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QTL analysis of main and epistatic effects for flour color traits in durum wheat

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Abstract The aim of this work was to map quantitative trait loci (QTLs) associated with flour yellow color (Fb*) and yellow pigment content (YPC) in durum wheat (*Triticum turgidum* L. var. *durum*). Additionally, QTLs affecting flour redness (Fa*) and brightness (FL*) color parameters were investigated. A population of 93 RILs (UC1113 × Kofa) was evaluated in three locations of Argentina over 2 years. High heritability values (>94%) were obtained for Fb* and YPC, whereas FL* and Fa* showed intermediate to high values. The main QTLs affecting Fb* and YPC overlapped on chromosome arms 4AL (4AL.2), 6AL (6AL.2), 7AS, 7AL, 7BS (7BS.2) and 7BL (7BL.2). The 7BL.1 QTL included the *Psy-B1* locus, but one additional linked QTL was detected. A novel minor QTL located on 7AS affected Fb*, with an epistatic effect on YPC. An epistatic interaction occurred between the 7AL and 7BL.2 QTLs. The 4AL.2 QTL showed a strong effect on Fb* and was involved in two digenic epistatic interactions. The 6AL.2 QTL explained most of the variation for Fb* and YPC. The main QTLs affecting FL* and Fa* were located on 2BS and 7BL, respectively. These results confirm the complex inheritance of flour color traits and open the possibility of developing perfect markers to improve pasta quality in Argentinean breeding programs.

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Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is used in pasta production due to the quality attributes of its grains, principally protein content, gluten strength and yellow color. Yellow pigment content (YPC) mainly caused by the xanthophyll lutein is the most important determinant of the bright yellow color in semolina (Hentschel et al. 2002). However, the final color of pasta is also influenced by the enzymatic degradation of carotenoids by lipoxygenases (Carrera et al. 2007), with at least two additional enzymes (peroxidases and polifenol oxidases) playing secondary roles (Borrelli et al. 2008). Grain debranning and manufacturing conditions are also key factors affecting yellowness (Matsuo and Dexter 1980; Borrelli et al. 2008). Carotenoids have an antioxidant role by reducing oxidative damage to biological membranes. As precursors of vitamin A, carotenoids are valuable nutritional components of pasta products.

Colorimetric determinations of semolina or flour are often used by breeders as tools to select materials with high yellow color. Alternatively, the quantification of carotenoid pigments and lipoxygenase activity in semolina through spectrophotometric determinations can be used. YPC in grains and yellow color of milling products are considered complex heritable traits controlled by several genomic regions (Clarke et al. 2006). The genetic architecture of these traits has been investigated through quantitative trait locus (QTL) analysis in durum and bread wheat using different mapping populations, and a large number of genomic regions were reported on chromosomes 1A (Patil et al. 2008; Zhang et al. 2009), 1B (Zhang et al. 2009), 2A (Pozniak et al. 2007; Blanco et al. 2011), 3A and 3B (Mares and Campbell 2001; Howitt et al. 2009; Blanco et al. 2011), 4A (Zhang et al. 2008), 5A (Hessler et al. 2002; Blanco et al. 2011), 5B (Patil et al. 2008), 6A (Mares and Campbell 2001; Zhang et al. 2006), 6B (Pozniak et al. 2007), 7A (Mares and Campbell 2001; Patil et al. 2008; He et al. 2008; Singh et al. 2009; Zhang et al. 2009; Blanco et al. 2011) and 7B (Elouafi et al. 2001; Pozniak et al. 2007; Zhang and Dubcovsky 2008). However, epistatic effects (digenic epistasis, QQ) and environmental interactions (QE or QQE) among QTLs affecting YPC and yellow color have been less frequently investigated. Zhang and Dubcovsky (2008) and Singh et al. (2009) reported analyses of epistatic interactions using two-way ANOVA. Several strategies were proposed to study QTL × environment interactions (Piepho 2000), or epistatic effects (Kao et al. 1999; Yi et al. 2003) separately, but it is also possible to integrate both effects into the framework of a mixed linear model (Wang et al. 1999; Yang et al. 2007).

Molecular characterization of the carotenoid biosynthetic pathway in wheat has recently been explored. Most of the effort was focused on the *Psy1* gene, coding for the enzyme phytoene synthase 1 (PSY1), which is considered the rate-limiting step of the pathway (Hirschberg 2001). QTL mapping analysis showed that the *Psy1* gene co-segregates with YPC and flour yellow color (Fb*) on homoeologous chromosomes 7BL (Pozniak et al. 2007; Zhang and Dubcovsky 2008) and 7AL (Howitt et al. 2009; Singh et al. 2009). Allelic variants of *Psy1* (*Psy-A1* and *Psy-B1*) were reported in durum (Singh et al. 2009; He et al. 2009a; Zhang and Dubcovsky 2008) and common (He et al. 2009b; Howitt et al. 2009; Crawford et al. 2011) wheat.

A second *Psy* functional gene (*Psy2*) was located on group 5 chromosomes of durum wheat through genetic and physical mapping, but it could not be associated with YPC (Pozniak et al. 2007; Blanco et al. 2009). In hexaploid wheat, full-length cDNAs for two additional enzymes (phytoene desaturase [PDS] and ζ-carotene desaturase [ZDS]) were cloned (Cong et al. 2009). These enzymes are responsible for four carotene desaturation steps of phytoene in the carotenoid biosynthesis pathway, producing lycopene. Cenci et al. (2004) mapped PDS and ZDS clones from a durum wheat BAC library on chromosome groups 4 and 2, respectively. Recently, Zhang et al. (2011) cloned the full-length DNA sequence of a ZDS gene, located on chromosome 2DL, and a functional marker was developed. Additionally, the lycopene ε-cyclase (ε-LCY) gene, responsible for cyclization of lycopene, was proposed by Howitt et al. (2009) as a candidate gene for a QTL on chromosome 3B affecting lutein content in common wheat (Mares and Campbell 2001).

In the present work, we focused on validation of QTLs associated with Fb* and YPC in Argentina, using a durum wheat mapping population derived from the cross UC1113 × Kofa previously evaluated in the USA, and searched for new and additional QTLs

expressed in these environments. We used single-locus and two-loci QTL analyses to detect main effects, digenic epistasis (QQ) and QTL × environment interactions (QE and QQE). Understanding the different genes/QTLs and their interactive effects is essential for determining optimal strategies for marker assisted selection (MAS). We also investigated the presence of QTLs associated with brightness and redness of flour. Additionally, the relationships between color traits and other quality and yield traits were explored. Our final objective was to use this information in Argentinean durum wheat breeding programs to improve flour color.

Materials and methods

Plant materials

A mapping population consisting of 93 recombinant inbred lines (RILs) was obtained by crossing the line UC1113 with the variety Kofa (Zhang et al. 2008). UC1113, a breeding line from the UC Davis Wheat Breeding Program, has excellent agronomic performance, but intermediate pasta quality parameters. Kofa, is a durum variety developed by West-Bred, has optimal semolina and pasta color, high protein content and strong gluten. Eight Argentinean durum cultivars were included as controls in all experiments (Buck Platino, Buck Topacio, Buck Esmeralda, Buck Cristal, Buck Ambar, Bonaerense INTA Facon, Bonaerense INTA Carilo and Bonaerense INTA Cumenay).

Field trials

The 93 RILs, along with the parental lines and control varieties, were evaluated over two consecutive years (2006 and 2007) at three locations in Argentina (Cabildo [CA] (39°36′S 61°64′W), Barrow [BW] (38°20′S 60°13′W) and Balcarce [BC] (37°45′S 58°18′W)). The experimental design consisted of randomized complete blocks with three replications using plots 3 m^2 in size (3 rows of 5 m length, 0.20 m evenly spaced). Seed was sown at an average of 150 per m^2. Each year × location combination was considered as an environment (E). Agronomic management was performed according to local practices at each location. Fertilization was performed in two applications, at pre-sowing/sown and tillering [described in Conti et al. (2011)].

Quality trait evaluations

Approximately 20–25 g of clean grain from each plot was ground to wholemeal flour in a UDY Cyclone Mill (Udy Corporation, Fort Collins, CO, USA) fitted with 1 mm sieve. Color parameters (CIE L*a*b*) were measured on the wholemeal flour within 48 h with a Minolta colorimeter (model CR310, Minolta Corp., Ramsey, NJ, USA). CIE L*a*b* tristimulus values were expressed within the tridimensional color space CIE 1976 (Commission Internationale de l'Éclairage, 1976). Positive values of CIE b* represent the yellow color (Fb*) of wholemeal flour, while positive values of CIE a* describe the redness of samples (Fa*). CIE L* values indicate the brightness of flour (FL*), ranging from 0 (black) to 100 (white).

