*gwm276-cfd6*	UC1113
7B	*0.15*	*0.13*	*0.10*	*0.13*	*0.15*	0.13	191	180–193	*wmc276-gwm146*	Kofa
PC										
1B	0.02	*0.11*	0.03	*0.08*	0.01	0.05	46	34–58	*wmc626-barc302*	Kofa
4B	*0.09*	*0.08*	0.02	0.02	0.01	0.04	13	6–17	*wmc617-BE446304*	Kofa
6A	*0.18*	*0.13*	*0.16*	*0.12*	*0.23*	0.16	67	64–69	*barc113-gwm570*	Kofa
7A	0.05	*0.16*	*0.15*	*0.20*	*0.21*	0.15	171	162–171	*gwm276-cfd6*	UC1113
7B	*0.16*	*0.13*	*0.18*	*0.14*	*0.10*	0.14	191	185–193	*barc1073-gwm146*	Kofa
TKW										
1A	NA	*0.10*	0.00	*0.11*	0.00	0.05	75	43–86	*wmc24-BM140362*	UC1113
5A	NA	*0.11*	0.02	*0.14*	0.06	0.08	10	0–18	*wmc350-barc101*	UC1113
5A	NA	*0.09*	*0.23*	0.07	*0.22*	0.15	62	37–75	*barc101-barc1182*	UC1113
TWT										
1B	NA	*0.10*	0.06	*0.12*	0.00	0.07	46	43–53	*wmc85-barc302*	UC1113
2B	NA	*0.19*	*0.15*	0.00	*0.22*	0.14	17	10–20	*wmc154-gwm429*	UC1113
GPC										
5AS	0.04	0.12	0.05	*0.11*	*0.16*	0.10	32	23–41	*gwm47-barc117*	Kofa
7B	0.00	*0.12*	*0.11*	0.01	0.05	0.06	191	184–199	*barc1073-cfa2257*	Kofa
WG										
5AS	*0.18*	*0.17*	*0.21*	*0.17*	*0.18*	0.18	32	23–42	*barc101-barc117*	Kofa
5AL	*0.12*	*0.21*	*0.22*	0.03	0.02	0.12	165	167–191	*BG607308-wmc577*	Kofa
CFN										
7B	0.00	*0.18*	*0.23*	0.03	*0.12*	0.11	8	0–14	*barc1005-gwm537*	Kofa
CL										
5A	*0.24*	0.03	*0.16*	0.08	0.21	0.14	41	33–51	*barc101-barc117*	UC1113
SDSS										
1A	*0.11*	0.02	0.04	*0.23*	0.05	0.09	70	59–81	*barc148-BM140362*	Kofa
1B	*0.20*	*0.15*	*0.13*	0.07	0.03	0.12	83	74–95	*barc181-psr162*	Kofa
5AL	0.01	*0.10*	0.01	*0.22*	0.01	0.07	165	143–173	*barc151-wmc110*	UC1113
6B	*0.18*	*0.12*	*0.23*	*0.14*	0.01	0.14	134	123–138	*gwm219-barc134*	Kofa
GluI										
1A	*0.28*	0.1	0.02	*0.19*	0.02	0.12	71	60–81	*barc148-BM140362*	Kofa
5AS	0.05	*0.14*	*0.14*	*0.12*	0.02	0.09	37	10–49	*gwm47-barc117*	UC1113
TTP										
1B	0.04	0.07	0.01	*0.10*	*0.14*	0.07	83	72–91	*barc181-psr162*	Kofa
5A	*0.16*	0.03	0.07	0.05	*0.10*	0.08	173	164–191	*BG607308-wmc577*	UC1113
PkHt										
6A	0.01	*0.07*	*0.11*	*0.10*	*0.17*	0.09	57	52–65	*barc116-wmc553*	Kofa
7A	0.02	*0.08*	*0.13*	0.01	0.01	0.05	73	59–74	*barc219-barc174*	UC1113
7A	0.01	*0.13*	*0.16*	*0.16*	*0.14*	0.12	100	97–101	*barc1034-wmc596*	Kofa

Table 1 continued

Chr	% of explained variation						Peak (cM)	Interval (cM)	Flanking markers	Allele high
	03D	04D	05I	06D	06I	Average				
PkWd										
6A	0.00	*0.13*	*0.12*	0.02	0.02	0.06	62	58–70	*barc116-wmc179*	Kofa
7B	0.00	0.03	0.02	*0.10*	*0.11*	0.05	62	60–63	*barc72-barc267*	Kofa
EdHt										
7B	0.00	0.04	*0.12*	*0.17*	*0.16*	0.10	62	54–67	*barc23-gwm333*	Kofa
Ash										
1B	0.00	NA	*0.08*	0.04	*0.13*	0.06	83	75–88	*barc181-psr162*	Kofa
6A	0.02	NA	*0.08*	0.01	*0.12*	0.06	67	60–74	*wmc256-wmc179*	Kofa

Values for columns 2–6 represent the % of variation (R^2) explained by the marker at the peak of the QTL, followed by the average of the five locations. The position of the peak of the QTL is followed by the coordinates of a 1-LOD confidence interval and the closest flanking markers. The allele contributing the larger values for each trait is indicated in the *last column*

