

# Molecular genetic analysis of flour color using a doubled haploid population in bread wheat (*Triticum aestivum* L.)

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**Abstract** Flour color is an important trait in the assessment of flour quality for the production of many end products. In this study, quantitative trait loci (QTLs) with additive effects, epistatic effects, and QTL × environment (QE) interactions for flour color in bread wheat (*Triticum aestivum* L.) were studied, using a set of 168 doubled haploid (DH) lines derived from a Huapei 3 × Yumai 57 cross. A genetic map was constructed using 283 simple sequence repeats (SSR) and 22 expressed sequence tags (EST)-SSR markers. The DH and parents were evaluated for flour color in three environments. QTL analyses were performed using QTLNetwork 2.0 software based on a mixed linear model approach. A total of 18 additive QTLs and 24 pairs of epistatic QTLs were detected for flour color, which were

distributed on 19 of the 21 chromosomes. One major QTL, *qa1B*, closely linked to *barc372* 0.1 cM, could account for 25.64% of the phenotypic variation of *a\** without any influence from the environments. So *qa1B* could be used in the molecular marker-assisted selection (MAS) in wheat breeding programs. The results showed that both additive and epistatic effects were important genetic basis for flour color, and were also sometimes subject to environmental modifications. The information obtained in this study should be useful for manipulating the QTLs for flour color by MAS in wheat breeding programs.

**Keywords** Doubled haploid · Epistatic effects · Flour color · Marker-assisted selection · Quantitative trait loci · Wheat (*Triticum aestivum* L.)

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## Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple crops worldwide. Flour color is an important trait in the assessment of flour quality for the production of many end products (Parker and Langridge 2000). Flour with high levels of yellow pigmentation is preferred for Chinese and Japanese alkaline noodle production. In many Asian countries, noodles are prepared from flour specifically selected to enhance the color of the final product (Kruger et al. 1994). Miskelly (1984) detected a significant positive

correlation between flour color and noodle sheet yellowness in both Chinese and Japanese noodles. However, other end products such as bread, steamed bread, dry white noodles, and dumplings require white flour with extremely low levels of yellow pigmentation. Color readings are usually expressed as  $L^*$  (lightness),  $a^*$  (red-green chromaticity), and  $b^*$  (yellow-blue chromaticity) of the flour sample (CIE 1976). Theoretically, a pure white flour should have zero values for  $a^*$  and  $b^*$ , and one hundred for  $L^*$  (Sun et al. 2002). Low values of  $L^*$  with high positive values of  $a^*$  result in a gray and dull color. Significant variations in flour color exist among genotypes of wheat. Moreover, environment and management practices may have impacts on flour color. Grain protein content, hardness, vitreousness, seed coat color, and kernel size and shape may all contribute to variations in flour color (Zhang and Tian 2008). The flour color from low-protein-content wheat is more white than the flour from high-protein-content wheat at the same milling extraction ratio. Flour from wheat with a red seed coat has higher  $a^*$  values than flour from the wheat with a white seed coat (Zhang and Tian 2008). Some recently released varieties show good agronomic characteristics and are high yielding but contain high levels of flour carotenoids, limiting the end product uses (Parker and Langridge 2000). It is of great value to understand the wheat cultivar's molecular genetic regulation and to select them effectively for genetic and plant breeding purposes. Flour color behaves as a typical quantitative trait. With conventional breeding methods, the selection efficiency for flour color is low.

Recently, the development of molecular markers such as restriction fragment length polymorphisms (RFLPs) and simple sequence repeats (SSRs), and their maps, have provided powerful tools for elucidating the genetic basis of quantitatively inherited traits, including most of the agriculturally important and quality traits, which makes them feasible to detect and map QTLs in a wheat population, even from a cross between two wheat cultivars (Huang et al. 2003). QTLs associated with important traits have been identified in wheat, including agronomic traits, grain yield, disease characters, and quality traits (Börner et al. 2002; Liu et al. 2002; Ma et al. 2005; Huang et al. 2006). Furthermore, DNA MAS has made it possible to develop near-isogenic lines and chromosome segment substitution lines for QTL regions affecting agronomic

and quality traits (Lin et al. 2000; Ma et al. 2002). There are several reports for flour color associated with markers. Parker et al. (1998) identified ten RFLP marker loci that showed significant associations with QTLs for flour color, located on chromosomes 3A and 7A, by using 150 single seed descent (SSD) lines. The estimated heritability of flour color was calculated as 0.68, indicating that a large part of the expression of this trait is genetically controlled, making it easier to manipulate at the genetic level in a breeding program. Mares and Campbell (2001) reported that xanthophyll content was very strongly associated with QTLs located on chromosomes 3B and 7A in 163 DH lines derived from Sunco  $\times$  Tasman. Zhang et al. (2006) identified eight QTLs for  $b^*$  located on chromosomes 1DS, 2DL, 3A, 4D, 5D, 6AL, 6D, and 7AL. Knott (1984) reported that a gene for  $b^*$  was linked to *Lr19* in 'Agatha' wheat. One SSR and sequence related amplified polymorphism (SRAP) marker closely linked to a *Lr19* resistance trait was obtained, and were named *wms44* and *M73* with genetic distances 0.9 and 2.6 cM, respectively (Li et al. 2005; Liu et al. 2007a). A co-dominant amplified fragment length polymorphism (AFLP) marker linked to a major locus controlling flour color in wheat has been converted to an sequence tagged sites (STS) marker for wider applicability in MAS (Parker and Langridge 2000). However, most previous studies have focused on  $b^*$ ; very few studies have reported on  $a^*$  and  $L^*$  values, which are also very important components of flour color in wheat.