Wholemeal flour samples were analyzed for YPC using the microtest described by Fares et al. (1991) during the week after milling. Yellow pigments were extracted from 1 g of wholemeal flour using 5 ml of water-saturated *n*-butanol, by shaking on a rocker table at room temperature for 3 h at 170 rpm. The extracted solution was filtered through Whatman No 1 paper and the absorbance was measured on the supernatant at 448 nm using a spectrophotometer (METROLAB 1600 PLUS, version 3.06b). YPC was expressed as μg/g using the formula c = [E (extinction at 448 nm) × Volume (ml) × 1,000]/[251 × g (sample weight) × s (optical path length)].

Additional quality and yield traits were included in order to examine their relationship with color traits. Thousand kernel weight (TKW) was obtained by weighing two samples of 100 seeds. Test weight (TW) was performed using a hectolitric balance and expressed in kg hl^{-1}. Grain yield (GY) of each plot was measured as the weight of clean grains from the entire plot area and the results were expressed in kg ha^{-1}. Grain protein content (GPC) and moisture were measured by the NIRT method using Infratec 1226 equipment (Tecator, Sweden). Gluten strength was estimated on wholemeal flour samples using the SDS sedimentation test (mm) modified by Dick and Quick (1983).

Statistical analysis

Statistical analysis was conducted using the SAS 9.0 software (SAS Institute Inc.; Cary, NC, USA). The normality of the residuals was confirmed by the Shapiro–Wilk test. Analyses of variance (ANOVA) were

performed using the PROC MIXED procedure, considering genotype, environments and interactions as random effects. Broad sense heritability (h^2) was estimated using the GENES software (http://www.ufv.br/dbg/genes/genes.htm). Pearson correlation coefficients between color traits with different yield and quality parameters were calculated using the PROC CORR procedure (Base SAS® 9.2 Procedures Guide, 2010).

QTL mapping

QTL mapping was performed using a genetic map of 269 markers spanning 2,140 cM and including molecular (230 SSR, 23 SNP, 10 RFLP and 3 STS), morphological (*Bla*) and protein markers (*Glu-B1* and *Gli-A2* loci) (Zhang et al. 2008). The X letter preceding the marker name that is generally used to indicate the marker type (McIntosh et al. 2003) was omitted from the text and tables. Main effect QTLs were detected for each environment using the Windows QTL-Cartographer software version 2.5 (Wang et al. 2004). Composite Interval Mapping (CIM) was implemented by the standard Model 6, with a 0.5 cM walking speed and a 10 cM window size. A QTL was declared significant when the LOD value was higher than the threshold calculated based on 1,000 permutations at significance level of 0.05 and suggestive at $p < 0.1$ (Churchill and Doerge 1994). The confidence intervals were calculated as the two-LOD drop off support intervals that confer a 95% confidence region (Van Ooijen 1992). To explore the genetic architecture of color traits the QTLNetwork version 2.0 (http://ibi.zju.edu.cn/software/qtlnetwork/) was used. Based on a mixed linear model (Wang et al. 1999), single-locus and two-loci QTL analyses were performed to examine main effects, epistatic effects (QQ) and QTL × environment interactions (QE and QQE). A genome scan configuration with a walk speed of 0.5 cM and a window size of 10 cM was selected. The critical threshold value of the *F*-statistic was determined by the 1,000 permutation test at a significance level of 0.05.

Results

Analysis of phenotypic data

The values of color parameters CIE L*a*b* and YPC showed transgressive bi-directional segregation in the six environments and in the pooled data (Table 1). Considering the average values of all six environments, the RILs ranged from 4.17 to 7.93 ppm for YPC and 14.27 to 17.71 color units for Fb*. Normal distributions of values for each trait were confirmed by Shapiro–Wilk tests in four environments. Kofa showed higher Fb* and YPC values than UC1113 in all environments. In most of them, values for Kofa were slightly lower than the corresponding values for Buck Topacio, the best local variety regarding this trait. In general, Buck Topacio, followed by BI Facon and BI Carilo had the highest Fb* and YPC (Tables 1 and S1). However, two promising RILs (numbers 32 and 42) performed better than Buck Topacio in most of the environments.

The combined ANOVA showed that genotype and environment effects were highly significant ($p < 0.0001$) for all color traits (Table S2). This analysis also revealed that genotype × environment interactions were highly significant for all traits. However, there were moderate to high correlations among environments (ranging from 0.61 to 0.78 for Fb* and 0.70 to 0.89 for YPC). The lowest Fb* and YPC values were observed in Balcarce (Table 1). Intermediate to high values were found in Barrow, whereas Cabildo showed the highest Fb* and YPC values (Table 1). High broad sense heritabilities (≥94%) for YPC and Fb* were obtained in all the environments, whereas FL* (brightness) and Fa* (redness) showed intermediate to high heritability values (Table 1).

The associations among color traits and with other quality and yield parameters (Table S3) were also investigated. Fb* and YPC were highly and positively correlated ($r = 0.83$, $p < 0.0001$). Both parameters, as well as Fa*, were highly significantly ($p < 0.0001$) and negatively correlated with GY (Fa*, $r = -0.32$; Fb*, $r = -0.11$; YPC, $r = -0.27$), TKW (Fa*, $r = -0.35$; Fb*, $r = -0.65$; YPC, $r = -0.55$) and TW (Fa*, $r = -0.58$; Fb*, $r = -0.49$; YPC, $r = -0.51$). Correlations between protein content (GPC) and color traits (YPC and Fb*) were highly significant, with moderately positive values ($r = 0.41$ and 0.51, $p < 0.0001$). There was an unexpected highly significant ($p < 0.0001$) negative correlation between red color (Fa*) and SDS test values ($r = -0.41$).

Identification of QTLs controlling Fb* and YPC

QTL mapping of the UC1113 × Kofa RIL population showed a high number of chromosomal regions involved in the expression of the correlated traits.

Table 1 Phenotypic data analysis and heritability values (h^2) of color parameters and YPC for the UC1113 × Kofa RIL population and parental lines across six environments in Argentina

Trait[a]	Environment[b]	RILs min.	RILs mean[c]	RIL máx.	SD[d]	Kofa	UC1113	Heritability (h^2)
FL*	CA 2006	79.06	81.38**c**	83.41	0.67	81.86	82.18	0.75
	BW 2006	78.28	80.49**e**	82.47	0.67	81.85	82.17	0.75
	BC 2006	78.13	80.87**d**	83.02	0.78	80.71	81.55	0.42
	CA 2007	80.21	82.33**b**	84.77	0.89	83.01	82.84	0.88
	BW 2007	79.33	81.39**c**	82.85	0.59	81.40	82.33	0.69
	BC 2007	80.38	82.98**a**	85.21	0.88	83.45	83.23	0.89
	Mean	80.53	81.57	82.33	0.39	82.04	82.38	0.68
Fa*	CA 2006	−0.18	0.62**a**	1.50	0.28	−0.18	0.11	0.87
	BW 2006	−0.46	0.46**b**	1.38	0.40	−0.16	0.19	0.94
	BC 2006	−0.63	0.31**c**	1.47	0.31	−0.25	−0.04	0.69
	CA 2007	−0.90	−0.06**d**	1.01	0.36	−0.70	−0.16	0.90
	BW 2007	−0.63	−0.04**d**	0.83	0.23	−0.06	0.01	0.73
	BC 2007	−0.89	−0.18**e**	0.53	0.29	−0.44	0.02	0.90
	Mean	−0.20	0.18	0.57	0.17	−0.30	0.02	0.63
Fb*	CA 2006	15.00	17.26**a**	19.54	0.82	18.13	16.76	0.95
	BW 2006	12.31	16.04**d**	18.50	0.93	16.78	15.17	0.96
	BC 2006	13.71	15.57**e**	17.66	0.78	16.70	15.11	0.95
	CA 2007	13.68	16.44**c**	18.50	0.83	16.91	15.95	0.95
	BW 2007	14.65	16.55**b**	18.71	0.87	17.56	15.62	0.94
	BC 2007	12.18	14.38**f**	16.24	0.84	15.45	13.65	0.96
	Mean	14.27	16.03	17.73	0.73	16.92	15.38	0.91
YPC	CA 2006	4.74	6.67**b**	9.36	0.92	7.56	5.60	0.97
	BW 2006	4.34	6.75**a**	9.66	0.98	7.66	5.66	0.97
	BC 2006	3.23	5.56**e**	7.43	0.86	6.16	4.45	0.98
	CA 2007	4.14	6.23**c**	8.43	0.84	7.03	5.57	0.98
	BW 2007	3.94	5.94**d**	8.13	0.86	7.02	5.08	0.97
	BC 2007	3.33	4.81**f**	6.67	0.69	5.68	3.86	0.98
	Mean	4.17	5.99	7.93	0.78	6.85	5.04	0.97