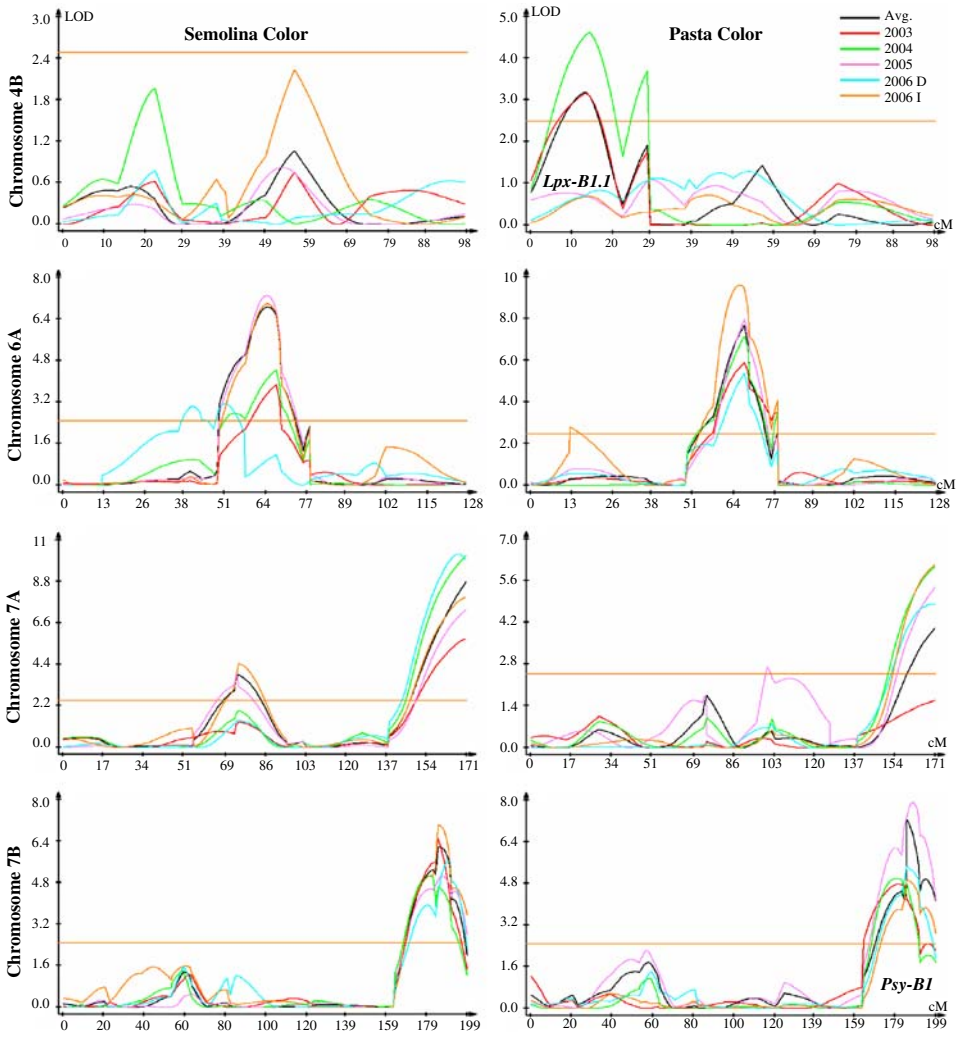


Fig. 2 QTL for semolina color (SC) and pasta color (PC) for chromosomes 4B, 6A, 7A and 7B. QTL for grain yellow pigment content (GYPC, data not shown) were similar to those for semolina color (Table 2)

the same region as a QTL for test weight (Fig. 1) suggesting possible dilution or concentration effects due to grain size.

The QTL on chromosome arm 4BS showed a significant peak at the *Lpx-B1* locus for PC but not for YP or SC (Fig. 2). A minor QTL for semolina color was detected in

Table 2 Analyses of variance for color traits

Variance component	Yellow pigment		Semolina color		Pasta color	
	Variation (%)	*P*	Variation (%)	*P*	Variation (%)	*P*
Environment	10.6	****	4.7	****	4.4	****
QTL						
1B (*barc240*)	8.8	****	10.7	****	10.3	****
4AS (*Lpx-A3*)	5.7	*	7.1	**	NS	NS
4AL (*psr573.1*)	0.6	***	1.0	****	0.0	**
4B (*Lpx-B1.1*,)	2.2	**	1.7	*	16.3	****
6A (*wmc553*)	13.9	****	15.9	****	21.0	****
7A (*wmc116*)	15.0	****	19.5	****	10.4	****
7B (*Psy-B1*)	7.7	****	12.3	****	14.1	****
Interactions						
ENV*4AL	0.6	*	NS	NS	NS	NS
ENV*7A	1.8	*	NS	NS	NS	NS
1B*4AL	0.0	*	0.0	*	NS	NS
1B*6A	0.0	***	NS	NS	NS	NS
1B*7A	2.6	***	NS	NS	NS	NS
4AS*4AL	0.0	****	0.0	****	0.7	****
4AS*6A	NS	NS	NS	NS	0.4	*
4AL*4B	0.0	*	NS	NS	NS	NS
4B*6A	0.0	**	NS	NS	NS	NS
4B*7B	NS	NS	NS	NS	0.0	*
6A*7A	0.9	*	2.2	**	2.9	***
6A*7B	4.6	*	NS	NS	NS	NS
Total explained variance	70		71		73	

The ANOVA model included environment, all major and minor QTL and all possible two way interactions. For each trait the *first column* indicates the percent of variation contributed by the factor (estimated by SAS PROC VARCOMP) and the *second column* the significance of the differences (*NS* $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$). Only the factors and interactions that were significant for at least one trait are shown. Complete ANOVA tables are available in Tables S2–S7

two environments on the distal region of chromosome 4AL. This region showed a significant peak for one environment for GYPC and non-significant peaks for PC (Fig. S1). Another QTL with small effects on all three traits showed a peak at the *Lpx-A3* locus on 4AS (Fig. S1).

Kofa showed higher GYPC, SC and PC values than UC1113 ($P < 0.01$), and contributed the alleles for improved GYPC, SC and PC for the QTL located on chromosomes 1B, 6A, and 7B. UC1113 contributed the positive alleles for the QTL on chromosome arms 4AL and 7AL. The QTL on chromosome 4BS showed a more complex pattern with Kofa contributing the allele with a large positive effect on PC, and UC1113 the allele with a small positive allele on GYPC and SC. The means of the UC1113 and Kofa alleles for each locus are described in Tables S3 (GYPC), S5 (SC) and S7 (PC).