Epistatic effects have been considered to be important for complex traits by many researchers (Cao et al. 2001; Fan et al. 2005; Ma et al. 2005, 2007). Determining the contribution of epistasis to complex traits is important for understanding the genetic basis of complex traits. Hence, genetic models for QTL mapping assuming no epistasis could lead to a biased estimation of QTL parameters. Large numbers of epistatic effects have recently been detected in rice and maize by using polymorphism markers in the whole genome (Cao et al. 2001; Fan et al. 2005; Ma et al. 2007). Ma et al. (2005) provided strong evidence for the presence of epistatic interactions on dough rheological properties in a wheat DH population. So far, no information has been found available on the role of epistasis in controlling flour color in wheat.

Genotype-by-environment interactions have received much attention in crop-breeding programs

(Dudley et al. 1996), which are important for MAS in crop improvement (Wang et al. 1999). A mixed-model-based composite interval mapping (Wang et al. 1999) methodology was proposed for mapping QTLs with additive effects and epistatic effects as well as their QE interactions, and the software QTLNetwork 2.0 (Yang and Zhu 2005) has been developed for analyzing the experimental data. This method has been successfully applied in recent QTL mapping studies (Liu et al. 2007b; Ma et al. 2007; Rebetzke et al. 2007).

In the present study, we investigated flour color for a DH population by using the software of QTLNetwork 2.0 (Yang and Zhu 2005), based on the method of mixed linear models (Wang et al. 1999). The primary goal of the study described here was to detect QTLs with additive effects, epistatic effects, and QE interactions for flour color ( $a^*$ ,  $b^*$ , and  $L^*$ ), with the aim of identifying markers that could be used for MAS. The results should be useful in relevant wheat breeding programs.

## Materials and methods

### Plant materials

A population of 168 DH lines was produced by hybridization with maize pollen of wheat F<sub>1</sub> hybrid plants from the cross between two Chinese bread wheats Huapei 3 × Yumai 57 and used for the construction of a genetic linkage map. Huapei 3 and Yumai 57 were registered by Henan in 2006 (Hai and Kang 2007) and by the State (China) in 2003 (Guo et al. 2004), respectively. Huapei 3 is a hard wheat with a higher grain protein content and wet gluten content. Yumai 57 is a soft wheat with higher gluten index and sodium dodecyl sulfate (SDS) sedimentation volume and is more productive under different ecological conditions (Hai and Kang 2007; Guo et al. 2004). The parents both have white seed coats.

### Field trials and flour color evaluation

Field trials were conducted under three environments during 2005 and 2006 in Taian, Shandong Province, and in 2006 in Suzhou, Anhui Province. The experimental design followed a completely randomized

block design with three replications at each location. In autumn 2005, all lines and parental lines were grown in 2-m-long three-row plots (25 cm apart), whereas in autumn 2006, they were grown in 2-m-long four-row plots (25 cm apart). Suzhou and Taian show large differences in climate, soil conditions, and day length. At Taian, there were remarkable differences in temperature and soil conditions between the years 2005 and 2006. Management was in accordance with local practice. The lines were harvested individually at maturity to prevent yield loss from overripening. Harvested grain samples per line from the three replicates at each environment were mixed and cleaned prior to conditioning and milling in order to maintain a manageable number of samples for quality testing.

One thousand grams of grain samples from each line at each environment were milled by using a Buhler experimental mill (model-MLU 300 M/s Buhler, Uzwil Switzerland). Color measurements of flour samples were carried out using a Minolta Color Meter 310 (Minolta Camera Co, Ltd, Japan) on the basis of  $L^*$ ,  $a^*$ , and  $b^*$  values (CIE 1976). The  $L^*$  value indicates the lightness, with 0–100 representing darkness to lightness. The  $a^*$  value gives the degree of the red–green color, with a higher positive  $a^*$  value indicating more red. The  $b^*$  value indicates the degree of the yellow–blue color, with a higher positive  $b^*$  value indicating more yellow (Hutchings 1994). Colorimeter scores were taken five times per sample. The mean values of flour color in each environment were used for statistical analyses.

### DNA marker assay and construction of genetic map

DNA was extracted from freeze-dried leaves as described by Ellis et al. (2005). A total of 2,002 polymorphism markers were determined using parental DNA. Primer sequences for 260 *Xgwm* (*wms*) and 82 *Xgdm* microsatellite markers were available from Röder et al. (1998) and Pestsova et al. (2000). Markers, 621 *Xwmc* and 480 *Xbarc*, were available from the Graingenes website (<http://www.wheat.pw.usda.gov/ggpapes/SSR/WMC/>) and the US wheat and barley scab initiative website ([http://www.scabusa.org/pdfs/BARC\\_SSRs\\_011101.html](http://www.scabusa.org/pdfs/BARC_SSRs_011101.html)), respectively. Thirty *BE* markers were available from Chen et al. (2005). Fifty *Xcfa*, 130 *Xcfd*, 48 *Xcwem*, and 301 *Xcfe* markers were

kindly provided by Dr. Xianchun Xia, Chinese Academy of Agricultural Sciences, Beijing, China.