[a] *FL** flour brightness, *Fa** flour redness, *Fb** flour yellow color, *YPC* yellow pigment content (μg g^{-1})

[b] *CA* Cabildo, *BW* Barrow, *BC* Balcarce, *Mean* pooled data of the six environments

[c] Mean values of RILs with the same letter are not significantly different according to the Duncan test at $p < 0.01$ (for each trait)

[d] *SD* standard deviation

Sixteen QTLs were detected across environments, and major overlapping QTLs (explaining more than 10% of the phenotypic variation) affecting Fb* and YPC were located on chromosome arms 4AL, 6AL, 7AS, 7AL, 7BS and 7BL (Tables 2, 3). However, only 2–5 QTLs per environment/trait combination were detected.

Chromosome arm 1BL

One QTL was detected on 1BL with positive alleles provided by Kofa. The *QYpc.cerz-1BL.1* peak was mapped between *BE443797_436* and *barc302*, and the QTL explained 10.2% of the phenotypic variation for YPC in BW 2006 (Table 3). Mapping analysis using the average of the six environments also detected a suggestive ($p < 0.1$) QTL in this region associated with Fb* (*QFb.cerz-1BL.1*), but failed to detect an effect in this region associated with YPC (Tables 2, 3; Fig. S1).

Chromosome arm 4AL

Two different QTLs were detected on 4AL, with one positive allele provided by UC1113 (4AL.1) and one by Kofa (4AL.2). *QYpc.cerz-4AL.1* lying between *Lpx-A3* and *wmc617* was associated with YPC in one environment (CA 2006) and with Fb* in two environments

Table 2 QTL mapping of flour yellow color (Fb*) in a RIL population (UC1113 × Kofa) in six environments in Argentina

Chromosome arm	QTL[a]	Flanking markers[b]	LOD[c]	Additive effect[d]	R^2 (%)[e]	Peak position (cM)	2-LOD support interval	Environment[f]
1BL	*QFb.cerz-1BL.1*	***BE443797_436***–*barc302*	2.8**s**	0.22	8.6	51.6	34.9–60.5	Mean
4AL	*QFb.cerz-4AL.1*	*dupw4*–***barc170***	3.4*	−0.24	9.1	45.7	31.6–57.8	CA 2006
4AL	*QFb.cerz-4AL.1*	*dupw4*–***barc170***	3.6*	−0.26	8.4	46.2	5.3–49.7	BW 2006
4AL	*QFb.cerz-4AL.2*	***wmc219***–*psr573.2*	3.2*	0.23	8.3	126.2	109.4–129.6	CA 2006
4AL	*QFb.cerz-4AL.2*	***wmc219***–*pr573.2*	3.5*	0.26	10.4	126.2	114.4–129.6	CA 2007
4AL	*QFb.cerz-4AL.2*	***wmc219***–*psr573.2*	3.0*	0.22	6.7	126.2	113.9–129.6	BW 2007
4AL	*QFb.cerz-4AL.2*	***wmc219***–*psr573.2*	4.0*	0.24	10.6	126.2	113.9–129.6	Mean
5AS	*QFb.cerz-5AS*	*wmc350*–***gwm47***	4.5*	−0.3	12.9	2.0	0.0–16.0	BW 2007
5AS	*QFb.cerz-5AS*	***wmc350***–*gwm47*	5.1*	−0.34	16.2	0.0	0.0–8.0	BC 2007
5BL	*QFb.cerz-5BL.1*	***barc74***–*gwm371*	4.3*	−0.29	12.2	42.7	23.1–53.2	BW 2007
6AL	*QFb.cerz-6AL.1*	***barc146***–*gwm132*	5.7*	0.37	16.1	36.2	23.3–47.4	BW 2006
6AL	*QFb.cerz-6AL.1*	***barc146***–*gwm132*	5.8*	0.35	18.4	36.2	22.8–39.7	CA 2007
6AL	*QFb.cerz-6AL.1*	***barc146***–*gwm132*	7.3*	0.39	21.4	36.2	26.3–39.7	BW 2007
6AL	*QFb.cerz-6AL.2*	*barc113*–***wmc553***	5.5*	0.34	17.1	65.4	57.9–70.8	CA 2006
6AL	*QFb.cerz-6AL.2*	*barc113*–***wmc553***	8.6*	0.4	28.3	64.9	57.9–68.8	BC 2006
6AL	*QFb.cerz-6AL.2*	*barc113*–***wmc553***	5.7*	0.32	17.9	62.4	50.7–70.3	Mean
6AL	*QFb.cerz-6AL.3*	*barc353*–***gwm169***	3.4*	0.29	12.4	90.4	78.9–117.2	CA 2007
6AL	*QFb.cerz-6AL.3*	***gwm169***–*BE483091_472*	3.9*	0.28	10.4	92.6	79.9–125.5	BW 2007
7AS	*QFb.cerz-7AS*	***wmc168***–*barc219*	3.1*	−0.29	12.6	39.1	15.5–72.6	CA 2006
7AS	*QFb.cerz-7AS*	*BQ170462_176*–***barc174***	4.2*	−0.26	11.7	74.1	64.4–84.8	BC 2006
7AL	*QFb.cerz-7AL*	*wmc116*–***cfd6***	5.8*	−0.43	22.5	165.8	151.3–171.1	BW 2006
7AL	*QFb.cerz-7AL*	*wmc116*–***cfd6***	3.4*	−0.26	9.8	171.1	149.8–171.1	BC 2007
7BS	*QFb.cerz-7BS.1*	*gwm537*–***gwm400***	2.9**s**	0.22	8.5	22.3	11.4–35.8	BC 2006
7BS	*QFb.cerz-7BS.2*	***barc72***–*gwm297*	4.4*	0.33	12.8	59.2	53.9–66.8	BW 2006
7BL	*QFb.cerz-7BL.1*	*Psy-B1*–***cfa2257***	4.3*	0.28	12.1	198.5	184.4–199.3	BC 2006
7BL	*QFb.cerz-7BL.2*	*cfa2040*–***barc1073***	3.6*	0.25	9.5	187.6	184.4. –199.3	BC 2006
7BL	*QFb.cerz-7BL.2*	*cfa2040*–***barc1073***	4.6*	0.35	14.0	184.6	170.9–190.3	BC 2007

[a] 1, 2, 3, different QTL positions on the same chromosome arm, *Fb* flour yellow color, *cerz* Centro de Recursos Naturales de la Zona Semiárida

[b] *Bold* closest marker at the peak of the QTL

[c] s and * threshold LOD at $p < 0.1$ (suggestive) and $p < 0.05$ (significant), respectively

[d] Positive values of additive effect indicate increasing effects of Kofa alleles; negative values indicate increasing effects of UC1113 alleles

[e] R^2 (%) percentage of phenotypic variance explained

[f] *CA* Cabildo, *BW* Barrow, *BC* Balcarce, *Mean* pooled data of the six environments

(between the markers *dupw4* and *barc170*), showing an overlap of the confidence intervals for both traits. The second QTL was located more distally on the long arm, associated with Fb* (*QFb.cerz-4AL.2*) in three environments (CA 2006, CA 2007 and BW 2007) and with YPC (*QYpc.cerz-4AL.2*) in one environment (BW 2006). The 4AL.2 QTL was strongly linked with marker *wmc219* for both traits (Tables 2, 3). QTL analysis using the average of all six environments also detected *QFb.cerz-4AL.2* (Fig. 1).