A factorial ANOVA including environment, major and minor QTL, and all possible two way interactions, explained a large portion of the variation in GYPC (70%), SC (71%) and PC (73%). The QTL on chromosome arms 6AL and 7AL explained the largest proportion of variation for all three color traits, followed by the QTL on chromosomes 1B and 7B (Table 2). The QTL on the distal region of chromosome 4AL was significant for all traits ($P < 0.01$, Fig. S1) but explained 1% or less of the variation (Table 2). The 4B QTL with a peak at the *Lpx-B1* locus explained a large proportion of the variation in pasta color (16.3%) but less than 3% of the variation in GYPC and SC. The *Lpx-A3* locus on chromosome 4A showed a significant effect ($P < 0.05$) on GYPC and SC but not on PC (Table 2). The combined two way interactions explained 18.1% of the variation in GYPC, 12.5% of the variation in SC and 11.0% of the variation in PC. The QTL interactions 6A × 7A and 4AS × 4AL were consistent across all traits (Table 2).

Test weight and thousand-kernel weight

Thousand-kernel weight and TWT were not recorded in 2003 but were measured in the next four field trials (2004–2006). A significant correlation was observed between the TWT and TKW values across the four environments ($R = 0.46$, $P < 0.0001$). This correlation was significant for each of the 4 years ($R = 0.25$–0.60, $P < 0.01$), suggesting a consistent relationship.

TKW

Thousand-kernel weight values for UC1113 were slightly higher (54.2 ± 2.0 g) than those from Kofa (51.7 ± 1.2 g) but the differences were not significant ($P > 0.05$). Only three QTL for TKW were consistent for at least two locations, one

on chromosomes 1A and two on chromosome 5A (Table 1; Fig. 1). The QTL on chromosome 1A has a peak at *barc83* in the centromeric region of the long arm. The peaks of the two QTL on chromosome 5A were more than 50 cM apart, one in the short arm between markers *wmc350* and *barc101* and the other one in the centromeric region between markers *barc101* and *barc1182* (Table 1; Fig. 1). UC1113 contributed the alleles for larger grains for the three QTL (Table S9).

The factorial ANOVA including QTL and environments showed highly significant effects for environment and loci ($P < 0.001$), but no significant interactions (Table S6). This model explained only 30% of the variation in TKW, with the QTL on chromosome 5A contributing a larger proportion of variation (5AS: 11.1%, 5A centromeric 8.6%, Table S8) than the one on chromosome 1A (2.0%).

TWT

Test weight values from UC1113 (83.1 ± 0.6 kg/hl) were significantly higher ($P = 0.017$) than those from Kofa (80.2 ± 0.4 kg/hl). Significant QTLs for TWT were identified on chromosomes 1BL (2 environments) and 2BS (3 environments, Table 1; Fig. 1). A factorial ANOVA including environment, loci, and all two way interactions explained 29% of the variation in TWT. The 2B QTL showed a larger effect (11.8% variation) than the 1B QTL (4.1% variation). The interaction between the two loci was highly significant ($P < 0.0001$) and explained a large proportion of the variation (12.6%, Table S10). UC1113 contributed the positive alleles for the two QTL (Table S11).

Grain protein content (GPC), wet gluten (WG), cooked firmness (CFN) and cooking loss (CL)

Traits GPC, WG, CFN and CL were highly correlated. Across the five environments GPC showed highly significant correlations ($P < 0.0001$) with WG ($R = 0.73$) and CFN ($R = 0.71$). The correlations between WG and CFN were also highly significant ($P < 000.1$, $R = 0.74$). The three previous parameters showed a significant ($P < 0.001$) negative correlation with CL (GPC $R = -0.42$, WG $R = -0.38$, and CFN $R = -0.46$). All these correlations were highly significant every year ($P < 0.01$) suggesting a consistent relationship between these parameters. Therefore, we used a common set of markers to analyze these four traits (Tables S12–19).

We identified four QTL for GPC, WG, CFN and CL, two on chromosome 5A and two on chromosome 7B (Table 1; Figs. 1, 3). The two QTL within each of these two chromosomes are more than 100 cM apart, and show similar peaks for GPC, WG and CFN (Fig. 3). For all four loci, Kofa contributed the alleles with positive effects (higher GPC, WG and CFN, and lower CL, Tables S13, S15, S17 and S19).

The ANOVA model including environment and loci explained 50–59% of the variation in GPC, WG, and CFN, but a lower proportion of the variation in CL (17%),