Amplification reactions were carried out in a 20  $\mu$ l reaction mixture, containing 3.6  $\mu$ l genomic DNA (20 ng/ $\mu$ l), 1.5  $\mu$ l MgCl<sub>2</sub> (25 mmol/l), 1.2  $\mu$ l dNTP mixtures (2.5 mmol/l), 2.0  $\mu$ l 10 $\times$  PCR buffer, 0.5  $\mu$ l primer (10  $\mu$ mol/l), 0.2  $\mu$ l *Taq* polymerase enzyme (5 units/ $\mu$ l), and 11.0  $\mu$ l ddH<sub>2</sub>O. The polymerase chain reactions (PCR) were performed in 96-well microtiter plates by using an Eppendorf AG 22331 Hamburg thermal cycler (Eppenf, Hamburg, Germany). DNA amplification was programmed at 95°C for 5 min, followed by 36 cycles of 95°C for 1 min, 50–65°C (depending on the primer combinations) for 50 s, 72°C for 1 min, and a final extension of 10 min at 72°C before cooling to 4°C. After amplification, the PCR products were mixed with 5.6  $\mu$ l loading buffer (2.5 mg/ml bromophenol blue, 2.5 mg/ml diphenylamine blue, 10 mmol/l EDTA, 95% (v/v) formamide), denatured for 5 min at 95°C, and chilled on ice for 5 min. The PCR products were separated using 6% (W/V) denatured polyacrylamide gel and were detected by silver staining (Karakousis et al. 2003).

### Statistical analysis

Analysis of variance (ANOVA) was carried out using the SPSS version 13.0 (SPSS, Chicago, USA) program. QTL analyses were performed using QTL-Network software version 2.0 (Yang and Zhu 2005) based on a mixed linear model (Wang et al. 1999).

Composite interval analysis was undertaken using forward–backward stepwise, multiple linear regression with a probability into and out of the model of 0.05, and window size set at 10 cM. QTL was declared if the phenotype was associated with a marker locus at  $P < 0.005$ . The final genetic model incorporated significant additive effects and epistatic effects as well as their environment interactions.

## Results

### Statistical analysis of the phenotypic assessments

Mean values of flour color for the parents Huapei 3 and Yumai 57, as well as the 168 DH lines under three environments in 2005 and 2006 cropping seasons, are shown in Table 1 and Fig. 1. Huapei 3 had a higher value for  $b^*$ , while Yumai 57 had higher values for both  $a^*$  and  $L^*$ . Transgressive segregants were observed for the three traits among the DH lines in the three environments. Three traits of the DH population segregated continuously and followed a normal distribution (Fig. 1), and both absolute values of skewness and kurtosis were less than 1.0 (Table 1), indicating its polygenic inheritance and suitability of the data for QTL analysis (Cao et al. 2001).

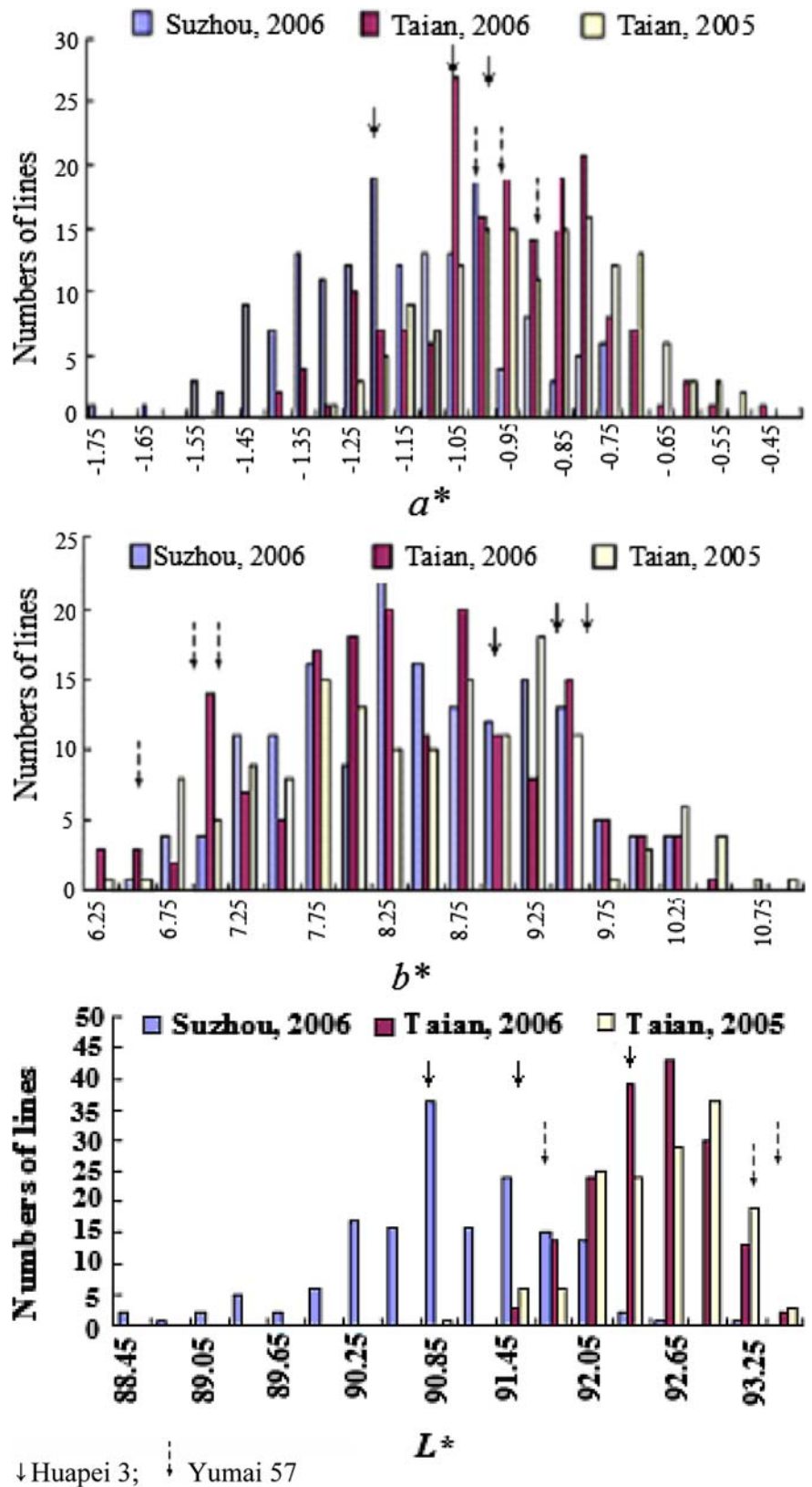
The correlations among the  $a^*$ ,  $b^*$ , and  $L^*$  values are shown in Table 2. Significantly negative correlations were detected between  $L^*$  and  $b^*$  ( $r = -0.559$ ), and between  $a^*$  and  $b^*$  ( $r = -0.494$ ).