Chromosome arms 5AS and 5AL

Single QTLs were detected on 5AS and 5AL (Fig. S1), with both positive alleles provided by UC1113. A QTL on 5AS was mapped between *wmc350* and

Table 3 QTL mapping of YPC in a RIL population (UC1113 × Kofa) grown in six environments in Argentina

Chromosome arm	QTL[a]	Flanking markers[b]	LOD[c]	Additive effect[d]	R^2 (%)[e]	Peak position (cM)	2-LOD support interval	Environment[f]
1BL	*QYpc.cerz-1BL.1*	***BE443797_436***–*barc302*	3.8*	0.31	10.8	52.1	38.1–63.0	BW 2006
2AS	*QYpc.cerz-2AS*	*wmc382*–***gwm296***	2.7**s**	−0.21	6.0	22.8	8.0–47.8	BW 2007
4AL	*QYpc.cerz-4AL.1*	*Lpx-A3*–***wmc617***	3.9*	−0.31	12.0	23.3	4.8–44.2	CA 2006
4AL	*QYpc.cerz-4AL.2*	***wmc219***–*psr573.2*	5.4*	0.34	12.0	126.2	122.6–129.6	BW 2006
5AS	*QYpc.cerz-5AS*	***wmc350***–*gwm47*	3.17**s**	−0.21	8.3	0.0	0.0–17.5	BC 2007
5AL	*QYpc.cerz-5AL*	*wmc727*–***cfa2149***	3.5*	−0.29	10.0	196.0	172.6–205.2	CA 2006
5BL	*QYpc.cerz-5BL.2*	*gwm499*–***BE495277_339***	3.8*	−0.26	9.0	73.3	54.5–85.8	BW 2007
6AL	*QYpc.cerz-6AL.1*	***barc146***–*gwm132*	7.5*	0.45	22.8	36.2	25.8–38.7	CA 2006
6AL	*QYpc.cerz-6AL.2*	*barc113*–***wmc553***	7.1*	0.41	16.8	66.4	58.4–68.8	BW 2006
6AL	*QYpc.cerz-6AL.2*	*barc113*–***wmc553***	7.2*	0.41	20.9	65.9	58.4–68.8	BC 2006
6AL	*QYpc.cerz-6AL.2*	*barc113*–***wmc553***	10.4*	0.49	33.4	64.9	58.4–68.6	CA 2007
6AL	*QYpc.cerz-6AL.2*	*barc113*–***wmc553***	13.7*	0.55	42.7	64.9	59.4–68.1	BW 2007
6AL	*QYpc.cerz-6AL.2*	*barc113*–***wmc553***	6.5*	0.3	18.6	65.4	57.4–70.8	BC 2007
6AL	*QYpc.cerz-6AL.2*	*barc113*–***wmc553***	10.5*	0.43	29.9	65.4	58.4–68.6	Mean
6AL	*QYpc.cerz-6AL.3*	*barc353*–***gwm169***	2.8**s**	0.29	9.8	88.4	77.6–122.2	CA 2006
7AS	*QYpc.cerz-7AS*	*BQ170462_176*–***barc174***	2.9**s**	−0.2	6.3	74.3	55.1–89.3	Mean
7AL	*QYpc.cerz-7AL*	*wmc116*–***cfd6***	2.9**S**	−0.24	7.0	171.1	152.8–171.1	BC 2006
7BS	*QYpc.cerz-7BS.2*	*barc23*–***barc72***	3.6*	0.27	9.6	58.9	43.7–66.8	CA 2007
7BS	*QYpc.cerz-7BS.2*	*barc23*–***barc72***	4.1*	0.27	9.5	59.2	52.7–62.5	BW 2007
7BL	*QYpc.cerz-7BL.1*	*Psy-B1*–***cfa2257***	3.4*	0.3	9.3	199.0	190.5–199.3	CA 2006
7BL	*QYpc.cerz-7BL.1*	*Psy-B1*–***cfa2257***	2.9**s**	0.24	7.9	195.5	165.9–199.3	Mean
7BL	*QYpc.cerz-7BL.2*	***cfa2040***–*barc1073*	5.6*	0.29	15.0	183.9	171.4–190.1	BC 2007
7BL	*QYpc.cerz-7BL.2*	*cfa2040*–***barc1073***	3.0**s**	0.23	6.6	184.6	165.9–199.3	Mean
7BL	*QYpc.cerz-7BL.2*	***wmc311***–*wmc276*	5.4*	0.42	16.9	170.4	163.0–190.1	BW 2006

[a] 1, 2, 3, different QTL positions on the same chromosome arm, *YPC/Ypc* yellow pigment content, *cerz* Centro de Recursos Naturales de la Zona Semiárida

[b] *Bold* closest marker at the peak of the QTL

[c] s and * threshold LOD at $p < 0.1$ (suggestive) and $p < 0.05$ (significant), respectively

[d] Positive values of additive effect indicate increasing effects of Kofa alleles; negative values indicate increasing effects of UC1113 alleles

[e] R^2(%) percentage of phenotypic variance explained

[f] *CA* Cabildo, *BW* Barrow, *BC* Balcarce, *Mean* pooled data of the six environments

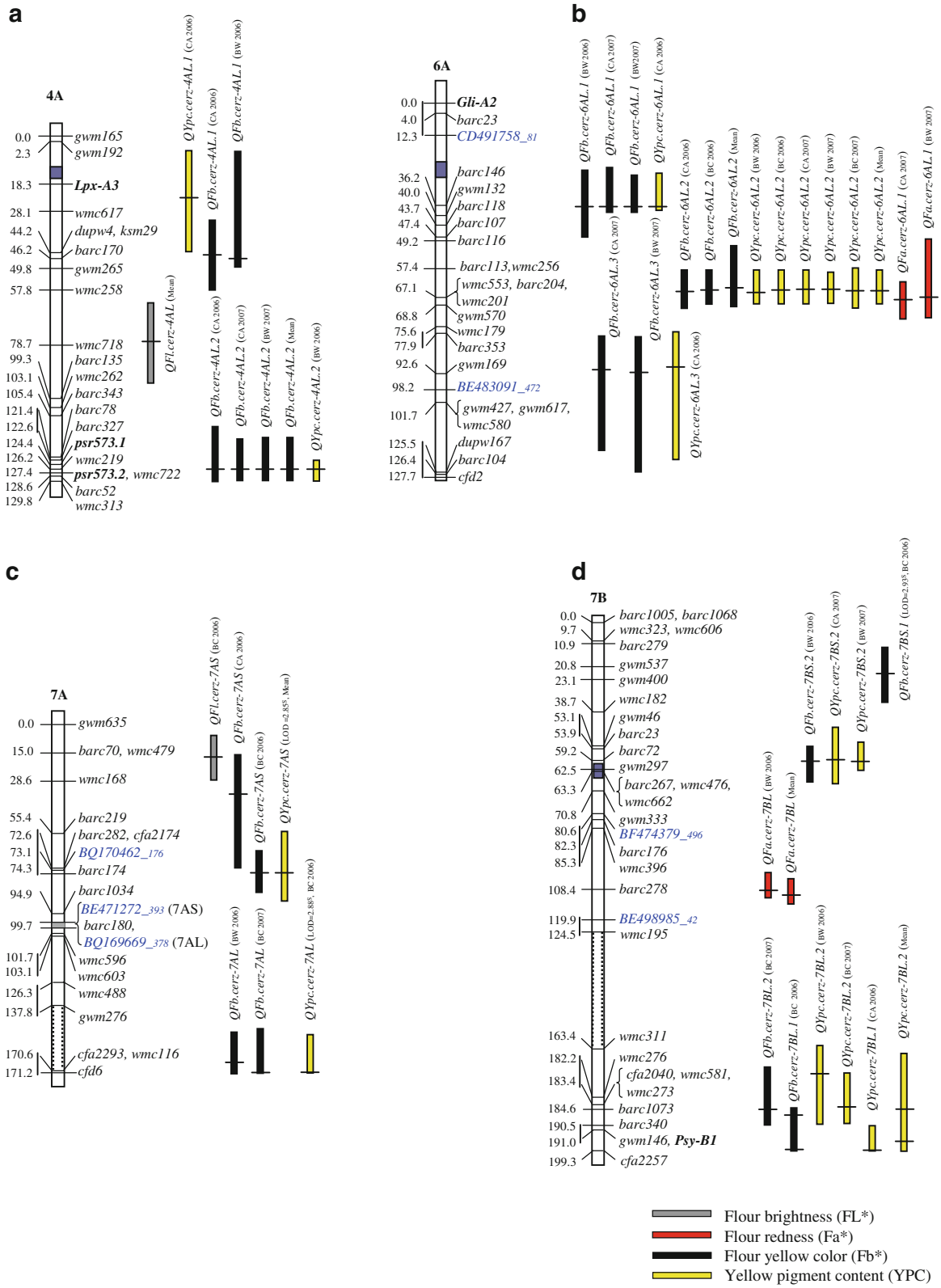
gwm47 for both traits (*QFb.cerz-5AS, QYpc.cerz-5AS*). *QYpc.cerz-5AL* was mapped between *wmc727* and *cfa2149* in one environment (CA 2006).