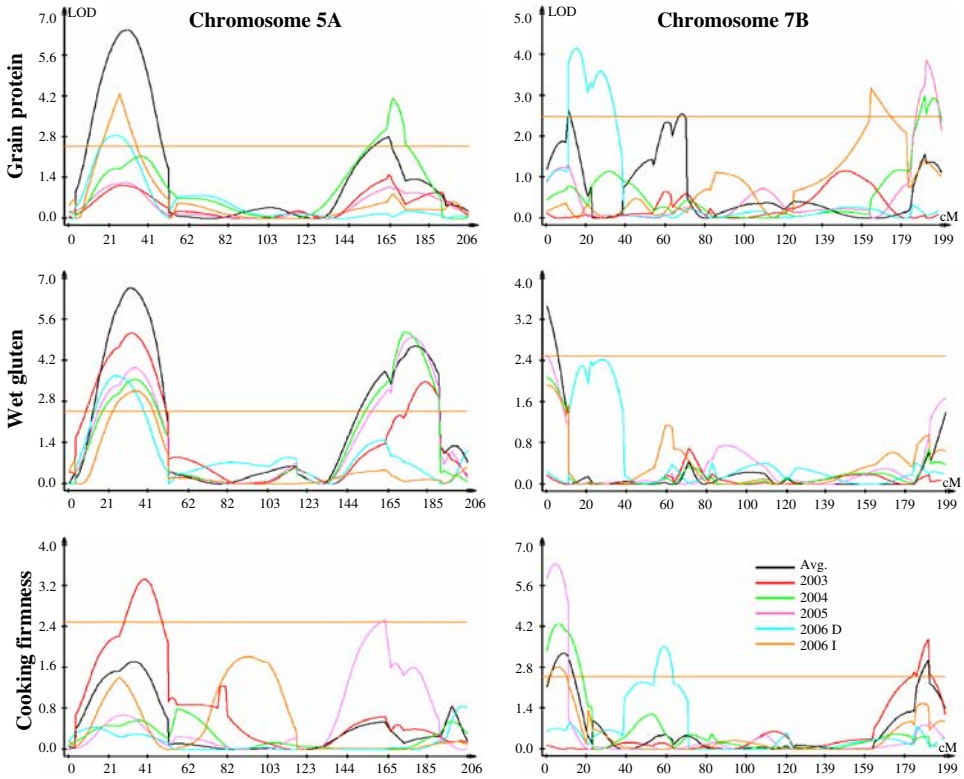


Fig. 3 QTL for grain protein content (GPC), wet gluten (WG), and cooked firmness (CFN) for chromosomes 5A and 7B. For QTL statistics see Table 3

suggesting that the last parameter is more variable (Table 3). The four loci included in these analyses (except the 7BS QTL for WG) showed significant differences between UC1113 and Kofa for GPC, WG and CFN (Table 3). Differences in CL were significant only for the QTL on 7BL, which explained the highest proportion of variation for the other three traits (Table 3 and S18).

To analyze the effect of protein on CFN and CL in more detail we performed an analysis of covariance (ANCOVA) using GPC as a covariable (Table S16). The ANCOVA analysis removes the effect of GPC. The significant differences in CFN between alleles found at the 5AL and 5AS loci were not significant in the ANCOVA. In addition the significance of the 7BL QTL was reduced (Table S16). This was also observed for CL (Table S18). These results further confirm the dependence of the CFN and CL QTL on the GPC differences. An additional example of the close relationship between CFN and protein content (GPC and WG) was the presence of a strong QTL for GPC, WG and CFN (LOD > 6) on the same region of the short arm of chromosome 3B (10–18 cM) only for year 2003 (Fig. S2).

Gluten index (GI) and SDS micro-sedimentation (SDSS)

Kofa showed significantly higher SDSS ($P = 0.002$, 30% increase) and GI ($P = 0.03$, 50% increase) than UC1113. The correlation between these two parameters over the five environments was highly significant ($R = 0.59$, $P < 0.0001$).

Four QTL that were significant for at least two environments were detected on chromosomes 1A, 1B, 5AL and 6B for SDSS and on chromosomes 1A and 5AS for GI (Fig. 1; Table 1). Kofa contributed the alleles for stronger gluten for chromosomes 1A, 1B and 6B, whereas UC1113 contributed the positive alleles for the two QTL on chromosome 5A (Tables S21 and S23). The peak of the QTL on chromosome 1B was located at the high molecular weight glutenin locus *Glu-B1*, and the peaks for the two QTL on chromosome 5 were in similar locations to those reported for GPC and WG above.

An ANOVA model including environment and loci explained 63% of the variation in SDSS and 65% of the variation in GI. Both traits showed highly significant differences between the UC1113 and Kofa alleles for the QTL on chromosomes 1A, 1B, 5AL, and 6B, but only SDSS was significant for the 5AS QTL (Table 4). The QTL on chromosomes 1A, 1B and 6B explained the largest proportion of variation on SDSS (11–15%) and GI (4–8%, Table 4). The significant interactions explained only a small proportion of the variation (<5%, Table 4).

The ANCOVA analyses for SDS and GI using GPC as covariable (Tables S20 and S22) showed almost identical *P* values in the ANOVA and ANCOVA indicating a limited effect of GPC on the QTLs detected for SDS and GI (Tables S20 and S22). Only the interaction between the QTL on 5AS and 5AL changed form marginally significant in the ANOVA ($P = 0.0454$) to marginally non-significant in the ANCOVA ($P = 0.0608$, Table S20).

Mixogram parameters

Time to peak (TTP)

Two consistent QTL were detected on chromosomes 1B and 5A for TTP (Fig. 1; Table 1). The QTL on chromosome

Table 3 Analyses of variance for protein content and cooking quality traits

Source	GPC		WG		CFN		CL	
	Variation (%)	*P*	Variation (%)	*P*	Variation (%)	*P*	Variation (%)	*P*
Environment	36.4	****	38.8	****	36.7	****	8.9	****
QTL								
5AS (*barc101*)	3.1	**	6.5	****	2.4	*	NS	NS
5AL (*barc355*)	3.3	**	6.7	****	1.3	**	NS	NS
7BS (*barc279*)	0.4	*	NS	NS	3.4	**	NS	NS
7BL (*Psy-B1*)	8.3	****	12.0	****	9.8	****	6.4	****
Interactions								
5AS*7BS	1.5	*	3.6	***	NS	NS	NS	NS
5AS*7BL	3.7	*	NS	NS	NS	NS	0.0	*
5AL*7BS	0.0	*	0.0	*	NS	NS	NS	NS
7BL*7BS	NS	NS	0.5	*	NS	NS	2.7	*
Explained var.	54		59		50		17	

The ANOVA model included environment all major and minor QTL and all possible two way interactions. For each trait the *first column* indicates the percent of variation contributed by the factor and the *second column* the significance of the differences (*NS* $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$). Only the factors and interactions that were significant for at least one trait are shown. Complete ANOVA tables are available in Tables S12–S19