**Table 1** Phenotypic of flour color ( $a^*$ ,  $b^*$ , and  $L^*$ ) for Huapei 3, Yumai 57, and their DH progenies evaluated at three environments in the 2005 and 2006 cropping seasons

Trait	Environment	Parents		DH population						
		Huapei 3	Yumai 57	Mean	SE	Max.	Min.	SD	Skew.	Kurt.
$a^*$	Env1: 2006 Suzhou	-1.16	-0.96	-1.17	0.02	-0.71	-1.78	0.20	-0.04	-0.24
	Env2: 2006 Taian	-1.05	-0.95	-0.97	0.01	-0.49	-1.44	0.17	-0.24	0.35
	Env3: 2005 Taian	-1.00	-0.92	-0.91	0.01	-0.50	-1.31	0.18	-0.01	-0.60
$b^*$	Env1: 2006 Suzhou	9.29	7.15	8.32	0.07	10.66	6.39	0.86	0.10	0.55
	Env2: 2006 Taian	9.13	6.67	8.22	0.07	10.21	6.01	0.93	-0.10	-0.47
	Env3: 2005 Taian	9.27	7.12	8.35	0.08	10.84	6.07	1.02	0.10	-0.60
$L^*$	Env1: 2006 Suzhou	90.85	91.08	90.78	0.07	93.36	88.16	0.83	-0.50	0.89
	Env2: 2006 Taian	92.10	93.32	92.41	0.04	93.44	91.17	0.48	-0.07	-0.67
	Env3: 2005 Taian	91.45	93.25	92.46	0.05	93.53	90.64	0.58	-0.52	-0.09

Mean, SE, Max., Min., SD, Skew., and Kurt. are the average, standard error, maximum, minimum, standard deviation, skewness, and kurtosis of all observations for the DH population in the three environments

**Fig. 1** Phenotypic summary distribution of DH progeny from a cross Huapei 3/Yumai 57 for  $a^*$ ,  $b^*$ , and  $L^*$ . DH lines and parental means are indicated



**Table 2** Coefficients of pairwise correlations of flour color ( $a^*$ ,  $b^*$ , and  $L^*$ ) for the DH progenies evaluated at three environments in the 2005 and 2006 cropping seasons

Trait	$a^*$	$b^*$
$b^*$	-0.494**	
$L^*$	-0.181*	-0.559**

\* Significance at the 0.05 level of probability

\*\* Significance at the 0.01 level of probability, respectively

### Construction of the genetic map

A genetic map of the DH population with 305 SSR markers, including 283 SSR and 22 ESTs loci, was constructed, covering a total length of 2141.7 cM, with an average distance of 7.02 cM between adjacent markers in the genome. Ten SSR markers remained unlinked. These markers formed 24 linkage groups at logarithm of the odds ratio (LOD)  $\geq 3.0$ . The chromosomal locations and marker orders in the map were in accordance with the microsatellite map for *Triticum aestivum* L. (Somers et al. 2004). The recommended map distance for genome-wide QTL scanning is an interval length less than 10 cM (Doerge 2002). Therefore the map is suitable for genome-wide QTL scanning.

### QTLs for flour color

Eighteen QTLs with additive effects and/or additive  $\times$  environment (AE) effects were detected for flour color in three environments (Table 3, Fig. 2), ranging from four to eight QTLs for each trait, and were distributed on 12 of the 21 chromosomes.

Twenty-four pairs of QTLs with epistatic effects and/or epistasis  $\times$  environment (AAE) effects were detected for flour color in the three environments (Table 4, Fig. 2), ranging from four to ten QTLs for each trait. The highest numbers of QTLs with epistatic effects were found in  $b^*$  and  $L^*$  with ten pairs of QTLs. In contrast, only four pairs of QTLs were detected for  $a^*$ .

### QTLs for $a^*$

Six additive QTLs were detected for  $a^*$ , on chromosomes 1B, 3B, 5D, 6A, 6D, and 7D. They increased  $a^*$  from 0.03 to 0.10, explaining phenotypic variance from 2.24% to 25.64%. *qa1B* had the most significant effect, accounting for 25.64% of the phenotypic variance.