Chromosome arm 5BL

Two QTLs located on chromosome arm 5BL (5BL.1 and 5BL.2) were 32.1 cM apart (Fig. S1). The peak of the 5BL.1 QTL associated with Fb* (*QFb.cerz-5BL.1*) was close to SSR *barc74* in BW 2007, whereas the 5BL.2 peak was located between *gwm499* and *BE495277_339* and associated with YPC (*QYpc.cerz-5BL.2*) in BW 2007.

Chromosome arm 6AL

Three QTLs were detected on 6AL (6AL.1, 6AL.2 and 6AL.3) with all positive alleles provided by Kofa


a
4A
0.0 gwm165
2.3 gwm192
18.3 Lpx-A3
28.1 wmc617
44.2 dupw4, ksm29
46.2 barc170
49.8 gwm265
57.8 wmc258
78.7 wmc718
99.3 barc135
103.1 wmc262
105.4 barc343
121.4 barc78
122.6 barc327
124.4 psr573.1
126.2 wmc219
127.4 psr573.2, wmc722
128.6 barc52
129.8 wmc313
QYpc.cerz-4AL.1 (CA 2006)
QFb.cerz-4AL.1 (CA 2006)
QFb.cerz-4AL.1 (BW 2006)
QFl.cerz-4AL (Mean)
QFb.cerz-4AL.2 (CA 2006)
QFb.cerz-4AL.2 (CA 2007)
QFb.cerz-4AL.2 (BW 2007)
QFb.cerz-4AL.2 (Mean)
QYpc.cerz-4AL.2 (BW 2006)
6A
0.0 Gli-A2
4.0 barc23
12.3 CD491758_81
36.2 barc146
40.0 gwm132
43.7 barc118
47.4 barc107
49.2 barc116
57.4 barc113,wmc256
67.1 wmc553, barc204, wmc201
68.8 gwm570
75.6 wmc179
77.9 barc353
92.6 gwm169
98.2 BE483091_472
101.7 gwm427, gwm617, wmc580
125.5 dupw167
126.4 barc104
127.7 cfd2
b
QFb.cerz-6AL.1 (BW 2006)
QFb.cerz-6AL.1 (CA 2007)
QFb.cerz-6AL.1 (BW2007)
QYpc.cerz-6AL.1 (CA 2006)
QFb.cerz-6AL.3 (CA 2007)
QFb.cerz-6AL.3 (BW 2007)
QYpc.cerz-6AL.3 (CA 2006)
QFb.cerz-6AL.2 (CA 2006)
QFb.cerz-6AL.2 (BC 2006)
QFb.cerz-6AL.2 (Mean)
QYpc.cerz-6AL.2 (BW 2006)
QYpc.cerz-6AL.2 (BC 2006)
QYpc.cerz-6AL.2 (CA 2007)
QYpc.cerz-6AL.2 (BW 2007)
QYpc.cerz-6AL.2 (BC 2007)
QYpc.cerz-6AL.2 (Mean)
QFa.cerz-6AL.1 (CA 2007)
QFa.cerz-6AL.1 (BW 2007)
QFa.cerz-6AL.2 (CA2007)
c
7A
0.0 gwm635
15.0 barc70, wmc479
28.6 wmc168
55.4 barc219
72.6 barc282, cfa2174
73.1 BQ170462_176
74.3 barc174
94.9 barc1034
99.7 BE471272_393 (7AS) barc180, BQ169669_378 (7AL)
101.7 wmc596
103.1 wmc603
126.3 wmc488
137.8 gwm276
170.6 cfa2293, wmc116
171.2 cfd6
QFl.cerz-7AS (BC 2006)
QFb.cerz-7AS (CA 2006)
QFb.cerz-7AS (BC 2006)
QYpc.cerz-7AS (LOD =2.85*, Mean)
QFb.cerz-7AL (BW 2006)
QFb.cerz-7AL (BC 2007)
QYpc.cerz-7AL (LOD=2.885, BC 2006)
d
7B
0.0 barc1005, barc1068
9.7 wmc323, wmc606
10.9 barc279
20.8 gwm537
23.1 gwm400
38.7 wmc182
53.1 gwm46
53.9 barc23
59.2 barc72
62.5 gwm297
63.3 barc267, wmc476, wmc662
70.8 gwm333
80.6 BF474379_496
82.3 barc176
85.3 wmc396
108.4 barc278
119.9 BE498985_42
124.5 wmc195
163.4 wmc311
182.2 wmc276
183.4 cfa2040, wmc581, wmc273
184.6 barc1073
190.5 barc340
191.0 gwm146, Psy-B1
199.3 cfa2257
QFa.cerz-7BL (BW 2006)
QFa.cerz-7BL (Mean)
QFb.cerz-7BS.2 (BW 2006)
QYpc.cerz-7BS.2 (CA 2007)
QYpc.cerz-7BS.2 (BW 2007)
QFb.cerz-7BS.1 (LOD=2.935, BC 2006)
QFb.cerz-7BL.2 (BC 2007)
QFb.cerz-7BL.1 (BC 2006)
QYpc.cerz-7BL.2 (BW 2006)
QYpc.cerz-7BL.2 (BC 2007)
QYpc.cerz-7BL.1 (CA 2006)
QYpc.cerz-7BL.2 (Mean)
Flour brightness (FL*)
Flour redness (Fa*)
Flour yellow color (Fb*)
Yellow pigment content (YPC)

◀ **Fig. 1** QTLs associated with brightness (FL*), *redness* (Fa*), *yellow color* (Fb*) and yellow pigment content (YPC) mapped in chromosomes 4A (**a**), 6A (**b**), 7A (**c**) and 7B (**d**) of the UC1113 × Kofa RIL population. Centromeres are indicated by *grey squares*. Markers are on the *right* and corresponding cumulative genetic distances are indicated on the *left*. *Vertical bars* represent QTL confidence intervals with 2-LOD drop offs with the name of the environment at the *top*. The positions of peaks are indicated by *horizontal lines* within the QTLs (genetic map from Zhang et al. 2008)

(Fig. 1). These three QTLs individually explained the highest percentages of variation in YPC and Fb* in the environment in which they were detected. The peak of the 6AL.2 QTL lay between SSRs *barc113* and *wmc553*. This was the most important QTL, displaying a strong and consistent effect on YPC (*QYpc.cerz-6AL.2*) and Fb* (*QFb.cerz-6AL.2*) across all six environments, and, in terms of mean values, explaining the highest percentage of the variation in both traits (Tables 2, 3). *QFb.cerz-6AL.1*, linked to *barc146*, was associated with Fb* in three environments (BW 2006, CA 2007 and BW 2007), whereas *QYpc.cerz-6AL.1* showed an effect on YPC only in CA 2006 (Tables 2, 3). Finally, *QFb.cerz-6AL.3*, linked to *gwm169*, was significantly associated with Fb* in two environments (Table 2).

Chromosome arm 7AS

One QTL was detected on 7AS, with the positive allele provided by UC1113 (Fig. 1). *QFb.cerz-7AS* between *wmc168* and *barc219* was significantly associated with Fb* in CA 2006 (Table 2) and between the markers *BQ170462_176* and *barc174* in BC 2006. Both peak positions gave overlapping confidence intervals. This QTL was also associated with YPC (*QYpc.cerz-7AS*) as suggestive ($p < 0.1$) from the pooled data, indicating a minor but relatively stable effect on YPC (Tables 2, 3). In addition, this 7AS QTL was detected with LOD values between 2.2 and 2.7 in three environments for YPC and two environments for Fb*.

Chromosome arm 7AL

A single QTL on 7AL, with the positive allele provided by UC1113, was flanked by *wmc116* and *cfd6* (Fig. 1). *QFb.cerz-7AL* was significantly associated with Fb* in two environments (BW 2006 and BC 2007), but 7AL was only suggestively associated with YPC (*QYpc.cerz-7AL*) in BC 2006 (Tables 2, 3).