Table 4 Analyses of variance for gluten strength traits

	SDSS		GI	
	Variation (%)	*P*	Variation (%)	*P*
Environment	28.0	****	43.1	****
QTL				
1A (*wg983*)	10.6	****	9.8	****
1B (*Glu-B1*)	8.2	****	1.2	****
5AS (*barc117*)	0.3	**	NS	NS
5AL (*barc355*)	0.3	***	0.8	***
6B (*wmc621*)	16.2	****	6.2	****
Interactions				
ENV*5AS	NS	NS	2.2	*
1A*1B	NS	NS	0.0	*
1A*6B	NS	NS	0.0	**
1B*5AL	3.8	***	NS	NS
5AS*5AL	0.7	*	2.1	**
5AL*6B	NS	NS	0.0	**
Explained var.	63		65	

The ANOVA model included environment all major and minor QTL and all possible 2 way interactions. For each trait the first column indicates the percent of variation contributed by the factor and the second column the significance of the differences (NS = $P > 0.05$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, and **** = $P < 0.0001$). Only the factors and interactions that were significant for at least one trait are shown. Complete ANOVA tables are available in Tables S20–23

1B showed a peak at the *Glu-B1* locus and the one at chromosome 5A at the *wmc110* locus. Kofa contributed the allele for high TTP for the QTL on chromosome 1B and UC1113 the one for the QTL on chromosome 5A (Table S25). These two QTL were at similar locations to those detected for SDSS, explaining the good correlation observed between TTP and SDSS ($R = 0.44$, $P < 0.0001$ across all years).

Although the ANOVA including environment and loci explained 49% of the variation in TTP (Table S24), most of this variation was originated in differences among environments. The QTL on chromosome 1B explained 4.2% of the variation and the one on chromosome 5A only 1.6% of the variation (Table S24). The interaction between these two loci was marginally significant ($P = 0.04$).

Peak height (PkHt), peak width (PkWd), and end height (EdHt)

Peak height was highly correlated with PkWd ($R = 0.71$, $P < 0.0001$) and EdHt ($R = 0.75$, $P < 0.0001$). A significant correlation ($R = 0.72$, $P < 0.0001$) was also observed between the last two parameters. Therefore, all the markers showing significant QTL in any of these markers were included in the combined ANOVA analysis.

We observed one QTL on chromosomes 6A, one on 7B, and three separate peaks on chromosome 7A (Fig. S3). The QTL peak on chromosome 6A was similar for all three traits, but only PkHt and PkWd were significant for more than 2 years (Table 1, Fig. S3). The QTL on chromosome 7B was significant for more than 2 years for PkWd and EdHt (Table 1, Fig. S3).

The QTL for PkHt on the long arm of chromosome 7A was significant for four of the five environments (Table 1). Peaks for the other two traits were detected at the same position but were significant only for 2004 (Table 1, Fig. S3). Two additional peaks were detected on the short arm of chromosome 7A and were designated Q7AS1 (peak at 73 cM) and Q7AS2 (peak at 29 cM). Although only Q7AS1 was significant in two environments, Q7AS2 was retained for the ANOVA analyses because of the consistency of the peaks across years and environments for all three traits (Fig. S3). Kofa contributed the alleles for higher PkHt, PkWd and EdHt for all the loci except for Q7AS1 (Tables S27, S29, and S31).

The complete ANOVA model including environment, loci and interactions explained only a limited proportion of the variation in PkHt (34%), PkWd (26%), and EdHt (39%). The QTL on 7AL and 7AS2 and 7B explained the largest proportion of the variation (5–16%) with the QTL on chromosomes 6A and 7AS1 playing a smaller role (<6%). Few interactions were significant, and they contributed individually less than 2% of the variation (Tables S26, S28, and S30).

The ANCOVA analyses for the mixogram parameters using GPC as covariable (Tables S24, S26, S28 and S30) showed almost identical *P* values in the ANOVA and ANCOVA indicating a limited effect of GPC on the QTLs detected for TTP, PkHt, PkWd, and EdHt (Tables S24, S26, S28 and S30). The *P* value for the effect of environment on PkHt was the only one that showed altered significance in the ANCOVA relative to the ANOVA.

Ash content

Two QTL were detected for ASH content on chromosomes 1B and 6A. The 1B QTL had a peak on *cfa2129b* and overlapped with the QTL for SDSS and mixing time to peak. The 6AQTL showed a peak on *wmc553* and overlapped with QTL for mixing peak height and width.

An ANOVA including both QTL and environments explained only 31% of the variation, and most of it was due to differences between environments. The QTL on 1B explained 6.2% of the variation and the one in 6A only 2.2% and the interaction between these QTL was not significant (Table S33). Kofa contributed the alleles for high ASH content for both QTL (Table S34).

Discussion

Genetic map and SNP markers

The genetic map presented here complements the information of previously published durum maps (Elouafi and Nachit 2004; Maccaferri et al. 2008; Nachit et al. 2001; Patil et al. 2008; Peleg et al. 2008; Pozniak et al. 2007) and adds 22 microsatellite markers not mapped before in common or durum maps. More importantly, this map integrates for the first time SNP and microsatellite markers in durum wheat. Gene-derived SNP markers, as the ones used in this study, are particularly valuable because they can be used as anchor points for comparative studies with the sequenced genomes of other grasses as previously done with RFLP markers (Sorrells et al. 2003). These comparative maps are useful to generate markers in targeted regions and to identify candidate genes (Yan et al. 2003).

Out of the 275 SNP markers screened, we were able to find polymorphisms and map only 23 of them, indicating a low level of polymorphisms (8.4%) relative to SSR markers (18.7%). The level of SNP polymorphism detected between Kofa and UC1113 was similar to the one observed for ND durum parental lines Rugby and Maier using the same set of SNPs (8.8% polymorphism) in a screening of different US varieties (S. Chao unpublished), suggesting that these are not unusual values for durum crosses between adapted lines. The level of SNP polymorphisms observed between pairs of durum accessions was almost half of that found between adapted hexaploid wheat lines (16.4%) using a set of 359 SNPs which included the 275 used in this study (Chao et al. 2008). One factor that might have contributed to this lower level of polymorphisms in durum wheat is that this set of SNP markers was pre-selected for polymorphisms discovered in a set of hexaploid wheat varieties. An unbiased comparison between SNP polymorphism in pasta and bread wheat would require the use of a set of SNP markers selected for polymorphism in both pasta and common wheat.