Three QTLs (*qa1B*, *qa5D*, and *qa6D*) had negative effects on  $a^*$  and were contributed by Yumai 57 alleles while the other loci had positive effects on  $a^*$  and were transmitted by Huapei 3 alleles. This suggested that alleles which increased  $a^*$  were dispersed within the two parents, resulting in small differences of phenotypic values between the parents and the transgressive segregants among the DH population. The total contribution of all the six QTLs to  $a^*$  was 41.29%. *qa5D* was also involved in AE interactions in two environments. The AE interactions explained 2.90% of the phenotypic variance of  $a^*$ .

Four pairs of epistatic effects were identified for  $a^*$  and located on chromosomes 1A–7B, 2A–4B, 4D–6A, and 5B–7B. They explained the phenotypic variance ranging from 0.60% to 2.35%. *qa4D/qa6A* had a positive effect of 0.03. However, the other three pairs showed negative effects on  $a^*$ . The general contribution of four pairs of epistatic QTLs was 7.10%, while no main-effect QTL was detected in epistatic effects. No AAE interactions were identified for  $a^*$ .

For  $a^*$ , the total QE interactions could explain 2.90% of the phenotypic variance.

### QTLs for $b^*$

Four QTLs with additive effects significantly influenced  $b^*$  were located on chromosomes 2B, 3D, 4D, and 5A. The *qb5A* made the highest contribution and explained 4.30% of the phenotypic variance. All four QTLs could account for 11.21% of the phenotypic variance and were derived from Huapei 3 alleles, which was in accordance with Huapei 3 having much larger  $b^*$ . No AE interactions were identified for  $b^*$ .

Ten pairs of epistatic effects were resolved for  $b^*$  and were located on chromosomes 3D–5B, 1A–1B, 2A–3D, 2B–7A, 3A–5B, 3D–4D, and 6B–7D. Among them, *qb3A/qb5B* had the highest contribution and accounted for 6.15% of the phenotypic variance. Three pairs of epistatic QTLs (*qb3D-2/qb5B*, *qb3A/qb5B*, and *qb6B/qb7D*) had negative effects on  $b^*$ . However, the other seven pairs showed positive effects on  $b^*$ . The total contribution of ten pairs of epistatic QTLs was 31.88%, so epistasis played a strong effect on  $b^*$ . One main-effect QTL, *qb3D-2*, was involved in three pairs of epistatic effects. No AAE interactions were identified for  $b^*$ .

For  $b^*$ , no QE interactions were detected in this study.

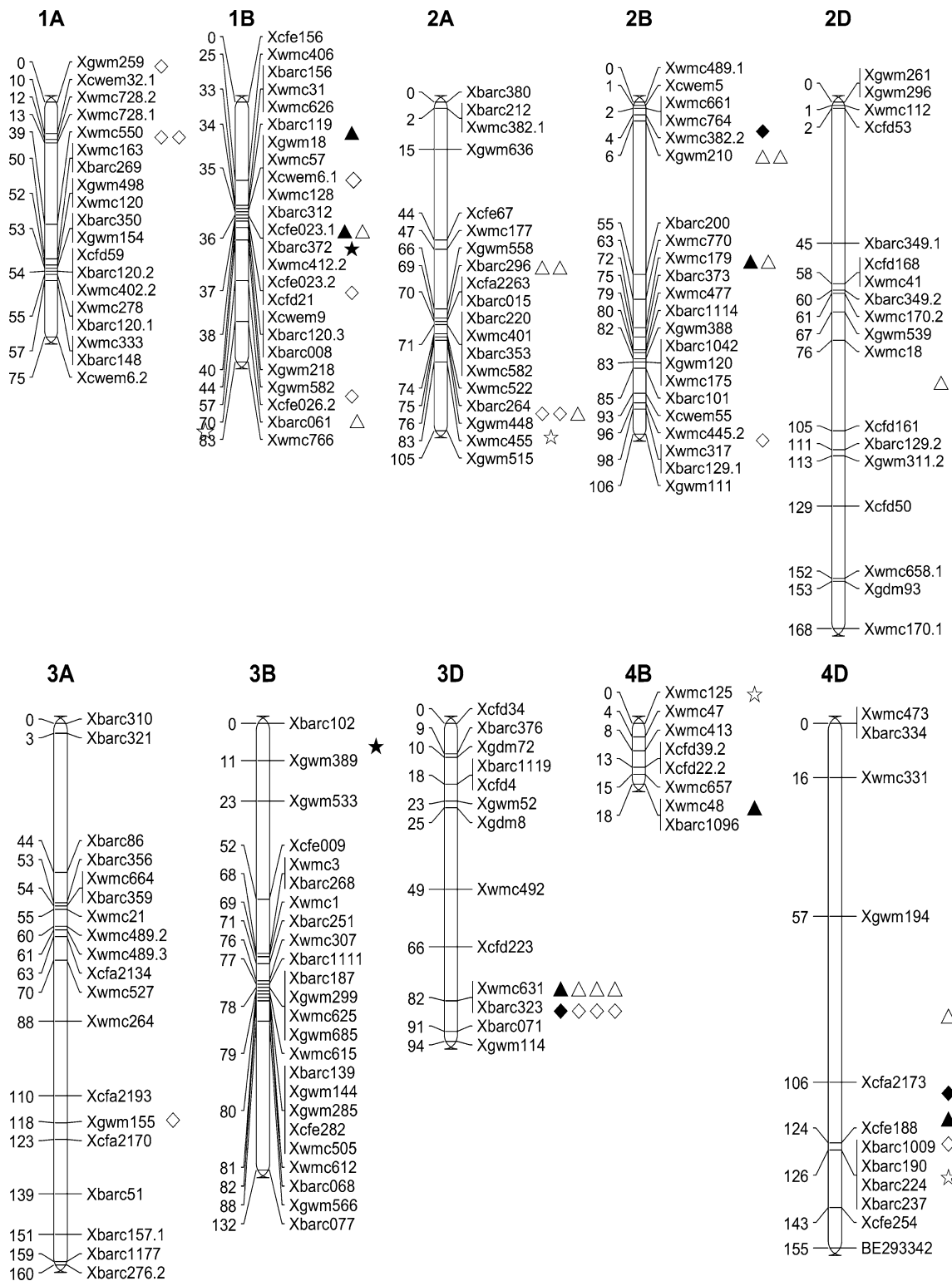
**Table 3** Estimated additive (A) and additive × environment (AE) interactions of QTLs for flour color ( $a^*$ ,  $b^*$ , and  $L^*$ ) at three environments in the 2005 and 2006 cropping seasons