Chromosome arm 7BS

Two linked QTLs were detected on 7BS, in a region apparently homoeologous to that on 7AS (Fig. 1). Positive alleles for these QTLs were provided by Kofa. *QFb.cerz-7BS.1* was associated with Fb* only as suggestive ($p < 0.1$) in BC 2006, between the markers *gwm537* and *gwm400*. The 7BS.2 QTL was significantly associated with Fb* and closely linked to *barc72* (*QFb.cerz-7BS.2*) in one environment (BW 2006), and associated with YPC (*QYpc.cerz-7BS.2*) in two environments. Although not significant, the 7BS.2 peak showed a LOD of 2.65 in the pooled data of Fb*.

Chromosome arm 7BL

Two closely linked QTLs with positive alleles provided by Kofa were detected in a 35 cM interval on the distal portion of 7BL (Fig. 1). The 7BL.1 peak was flanked by the markers *Psy-B1* and *cfa2257* and significantly associated with Fb* (*QFb.cerz-7BL.1*) in BC 2006 and with YPC (*QYpc.cerz-7BL.1*) in CA 2006 (Tables 2, 3). The 7BL.2 QTL was located between *cfa2040* and *barc1073*, at a distance of 7.9–15 cM from the 7BL.1 peak. *QFb.cerz-7BL.2* was associated with Fb* in two environments (BC 2006 and BC 2007) and with YPC (*QYpc.cerz-7BL.2*) in BC 2007. In BW 2006, the peak of the 7BL.2 QTL was flanked by *wmc311* and *wmc276* and significantly associated with YPC, with a closely linked second peak between *cfa2040* and *barc1073*. Mapping analysis on the average data detected both QTLs, *QYpc.cerz-7BL.1* (LOD = 2.9) and *QYpc.cerz-7BL.2* (LOD = 3.0) ($p < 0.1$).

Fb* and YPC QTLs with epistatic effects or environmental interactions

According to the *F*-statistic, tested on any pair of loci in the genome, a total of 10 digenic epistatic QTLs were detected across nine chromosomes (Table 4). However, no QE or QQE interactions were identified in our analyses. The most important digenic epistatic additive effect was found between the *QFb.cerz-1BL.1* and *QFb.cerz-1BL.2* QTLs. The new QTL, *QYpc.cerz-7AS.2*, showed QQ interaction for YPC with a QTL on

Table 4 Epistatic QTLs detected using two-loci QTL analysis

Trait[a]	QTL_i[b]	Flanking $markers_i$	Peak $position_i$	QTL_j[b]	Flanking $markers_j$	Peak $position_j$	Q_iQ_j effect[c]
YPC	*QYpc.cerz-1AL*	*barc213–cdo393*	147	*QYpc.cerz-6BL*	*gwm219–wmc621*	115.5	−0.16
	QYpc.cerz-6BL	*gwm219–wmc621*	115.5	***QYpc.cerz-7AS***	*barc174–barc1034*	74.3	−0.12
	QYpc.cerz-2AS	*gwm249–gwm71*	71.3	***QYpc.cerz-7BS.1***	*gwm537–gwm400*	22.3	−0.11
	QYpc.cerz-6AL.2	*barc1165–barc113*	49.2	***QYpc.cerz-7BS.2***	*barc267–gwm333*	65.3	−0.12
Fb*	***QFb.cerz-4AL.2***	*wmc219–psr573.2*	126.2	***QFb.cerz-6AL.1***	*CD491758_81–barc146*	31.8	−0.15
	QFb.cerz-4AL.2	*wmc219–psr573.2*	126.2	***QFb.cerz-7AL***	*wmc116–cfd6a*	171.1	0.10
	QFb.cerz-6AL.1	*CD491758_81–barc146*	31.8	***QFb.cerz-7AL***	*wmc116–cfd6a*	171.1	0.16
	QFb.cerz-7AL	*wmc116–cfd6a*	171.1	***QFb.cerz-7BL.2***	*barc1073–barc340*	188.1	0.13
	QFb.cerz-1BL.1	*BE443797_436–barc302*	50.6	*QFb.cerz-1BL.2*	*GluB1–cfa2129b*	82.6	−0.20
	QFb.cerz-5BL.2	*BE495277_339–gwm408*	98.8	*QFb.cerz-7BL*	*barc176–wmc396*	82.3	−0.10

[a] *Fb*/Fb* flour yellow color, *YPC/Ypc* yellow pigment content

[b] *Bold* QTL with significant main effect

[c] Q_iQ_j *effect,* epistatic additive effect between $QTL_i \times QTL_j$, a negative number indicates decreased trait value; a positive number indicates increased trait value

6BL; the latter was not detected as a main effect QTL for this trait. The main QTL associated with YPC (*QYpc.cerz-6AL.2*) identified by CIM also showed a QQ effect with another main effect QTL (*QYpc.cerz-7BS.2*), whose peak position varied for the epistatic effects from 61.2 to 69.3 cM on 7B. This analysis showed an important QQ effect for Fb* involving QTLs on chromosomes 7AL (*QFb.cerz-7AL*) and 7BL (*QFb.cerz-7BL.2*), and the former QTL also showed epistatic interaction with two additional important genomic regions, viz *QFb.cerz-6AL.1* and *QFb.cerz-4AL.3*.

QTL analysis of the FL* color parameter

A genome scan for the FL* trait using average phenotypic data found four stable QTLs (*QFl.cerz-2BS, QFl.cerz-3AS, QFl.cerz-3BS* and *QFl.cerz-4AL*). The *QFl.cerz-2BS* QTL was identified in three environments (Table 5). However, considering the peak position of this QTL in individual environments, we discarded the possibility of two putative QTLs. This QTL individually explained most of the phenotypic variation in environments CA 2006, BW 2006 and BW 2007, as well as for the mean environmental values (Table 5). *QFl.cerz-5BL* detected in BC 2007 explained 20.6% of the variation. The presence of *QFl.cerz-7AS* was detected in BC 2006.

QTL analysis of the Fa* color parameter

Based on the analysis of the mean values of all six environments, three QTLs associated with Fa* on chromosomes 1AL, 1BL and 7BL (*QFa.cerz-1AL*, *QFa.cerz-1B.2* and *QFa.cerz-7B*) explained 12.5, 11.3 and 20.2% of the phenotypic variation, respectively (Table 5). A QTL was also detected in environment BW 2006 (*QFa.cerz-7BL*).

With regard to individual environments, *QFa.cerz-6AL.1* was found in two environments (CA 2007 and BW 2007), and *QFa.cerz-6AL.2* was detected in CA 2007. These two QTLs showed pleiotropic effects for both Fa* and Fb* (Fig. 1).

Discussion

ANOVA indicated strong genotypic effects for all traits within each environment, with higher mean square values for YPC and Fb* than for Fa* and FL* (Table S2). Environmental effects and genotype × environment interactions were detected for all traits (Table S2), indicating that genetic control of flour color in durum wheat is markedly affected by genotype × environment interaction. However, high broad sense heritability values (>94%) for YPC and Fb* were observed (Table 1), confirming previous

Table 5 QTL mapping of flour redness (Fa*) and brightness (FL*) in a RIL population (UC1113 × Kofa) grown in six environments of Argentina