Pasta color

Grain yellow pigment content is relatively easy to determine and therefore several studies have analyzed the genetic control of this trait in pasta wheat (Elouafi et al. 2001; Patil et al. 2008; Pozniak et al. 2007; Zhang and Dubcovsky 2008). However, GYPC is just a predictive test of the final pasta color, which to our knowledge, has not been directly analyzed in previous QTL studies. As shown in this study, the direct analysis of pasta color has the potential to reveal additional loci not detected by GYPC.

Pasta color is dependent not only of the accumulation of lutein and other carotenoid pigments, but also of their oxidative degradation by lipoxygenase activity (Borrelli et al. 1999; Troccoli et al. 2000). We have recently shown that a deletion of the *Lpx-B1.1* copy in Kofa is associated with a 4.5-fold reduction in lipoxygenase activity in the grain (Carrera et al. 2007). Since the *Lpx-B1* locus maps to the peak of the 4B QTL for pasta color, we hypothesize that the improved pasta color associated with the Kofa allele might be the result of reduced pigment degradation during pasta processing. A final validation of the causal role of *Lpx-B1* on this PC QTL will require the analysis of mutants or transgenic lines for this gene. It is currently not possible to determine if the small increase in GYPC and SC associated with the presence of the UC1113 allele at this locus (Tables S3 and S5) is a pleiotropic effect of *Lpx-B1.1* or the result of a separate polymorphism in the duplicated *Lpx-B1.2* copy or in other closely linked gene (Carrera et al. 2007).

A second lipoxygenase locus, *Lpx-A3*, was mapped on the homoeologous region on chromosome 4A. This locus has a small effect on GYPC and SC (6–7% of the variation, Table 2) but the effect on pasta color was not significant ($P = 0.08$, Table 2).

The other four QTL for pasta color (1B, 6A, 7A and 7B) were all collocated with QTL for GYPC, SC, suggesting that they are all the result of a larger accumulation of carotenoid pigments in the grain. The QTL for the three color traits on chromosome 1B were in a similar region as the QTL for TWT (and TKW for 1 year, data not shown), suggesting a dilution effect associated with the differences in GYPC, SC and PC. Kofa contributed the allele for improved color but also the allele for smaller TWT, limiting its use for breeding purposes. The QTL on chromosomes 6A, 7A and 7B showed the largest contribution to the variation in pasta color and were not associated with differences in grain size or shape, suggesting a more direct effect on the accumulation of carotenoid pigments.

The distal region of the long arm of chromosome 7 has been repeatedly associated with differences in GYPC. Significant effects on this region have been reported in tetraploid wheat (Elouafi et al. 2001; Patil et al. 2008; Pozniak et al. 2007; Zhang and Dubcovsky 2008), hexaploid wheat (He et al. 2008; Mares and Campbell 2001; Parker et al. 1998; Zhang and Dubcovsky 2008), *Lophopyrum ponticum* (Zhang and Dubcovsky 2008; Zhang et al. 2005), and *Hordeum chilense* (Atienza et al. 2007), suggesting the presence of a major gene affecting GYPC in the Triticeae. Pozniak et al. (2007) suggested that these differences could be the result of variation at the *Phytoene synthase 1* (*Psy-1*) gene, which maps on this chromosome region. The *Psy-1* enzyme is a critical and limiting enzyme in the carotenoid biosynthetic pathway, and its transcript accumulation is also correlated with GYPC in other grasses (Gallagher et al. 2004).

We have recently shown that an ethylmethane sulfonate (EMS) mutant line of the 7EL chromosome from *T. ponticum*

selected for its reduced GYPC content carries a mutation in the *Psy-E1* gene, which codes for an altered amino acid in a conserved position of the protein (Zhang and Dubcovsky 2008). This result provides strong support to Pozniak et al.'s (2007) hypothesis for an important role of *Psy-1* in the determination of GYPC. Additional support to this hypothesis is provided in this study by the mapping of the peak of the 7B QTL for SC and PC on the *Psy-B1* locus. The ANOVA model for SC and PC showed maximum *F* values for the 7B QTL when *Psy-B1* was used as the classification variable, and smaller *F* values for flanking markers *barc340* and *cfa2257* (data not shown). However, GYPC showed a slightly higher *F* value for *barc340* than for *Psy-B1*, which is only 0.5 cM apart (Fig. 1). This slight displacement of the GYPC QTL peak is likely the result of random variation since only one RIL has recombination between these two markers. The Kofa *Psy-B1* allele associated with the improved SC and PC is very characteristic, since it is a hybrid allele originated from a homoeologous conversion event between the *Psy-A1* and *Psy-B1* genes (Zhang and Dubcovsky 2008).

Although the role of *Psy-1* on grain semolina and pasta color seems to be well documented, there seems to be a second linked locus affecting the same traits. This hypothesis is supported by the identification of an independent white endosperm mutant of the line carrying the 7EL translocation, which showed no sequence differences at the *Psy-E1* gene with the wild type allele (Zhang and Dubcovsky 2008). An independent observation supporting the existence of a second gene affecting GYPC is the absence of sequence differences between Kofa and UC1113 in the *Psy-A1* coding and promoter regions (Zhang and Dubcovsky 2008) in spite of the presence of a strong QTL for GYPC, SC and PC QTL on this chromosome region (Fig. 3). It is interesting to point out, that the beneficial allele for this second 7AL locus was contributed by the parent UC1113 with low GYPC, SC and PC.