Trait	QTL	Flanking marker	Site (cM)	F-value	P	A <sup>a</sup>	H <sup>2</sup> (A, %) <sup>b</sup>	AE <sub>1</sub>	H <sup>2</sup> (AE <sub>1</sub> , %)	AE <sub>2</sub>	H <sup>2</sup> (AE <sub>2</sub> , %)	AE <sub>3</sub>	H <sup>2</sup> (AE <sub>3</sub> , %)
General contribution—additive: H <sup>2</sup> (A) = 41.29%; QE interactions—H <sup>2</sup> (AE) = 2.90%													
<i>a</i> *	<i>qa1B</i>	<i>Xbarc372-Xwmc412.2</i>	0.1	49.11	0.000	-0.10	25.64						
	<i>qa3B</i>	<i>Xbarc102-Xgwm389</i>	3.0	5.68	0.000	0.04	3.94						
	<i>qa5D</i>	<i>Xwmc215-Xgdm63</i>	9.0	7.92	0.000	-0.03	2.24	0.02	1.68	-0.02	1.22		
	<i>qa6A</i>	<i>Xgwm459-Xgwm334</i>	4.0	6.38	0.000	0.03	2.49						
	<i>qa6D</i>	<i>Xcfa2129-Xbarc080</i>	16.4	5.94	0.000	-0.04	3.97						
	<i>qa7D</i>	<i>Xgwm295-Xgwm676</i>	19.0	4.14	0.000	0.04	3.01						
General contribution—additive: H <sup>2</sup> (A) = 11.21%; QE interactions—H <sup>2</sup> (AE) = 0.0%													
<i>b</i> *	<i>qb2B-1</i>	<i>Xwmc764-Xwmc382.2</i>	0.7	5.09	0.036	0.07	0.51						
	<i>qb3D-2</i>	<i>Xwmc631-Xbarc323</i>	5.2	4.45	0.000	0.17	2.95						
	<i>qb4D-1</i>	<i>Xcfa2173-Xcfe188</i>	2.8	10.83	0.000	0.17	3.45						
	<i>qb5A</i>	<i>Xbarc358.2-Xgwm186</i>	1.40	5.66	0.000	0.19	4.30						
General contribution—additive: H <sup>2</sup> (A) = 22.28%; QE interactions—H <sup>2</sup> (AE) = 3.01%													
<i>L</i> *	<i>ql1B-1</i>	<i>Xbarc119-Xgwm18</i>	0.5	15.19	0.000	0.12	3.48	0.07	1.19				
	<i>ql2B-2</i>	<i>Xwmc179-Xbarc373</i>	5.9	8.83	0.000	-0.11	2.67						
	<i>ql3D</i>	<i>Xwmc631-Xbarc323</i>	3.2	5.61	0.000	-0.12	3.49	-0.09	1.82				
	<i>ql4B</i>	<i>Xwmc48-Xbarc1096</i>	1.7	4.62	0.004	0.06	0.82						
	<i>ql4D-2</i>	<i>Xcfa2173-Xcfe188</i>	11.8	10.61	0.000	-0.16	6.14						
	<i>ql5D-2</i>	<i>Xcfd226-Xwmc765</i>	8.0	5.54	0.005	-0.07	0.98						
	<i>ql7B</i>	<i>Xgwm333-Xwmc10</i>	11.0	5.20	0.000	0.11	2.83						
	<i>ql7D</i>	<i>Xgwm295-Xgwm676</i>	13.0	5.42	0.001	-0.09	1.87						

<sup>a</sup> The additive effect of putative main-effect QTL, a positive value indicates that the Huapei 3 allele has a positive effect on that trait

<sup>b</sup> Contribution explained by putative main-effect QTL

E<sub>1</sub>: Suzhou, 2006; E<sub>2</sub>: Taian, 2006; E<sub>3</sub>: Taian, 2005



**Fig. 2** A genetic linkage map of wheat showing QTLs of flour color with additive effects and epistatic effects. ★, additive QTL of *a\**; ☆, epistatic QTL of *a\**; ◆, additive QTL of *b\**; ◇, epistatic QTL of *b\**; ▲, additive QTL of *L\**; △, epistatic QTL of *L\**