Trait[a]	Chromosome arm	QTL[b]	Flanking markers[c]	LOD[d]	Additive effect[e]	R^2 (%)[f]	Peak position (cM)	2-LOD support interval	Environment[g]
Fa*	1AL	*QFa.cerz-1AL*	***barc83***–*gwm135*	4.4*	–0.06	12.5	75.2	65.5–84.9	Mean
	1BL	*QFa.cerz-1BL.2*	***cfa2129b***–*psr162*	3.0*	–0.06	11.3	90.8	84.1–123.6	Mean
	2AS	*QFa.cerz-2AS*	***gwm249***–*gwm71*	3.0*	0.08	11.4	71.3	58.9–86.7	BC 2006
	3BS	*QFa.cerz-3BS*	***ksm45***–*wmc43*	3.1*	0.09	12.8	37.8	26.0–74.4	CA 2006
	6AL	*QFa.cerz-6AL.1*	***wmc553***–*gwm570*	5.7*	–0.17	21.1	67.6	61.9–73.8	CA 2007
	6AL	*QFa.cerz-6AL.1*	***wmc553***–*gwm570*	3.8*	–0.07	11.0	67.1	48.9–72.8	BW 2007
	6AL	*QFa.cerz-6AL.2*	*barc353*–***gwm169***	4.2*	0.17	18.3	90.9	81.9–98.1	CA 2007
	7BL	*QFa.cerz-7BL*	***barc278***–*BE498985_42*	3.3*	0.14	12.5	108.4	95.8–115.9	BW 2006
	7BL	*QFa.cerz-7BL*	***barc278***–*BE498985_42*	5.9*	0.08	20.2	110.4	99.3–117.9	Mean
FL*	1AS	*QFl.cerz-1AS*	*wmc24*–***barc148***	3.2*	0.2	13.9	47.5	39.1–68.0	CA 2006
	2BS	*QFl.cerz-2BS*	*cfa2201*–***gwm429***	4.2*	–0.24	18.8	20.8	8.5–33.2	CA 2006
	2BS	*QFl.cerz-2BS*	***wmc154***–*cfa2201*	3.5*	–0.21	14.1	3.5	0.0–17.3	BW 2006
	2BS	*QFl.cerz-2BS*	***wmc154***–*cfa2201*	5.3*	–0.2	17.5	0.0	0–9.5	BW 2007
	2BS	*QFl.cerz-2BS*	***BM140538_39***–*cnl158*	6.6*	–0.18	21.5	27.2	17.2–35.2	Mean
	3AS	*QFl.cerz-3AS*	***wmc532***–*gwm369*	3.9*	–0.14	12.1	0.0	0.0–5.0	Mean
	3AL	*QFl.cerz-3AL*	*ksm28*–***wmc428***	2.9[S]	–0.16	11.8	60.4	51.3–75.4	BW 2007
	3BS	*QFl.cerz-3BS*	***gwm493***–*cfd79*	5.8*	0.27	23.6	15.0	9.3–21.0	BC 2006
	3BS	*QFl.cerz-3BS*	***gwm493***–*cfd79*	3.1[S]	0.12	9.2	13.0	6.0–21.5	Mean
	4AL	*QFl.cerz-4AL*	*wmc258*–***wmc718***	3.6*	–0.14	11.9	77.3	62.3–92.7	Mean
	5BL	*QFl.cerz-5BL*	*gwm408*–***barc142***	4.2*	–0.35	20.3	117.8	106.3–127.5	BC 2007
	7AS	*QFl.cerz-7AS*	***barc70***–*wmc168*	3.5*	–0.19	12.7	16.5	5.5–28.5	BC 2006

[a] *Fa** flour redness (a* color parameter), *FL** flour brightness (L* color parameter)

[b] *cerz* Centro de Recursos Naturales de la Zona Semiárida. 1, 2 different QTL positions on the same chromosome arm

[c] *Bold* closest marker at the peak of the QTL

[d] s and * threshold LOD at $p < 0.1$ (suggestive) and $p < 0.05$ (significant), respectively

[e] Positive values of additive effect indicate increasing effects of Kofa alleles; negative values indicate increasing effects of UC1113 alleles

[f] R^2 (%) percentage of phenotypic variance explained

[g] *CA* Cabildo, *BW* Barrow, *BC* Balcarce, *Mean* pooled data of the six environments

studies showing strong genotypic effects of yellow pigment concentration in durum wheat (Clarke et al. 2006; Digesu et al. 2009; Patil et al. 2008). The high correlation coefficients for YPC and Fb* across environments suggest that crossover interaction was not important. The population showed transgressive bi-directional segregation for all traits, supported by the presence of genes/QTLs with positive and negative effects from both parents (Table 1). On the other hand, the negative correlation among quality and yield parameters suggests that the environmental effects on grain shape and size could be affecting grain composition and, indirectly, color expression (L*, a*, b*) and carotenoid content, as reported by Hessler et al. (2002) (Table S3).

QTLs associated with CIE b* and YPC

QTL analyses indicated that the correlated Fb* and YPC traits have complex patterns of inheritance with several genes/QTLs involved. QTLs affecting both traits located on the long arms of group 7 chromosomes were reported in several wheat populations (Pozniak et al. 2007; He et al. 2008; Patil et al. 2008; Howitt et al. 2009), and *Psy1* genes were proposed as candidate genes for those QTLs (Pozniak et al. 2007). In agreement with previous work using the present mapping population (Zhang and Dubcovsky 2008), the *Psy-B1* marker was associated with *QYpc.cerz-7BL.1* and *QFb.cerz-7BL.1* affecting YPC and Fb*, respectively. Although the peak of the 7BL.1 QTL was not coincident with the locus *Psy-B1*, it was near to this gene in some environments. Our analysis showed the peak of the 7BL.1 QTL was closely linked to *cfa2257* located 8.3 cM distal to *Psy-B1*. However, in the current study, a second QTL (*QYpc.cerz-7BL.2*; *QFb.cerz-7BL.2*) was detected between *cfa2040* and *barc1073* in some environments, instead of the QTL flanked by the *Psy-B1* marker. This QTL showed the highest LOD score (3.0) using the mean value of YPC. Based on the information obtained in the Argentinean environments, it was possible to differentiate more clearly these two linked QTLs located in the 7BL region. These results provide additional evidence to support the proposal of Zhang and Dubcovsky (2008) that at least one additional gene affecting flour color traits was present in the 7BL region. Recently, Blanco et al. (2011) reported similar results by detecting two linked QTL, associated with individual carotenoid compounds (β-cryptoxanthin and zeaxanthin) on chromosome 7BL. One of them (for zeaxanthin) was associated with the *wmc311*, the marker located at the peak of *QYpc.cerz-7BL.2* in our population in BW 2006. Using wheat deletion lines, Crawford et al. (2008) mapped the (Rab) geranylgeranyl transferase I α-subunit gene (RGGT) distally on the long arms of chromosomes 7B and 7D. This gene encoding enzymes involved in the xanthophyll biosynthetic pathway could be a potential candidate for the additional gene proposed by Zhang and Dubcovsky (2008) and in this study.

Kofa carried positive alleles for the QTLs on 7B, whereas UC1113 contributed positive alleles for the 7A QTLs. The QTL mapped on 7AL was reported in different mapping populations of durum and common wheat, explaining a large portion of the yellow pigment variation (He et al. 2008; Patil et al. 2008; Howitt et al. 2009; Blanco et al. 2011). This QTL was also associated with allelic variation of *Psy-A1* in durum (Singh et al. 2009; Blanco et al. 2011) and common wheat (He et al. 2008; Howitt et al. 2009). In our study, the *QFb.cerz-7AL* was significantly associated with Fb* (LOD between 3.8 and 5.4) and weakly associated with YPC, with LOD values between 2.0 and 3.0. Our results showed that the effect of the 7AL genomic region was environmentally unstable, in contrast to the report by Zhang et al. (2008). However, we found that the *QFb.cerz-7AL* was involved in epistatic interactions with the QTLs *QFb.cerz-6AL.1*, *QFb.cerz-7BL.2* and *QFb.cerz-4AL.3*. Singh et al. (2009) explored the interaction between *Psy-A1* and *Psy-B1* in durum wheat, without positive results. In the linkage map for the UC1113 × Kofa population, the *Psy-A1* locus could not be mapped since it was monomorphic in the cross. Interestingly, the markers flanking the 7AL QTL were coincident with the second QTL reported in this region by Singh et al. (2009). This information suggests that in the epistatic effect *QFb.cerz-7AL* × *QFb.cerz-7BL.2*, a second pair of orthologous genes different from *Psy1* located on 7L could be involved. Moreover, two linked QTLs affecting both YPC and YC on chromosome 7AL were reported by Blanco et al. (2011). One of these QTLs was flanked by a *Psy-A1* marker, further supporting the hypothesis of more than one gene on 7L associated with these traits.