Current efforts to improve pasta color at the UC Davis durum wheat breeding program are focused on the selection of Kofa alleles for the *Lpx-B1.1* deletion, the hybrid *Psy-B1* allele on 7BL, and the 6A QTL and the UC1113 alleles for the 7A QTL. The RILs having the four beneficial alleles showed Minolta b color values for pasta (48.6) that were 17% higher than those observed in the lines with the four opposite alleles (41.7). A model including only these major four QTL is sufficient to explain 56% of the variation in pasta color in this population. The complete ANOVA model for pasta color explained 73% of the variation, of which 68% was due to genetic differences (Table 2), confirming the high heritability of this trait.

Protein content and pasta firmness

High grain protein content is a critical parameter for pasta quality because of its positive effect on multiple pasta quality parameters (for a review see Troccoli et al. 2000). In our study GPC was significantly ($P < 0.0001$) and positively correlated with WG ($R = 0.73$), CFN ($R = 0.71$), and mixogram PkHt ($R = 0.28$), and negatively correlated with CL ($R = -0.42$), GI ($R = -0.26$), TTP ($R = -0.29$), and TWT ($R = -0.25$). These correlations are consistent with previous studies using different durum cultivars and breeding lines (Peña 2000). Also consistent with previous results is the identification of the QTL with largest effect on GPC (Table 3) on the distal region of the long arm of chromosome 7BL. A QTL explaining a large proportion of the variation in GPC (9.1%) was previously identified on the same chromosome region in a different cross (Blanco et al. 2006). The peak of the previous QTL was mapped at the *gwm577* locus, which has been mapped only 4 cM proximal to *barc340* (Song et al. 2005) located at the GPC QTL in this study (Fig. 1).

Particularly important among the correlations between GPC and pasta quality traits is the significant and positive correlation observed between CFN and GPC (also observed between WG and CFN, $R = 0.74$). Cooked firmness is a complex trait, and its evaluation requires large amounts of seed, pasta production, and cooking tests. Therefore, the determination of CFN predictive traits that can be performed with smaller amounts of seed and at lower costs is useful for pasta breeding programs. In this study, the four loci with significant effects on GPC and WG (ANOVA Tables S12 and S14) were also significant for CFN (Table S16, Fig. 2). The removal of GPC effects from the statistical analyses by using this trait as a covariable in an ANCOVA altered the significance of the CFN and CL QTL (Tables S16 and S18). The strong effect of loci affecting GPC and WG on CFN was further exemplified by the overlap of strong QTL for these three traits on chromosome 3B exclusively in the UC Davis experiment performed in 2003 (Fig. S2). A possible explanation for this QTL occurring only in 2003 is the high impact of stripe rust observed during this year. Differences in rust susceptibility are known to be associated with differences in GPC (Dimmock and Gooding 2002). This hypothesis was supported by the discovery of significant differences in stripe rust infection scores in flag leaves associated with the locus located at the peak of the GPC, WG, and CFN QTL (*gwm493* $P = 0.003$, J. Brevis and J. Dubcovsky unpublished). Regardless of the cause of the 2003 GPC and WG QTL, their perfect overlapping with the CFN QTL provides a compelling example of the association between these parameters.

The CFN values were inversely correlated with those for CL ($R = -0.46$), as expected by the lower losses observed

during cooking of firmer pasta varieties (Grzybowski and Donnelly 1979). Cooking loss was also negatively correlated with GPC and WG, but the absolute values of these correlations were not as strong as those observed for CFN. CL was more variable than CFN and the ANOVA model only explained 17% of the variation in this trait, and only one locus was significant (Table 3).

In summary, GPC and WG seem to be good predictors of CFN in this population and selection for the Kofa loci for higher protein are likely to result in beneficial effects in pasta cooking quality. However, the effect of these alleles on different genetic backgrounds and different pasta drying cycles (high and ultrahigh) remains to be tested. In addition, it should be pointed out that this result was obtained using a low temperature drying cycle and that high or ultrahigh temperature drying cycles can reduce the impact of GPC on CFN. High temperature drying denatures some of the protein in the gluten matrix and improves cooking quality (Novaro et al. 1993).

Gluten strength

One of the distinctive characteristics of Kofa is its very strong gluten. This is a positive characteristic for pasta quality because strong gluten cultivars usually produce pasta with greater after-cooking firmness (Pogna et al. 1990). Although, the gluten strength of UC1113 (GI: 62 ± 12, alveograph *W* value: 133 ± 8) is smaller than that of Kofa (GI: 94 ± 2, alveograph *W* value: 272 ± 24), it is still adequate for pasta production. The absence of alleles with large detrimental effects on gluten strength in this population may explain the smaller correlations observed between pasta cooking quality parameters (CFN and CL) and gluten strength parameters (SDSS $R = 0.04$ and GI $R = -0.28$) relative to ones observed with protein content parameters (GPC $R = 0.71$ and WG $R = 0.74$). The absence of highly contrasting alleles may also explain the lower proportion of variation in SDSS and GI explained by genetic factors relative to the one determined by the environment (Tables S20 and S22).

It has been suggested that GI has a greater range of variation than SDSS, making it a better predictive test for early generation screening (Cubadda et al. 1992). In this study, CFN showed a stronger correlation with GI ($R = -0.28$, $P < 0.0001$) than with SDSS ($R = 0.04$, $P = 0.37$) supporting the previous suggestions. Similarly, GPC showed a stronger negative correlation with GI ($R = -0.26$) than with SDSS ($R = -0.15$), as reported before (Peña 2000). In spite of these differences, a significantly positive correlation was observed between SDSS and GI ($R = 0.60$), and four out of five loci were significant for both traits (Tables S20 and S22), suggesting that both parameters reflect common aspects of the gluten strength. Removal of the GPC effect from the analyses using ANCOVA did not altered the significance of the discovered QTL (Tables S20 and S22) suggesting that these QTLs are more dependent on protein quality than protein quantity.