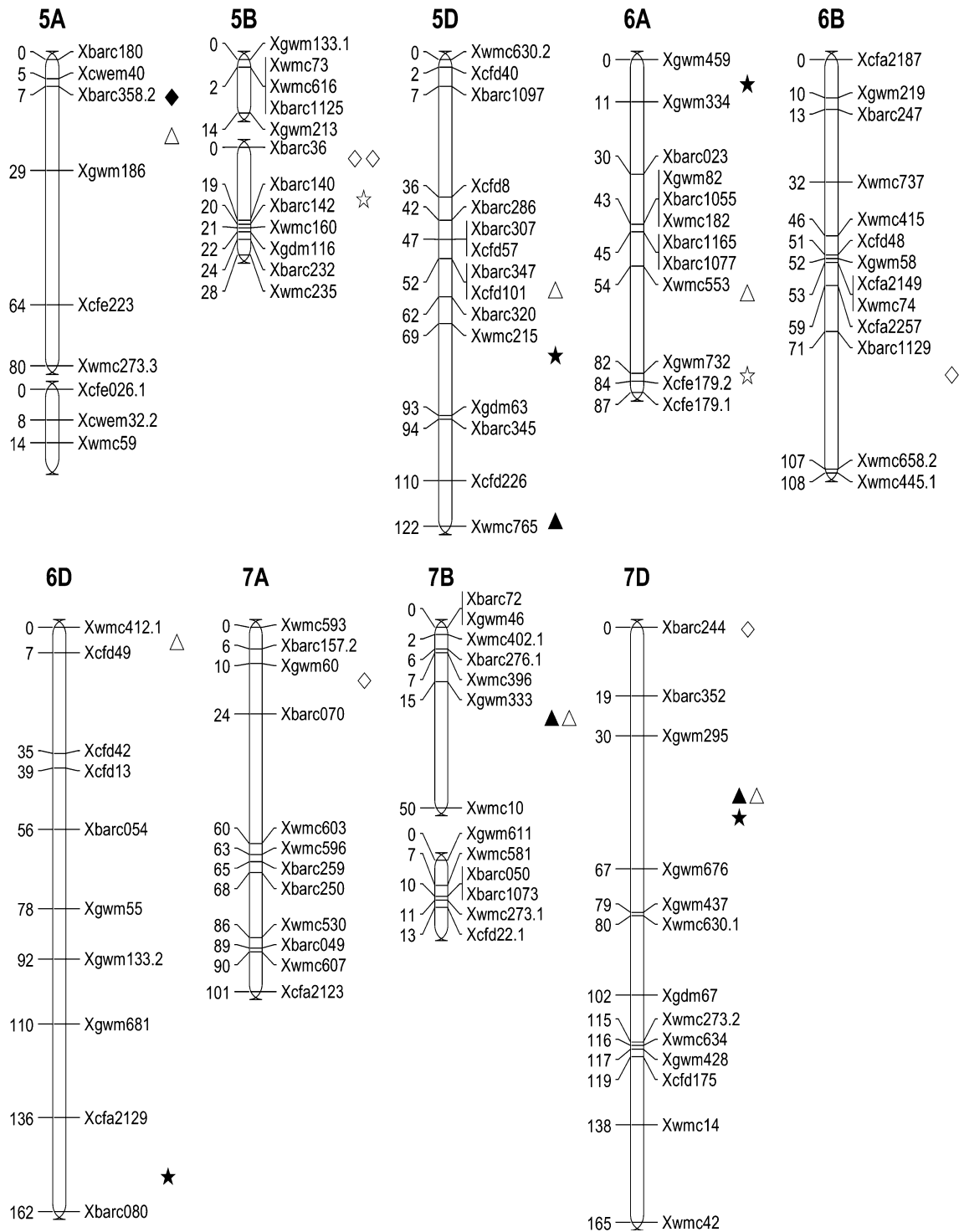


Fig. 2 continued

**Table 4** Estimated epistasis (AA) and epistasis × environment (AAE) interactions of QTLs for flour color ( $a^*$ ,  $b^*$ , and  $L^*$ ) at three environments in the 2005 and 2006 cropping seasons