In addition to the QTLs on 7L, two QTLs on 7S associated with traits Fb* and YPC were found. This

also agrees with previous reports. A minor QTL linked to *gwm46* on 7BS was reported by Patil et al. (2008). On our map, this SSR is located 6.1 cM from *barc72* flanking the 7BS.2 QTL and was mapped in the same position as in the consensus linkage map (Somers et al. 2004). Reimer et al. (2008) also identified QTLs on homeologous regions of 7AS and 7BS, flanked by markers linked to our QTLs based on the consensus linkage map (Somers et al. 2004). The QTLs mapped by us in the putative homoeologous region 7AS (*QFb.cerz-7AS*, *QYpc.cerz-7AS*) were not reported in previous work using biparental populations. We confirmed the main effect of this QTL on YPC for the overall analysis using QTLNetwork v.2 software (2D genome scan and MCMC options) with an *F* value of 8.25 (critical value, 4.51). In addition, the 7AS QTL was detected in three and four environments associated with YPC and YC, respectively, using this software. Crawford et al. (2008) reported that genes encoding the enzyme geranylgeranyl transferase II β-subunit, involved in the carotenoid biosynthetic pathway upstream of the *Psy* genes, were located in homoeologous regions of 7S chromosomes in common wheat. We can consider this group of genes as potential candidates for 7S QTLs. Although the 7S QTLs explained only a minor proportion of the variation, all 7S regions studied (7AS, 7BS.1 and 7BS.2) showed digenic epistatic interactions that contributed to the genetic variance. However, it is possible, because it was detected only as suggestive in only one environment, that the 7BS.1 QTL is not a true YC QTL, but rather the consequence of a pleiotropic effect.

In agreement with Zhang et al. (2008), we found *QYpc.cerz-4AL.1* associated with YPC flanking the *Lpx-A3* locus. For Fb*, the peak *QFb.cerz-4AL.1* was detected closer to *barc170*, sharing a common confidence interval. This marker was reported by Reimer et al. (2008) as associated with YPC using an association mapping strategy. The second QTL identified in this work, on the distal part of the long arm of the chromosome 4A (4AL.2), was also detected by Zhang et al. (2008) and recently reported in common wheat (Zhang et al. 2009). *QFb.cerz-4AL.2* showed a strong effect on Fb* and was involved in two digenic epistatic interactions, confirming the relevance of this genomic region in flour yellow color determination. In line with this argument, *QFb.cerz-4AL.2* and *QFb.cerz-6AL.2* were the only stable QTLs detected using Fb* pooled data.

A second *Psy* gene (*Psy2*) was assigned to homeologous group 5 chromosomes (Pozniak et al. 2007; Blanco et al. 2009). We detected one QTL on 5AS and two linked QTLs on 5BL (5BL.1 and 5BL.2). *Psy-A2* and *Psy-B2* were located by Blanco et al. (2009) on the short arms of 5A and 5B by linkage analysis and physical mapping. *Psy-A2* shows a similar position to our 5AS QTL. Our results showed that *QFb.cerz-5BL.1* was closely linked to the marker *barc74*, in the same region in which Blanco et al. (2009) positioned the *Psy-B2* gene. This finding suggests that the 5BL.1 QTL corresponds with the *Psy2* locus. Similar results were obtained by Pozniak et al. (2007). However, Ramya et al. (2010) found that *barc74* and *gwm371* were associated with a strong QTL involved in kernel width and TKW. Therefore more evidence is required to associate *Psy-B2* and the 5BL.1 QTL. In agreement with our results, Reimer et al. (2008) found two linked QTLs on 5B, the second one in a similar position to 5BL.2, based on the common marker *gwm408*. This marker was also reported flanking a QTL for YPC on 5B in durum wheat (Patil et al. 2008).

Based on our data, and in contrast to previous work (Elouafi et al. 2001; Pozniak et al. 2007; Patil et al. 2008; Zhang et al. 2008; Blanco et al. 2011), *QYpc.cerz-6AL.2* and *QFb.cerz-6AL.2* were the most relevant QTLs for YPC and Fb*, explaining the highest percentages of phenotypic variation for the mean data for both traits. However, three QTLs were detected on the 6AL chromosome arm. Considering individual environments, *QYpc.cerz-6AL.2* was strongly associated with YPC in five of them (Table 3), while the other two QTLs (*QFb.cerz-6AL.1* and *QFb.cerz-6AL.3*) jointly explained the highest percentage of variation in Fb* in two environments (Table 2). The *barc146* marker closely linked to the 6AL.1 QTL was reported by Reimer et al. (2008) as significantly associated with YPC. We also found that the interval *CD491758_81-barc146* showed an epistatic effect with the 7AL QTL, flanked by the *wmc116-cfd6* marker interval. In addition, *gwm169* linked to the 6AL.3 QTL was found by Zhang et al. (2006) to flank a QTL on chromosome 6AL that was strongly associated with flour yellowness (b*) in common wheat. Additionally, a QTL underlying phenotypic variation in grain flour color was also reported on 6BL (Pozniak et al. 2007), but this may be associated with a pleiotropic effect of TKW on grain

color, as suggested by the authors. Sun et al. (2009) found a TKW QTL in a similar region to the 6AL.3 QTL based on the Somers et al. (2004) consensus map. Interestingly, a molecular study of the carotenoid biosynthetic pathway reported genes encoding the enzyme ε-carotene hydroxylase on homoeologous chromosome arms 6AL, 6BL, 6DL in common wheat (Crawford et al. 2008). This enzyme is involved in the final step of lutein production and it may be a good candidate for the stable 6AL.2 QTL detected on 6AL in the current work. Moreover, further genetic studies should be done in order to determine if the gene encoding the enzyme ε-carotene hydroxylase is responsible for high yellow color, particularly given that the possibility of a QTL associated with grain size (TKW) having a pleiotropic effect on flour color (Pozniak et al. 2007) cannot be discarded.

QTL analyses of the Fa* and FL* color parameters

Our results showed a strong QTL associated with FL* located on chromosome arm 2BS (*QFl.cerz-2BS*). Mares and Campbell (2001) reported a QTL on 2B associated with the flour L* parameter and initial brightness (L*0 h) of noodle sheets in common wheat. In previous work, QTLs affecting polyphenol oxidase activity (PPO) were reported on homoeologous group 2 chromosomes (Mares and Campbell 2001; Watanabe et al. 2006). It is known that polyphenol oxidases play a major role in the darkening of noodles and other wheat products. Although the main QTLs for PPO were identified on chromosomes 2A in durum (Watanabe et al. 2006) and 2D in common wheat (Mares and Campbell 2001) we cannot rule out the possibility that PPO genes affected the FL* parameter in our mapping population. On the other hand, Zhang et al. (2011) located the ZDS gene, encoding the zeta-carotene desaturase enzyme associated with yellow pigment content, on chromosome arm 2DL. This enzyme could be causing an indirect effect on FL*. Both possibilities need to be investigated.

Correlation analysis showed a negative relationship between the Fa* color parameter and the SDS test ($r = -0.41$, Table S3). To our knowledge, there are no previous reports about this association and it would be valuable to confirm the possibility that the MINOLTA red color (Fa*) parameter is a moderate indicator of the SDS test and of gluten strength. Conti et al. (2011) detected four QTLs for SDS test with pleiotropic effects on the Fa* color parameter in the current work (*QFa.cerz-1AL*, *QFa.cerz-1BL.1*, *QFa.cerz-3BS* and *QFa.cerz-6AL.1*). These data suggest that gluten composition affects grain color on the Fa* axis of the color scale. In line with this hypothesis, a QTL for Fa* was associated with the *Glu-B3* locus on 1BS that encodes low molecular weight glutenin subunits and affects the SDS test in common wheat (Zhang et al. 2009). The *Glu-3* locus was not included in our map; however, *QFa.cerz-1BL.1* was linked to the *Glu-B1* locus, another storage protein locus associated with gluten strength (Conti et al. 2011), supporting the association between gluten composition and Fa* observed by Zhang et al. (2009).

Conclusions

In this work we detected several QTLs affecting flour color traits, particularly for Fb* and YPC, confirming the complex inheritance of these traits. We validated the main QTLs reported for this population in California by using contrasting environments in a different hemisphere (Argentina), allowing us to find additional QTLs and epistatic interactions involving the most important QTLs. This study allowed a thorough analysis of the environmental stability of QTLs involved in the particular genetic background evaluated.

A novel QTL affecting Fb* and YPC on chromosome arm 7AS was found. It showed an epistatic effect for YPC with a 6BL QTL of minor effect. It is remarkable that a second putative pair of genes/QTLs affecting Fb* on 7AL and 7BL showed epistatic interaction. Our results differed from previous reports in that the most important QTL affecting Fb* and YPC was located on chromosome 6AL (6AL.2), with stable performance across environments. This QTL, in addition to QTLs linked to the *Psy1* genes, could be a useful target for breeding programs aimed at improving durum wheat quality.

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