Protein analyses of the high molecular weight glutenin profiles showed that Kofa has the subunits 6 + 8 (*Glu-B1d* allele) and UC1113 the subunits 7 + 8 (*Glu-B1b* allele) (Conti 2007). SDSS and GI values were 8% higher for the RILs carrying the 6 + 8 subunits than in those carrying the 7 + 8 subunits ($P < 0.0001$, Table S20–S23). This result agrees with a previously published comparison between these two alleles in a different mapping population (Martinez et al. 2005). The *Glu-B1* locus was mapped at the peak of the 1B QTL for SDSS and GI suggesting that it might be a good candidate gene for these QTL. The *F* values for SDSS and GI had a maximum at the *Glu-B1* locus (SDSS $F = 69.6$, GI $F = 20.8$) and decreased at flanking loci *barc181* (SDSS $F = 21.5$, GI $F = 3.3$) and *cfa2129* (SDSS $F = 62.3$, GI $F = 19.3$). Based on these results it is possible to conclude that the locus responsible for the SDSS and GI QTL on chromosome 1B is either *Glu-B1* or a linked locus located between *barc181* and *cfa2129*.

Both Kofa and UC1113 have the null *Glu-A1* allele (Conti 2007). Therefore, the QTL for SDSS and GI found in this region cannot be attributed to differences at the *Glu-A1* locus. Interestingly, the QTL on chromosome 1A overlaps with a QTL for TKW suggesting that there might be some relationship between these effects.

Mixogram parameters

Time to peak (TTP) provides a measurement of the rate of hydration, whereas PkHt, PkWd and EdHt provide an indication of initial and ending dough strength. Therefore, it is not surprising that the correlations between TTP and PkHt ($R = -0.13$, $P = 0.005$), PkWd ($R = 0.07$, $P = 0.12$) and EdHt ($R = 0.29$, $P < 0.001$), were lower than the correlations among the last three parameters ($R = 0.71$–0.75, $P < 0.0001$). Similarly, the QTL for TTP were not overlapping the QTL for the other three parameters (Fig. 1).

The two QTLs for TTP overlapped with the QTL for SDSS suggesting that this parameter is mainly determined by gluten strength. This is further supported by positive and significant ($P < 0.0001$) correlations between TTP and SDSS ($R = 0.44$) and GI ($R = 0.34$, Table S32). In this population, TTP showed a negative correlation with GPC and WG ($R = -0.27$ to -0.29). However, the correlations between TTP and GPC should be interpreted with caution because they vary widely among mapping populations (Martinez et al. 2005). In this study, TTP was not a good predictor of CL and CFN (Table S3).

The other mixogram parameters (PkHt, PkWd, EdHt) showed greater correlations with CFN ($R = 0.21$–0.41) than TTP, suggesting that they are better predictive parameters for pasta cooking quality (Table S3). PkWd and EdHt showed greater correlations with SDSS and GI than TTP and PkHt, which may indicate that the first two parameters are more affected by gluten strength than the last two. Positive and significant correlations between SDSS and mixogram parameters TTP and PkHt have been also reported in other mapping populations (Clarke et al. 2000; Martinez et al. 2005; Ruiz and Carrillo 1995).

In summary, PkHt seems to have the highest predictive value among the mixogram parameters for pasta quality (CFN). TTP seems to be determined mainly by gluten strength and therefore selection for the Kofa allele at the 1B QTL and for UC1113 alleles at the 5AL QTL might result in simultaneous increases in SDSS and TTP. Selection for Kofa alleles at the PkHt, PkWd, EdHt peaks of the 6A, 7AS2, 7AL and 7B QTL and for UC1113 alleles at the 7AS1 QTL should result in simultaneous increases in these three mixogram parameters in this population.

Test weight and thousand kernel weigh

Although in our study TKW and TWT were correlated, TKW is known to be more related to kernel size whereas TWT is known to be more related to kernel shape (Troccoli et al. 2000). Variation in TWT is of particular interest to durum millers because it is usually positively correlated with semolina yield (Matsuo and Dexter 1980; Zeleny 1964), although these relationships can be affected by genotype and environment (Marshall et al. 1986; Troccoli and di Fonzo 1999).

In this study, both parental lines have high TWT values (Kofa 80.2 ± 0.4 kg/hl, UC1113 83.1 ± 0.7 kg/hl) with UC1113 contributing the positive alleles for the two QTLs. However, since the differences between the means of the two alleles at the 1B and 2B QTL were very small ($\leq 1\%$, Table S11), this information will have limited value for breeding programs. Given the small proportion of variation explained by these QTL it is not surprising that none of them overlaps with QTL for TKW or TWT found in a different QTL study (Elouafi and Nachit 2004).

The differences in TKW between Kofa and UC1113 were relatively larger than the ones observed in TWT. UC1113 contributed the alleles for larger kernel weight to the three QTLs located on chromosomes 1A, 5AS and 5AL; and RILs carrying the UC1113 allele (54.3 g) were 13% higher on average than RILs carrying Kofa allele (48.2 g). These results suggest that selection for the three UC1113 alleles may result in significant increases in TKW, in this population.

Ash content

Ash content is an important quality parameter for the durum milling industry. In Italy, for example, ash content must not exceed 0.9% for first grade commercial semolina (Troccoli et al. 2000). The two parents used in this study are below this limit (Kofa 0.69 ± 0.01, UC1113 0.62 ± 0.02) and only a small proportion of the variation in this trait is explained by the QTL detected on chromosomes 1B (6.2% of the variation) and 6A (1.5% of the variation). The Kofa allele for the 1B QTL is associated with a 4.5% increase in average semolina ash content limiting the use of the favorable effect of this region on SDSS and TTP in breeding efforts aimed to improve pasta quality.

In summary, the QTL analysis of the different pasta quality parameters provided an integrated picture of the genetic basis of pasta quality and generated valuable information for durum wheat breeding programs.

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