Trait	QTL	Flanking marker	Site (cM)	QTL	Flanking marker	Site (cM)	P	AA <sup>a</sup>	H <sup>2</sup> (AA, %) <sup>b</sup>	AAE <sub>1</sub>	H <sup>2</sup> (AAE <sub>1</sub> , %)	AAE <sub>2</sub>	H <sup>2</sup> (AAE <sub>2</sub> , %)	AAE <sub>3</sub>	H <sup>2</sup> (AAE <sub>3</sub> , %)
General contributions—epistasis: H <sup>2</sup> (AA) = 7.10%; QE interactions—H <sup>2</sup> (AAE) = 0.0%															
$a^*$	qa1A	Xwmc333-Xbarc148	1.6	qa7B	Xwmc273.1-Xcfd22.1	2.0	0.000	-0.03	2.12						
	qa2A	Xgwm448-Xwmc455	1.5	qa4B	Xwmc125-Xwmc 47	0.0	0.000	-0.03	2.35						
	qa4D	Xbarc190-Xbarc224	0.3	qa6A	Xcfe179.2-Xcfe179.1	2.0	0.000	0.03	2.03						
	qa5B	Xbarc140-Xbarc142	1.4	qa7B	Xwmc273.1-Xcfd22.1	2.0	0.016	-0.02	0.60						
General contributions—epistasis: H <sup>2</sup> (AA) = 31.88%; QE interactions—H <sup>2</sup> (AAE) = 0.0%															
$b^*$	qb3D-2	Xwmc631-Xbarc323	5.2	qb5B	Xbarc36-Xbarc140	0.0	0.024	-0.07	0.60						
	qb1A-1	Xgwm259-Xcwem32.1	4.0	qb1B-1	Xcwem6.1-Xwmc128	0.4	0.001	0.11	1.54						
	qb1A-2	Xwmc728.1-Xwmc550	7.0	qb1B-2	Xcfe023.2-Xcfd21	0.9	0.006	0.11	1.37						
	qb1A-2	Xwmc728.1-Xwmc550	6.0	qb1B-3	Xgwm582-Xcfe 026.2	5.0	0.002	0.12	1.85						
	qb2A	Xbarc264-Xgwm448	1.2	qb3D-2	Xwmc631-Xbarc323	5.2	0.000	0.17	3.48						
	qb2A	Xbarc264-Xgwm448	1.2	qb3D-1	Xbarc376 -Xgwm72	0.4	0.000	0.18	3.74						
	qb2B-2	Xwmc445.2-Xwmc317	1.5	qb7A	Xgwm60-Xbarc070	5.0	0.000	0.18	3.96						
	qb3A	Xcfa2193-Xgwm155	4.0	qb5B	Xbarc36-Xbarc140	0.0	0.000	-0.23	6.15						
	qb3D-2	Xwmc631-Xbarc323	5.2	qb4D-2	Xcfe188-Xbarc1009	1.6	0.000	0.18	3.94						
	qb6B	Xbarc1129-Xwmc658.2	9.0	qb7D	Xbarc244-Xbarc352	0.0	0.000	-0.21	5.25						
General contributions—epistasis: H <sup>2</sup> (AA) = 21.36%; QE interactions—H <sup>2</sup> (AAE) = 3.88%															
$L^*$	q12B-2	Xwmc179-Xbarc373	5.9	q17B	Xgwm333-Xwmc10	11.0	0.000	0.09	1.82						
	q13D	Xwmc631-Xbarc323	3.2	q17D	Xgwm295-Xgwm676	13.0	0.000	-0.11	2.82						
	q11B-2	Xcfe023.1-Xbarc372	0.1	q12B-1	Xwmc382.2-Xgwm210	2.9	0.002	-0.07	1.01						
	q11B-2	Xcfe023.1-Xbarc372	0.1	q16A	Xwmc553-Xgwm732	6.2	0.000	-0.07	1.25						
	q11B-3	Xcfe026.2-Xbarc061	5.0	q12B-1	Xwmc382.2-Xgwm210	2.9	0.000	-0.09	1.89						
	q12A-1	Xgwm558-Xbarc296	1.9	q13D	Xwmc631-Xbarc323	3.2	0.000	-0.09	1.78						
	q12A-1	Xgwm558-Xbarc296	1.9	q16D	Xwmc412.1-Xcfd49	4.0	0.012	0.06	0.80						
	q12A-2	Xbarc264-Xgwm448	1.2	q13D	Xwmc631-Xbarc323	3.2	0.000	-0.08	1.45					-0.06	0.92
	q12D	Xgwm311.2-Xcfd50	11.6	q15D-1	Xbarc347-Xcfd101	0.6	0.000	0.10	2.20						
	q14D-1	Xwmc331-Xgwm194	28.8	q15A	Xbarc358.2-Xgwm186	9.0	0.000	-0.17	6.34					0.08	1.31

<sup>a</sup> Epistatic effects, a positive value indicates that the parental two-locus genotypes have a positive effect and that the recombinants have a negative effect

<sup>b</sup> Contribution explained by QTL

E<sub>1</sub>: Suzhou, 2006; E<sub>2</sub>: Taian, 2006; E<sub>3</sub>: Taian, 2005

## QTLs for $L^*$

Eight main-effect QTLs were identified for  $L^*$ . These QTLs increased the  $L^*$  from 0.06 to 0.16, and accounted for the phenotypic variance ranging from 0.82% to 6.14%. The total contribution of the main-effect QTLs could explain 22.28% of the phenotypic variance of  $L^*$ . Among them, three alleles (*ql1B-1*, *ql4B*, and *ql7B*) came from the parent Huapei 3 and the rest derived from the parent Yumai 57 alleles. This suggested that alleles for increased  $L^*$  were dispersed within the two parents. This result was also in accordance with the presence of a wide range of variation and transgressive segregations of  $L^*$  in the DH population. Two QTLs were involved in AE interactions, which could explain 3.01% of the phenotypic variance of  $L^*$ .

Ten pairs of epistatic QTLs for  $L^*$  were resolved, explaining the phenotypic variance ranging from 0.80% to 6.24%. *ql4D-1/ql5A* had the largest effect, with a negative effect of  $-0.17$ , accounting for 6.34% of the phenotypic variance. The total contribution of epistatic QTLs was 21.36%. Three main-effect QTLs (*ql3D*, *ql7B*, and *ql7D*) were detected in epistatic effects. Two pairs of epistatic QTLs were also involved in AAE interactions, accounting for 3.88% of the phenotypic variance.

For  $L^*$ , the total QE interactions could explain 6.89% of the phenotypic variance.

## Discussion

Epistasis is an important genetic basis of flour color in wheat

Epistasis as an important genetic basis of complex traits has been well demonstrated in recent QTL mapping studies (Cao et al. 2001; Fan et al. 2005; Ma et al. 2005, 2007). Ma et al. (2005) provided strong evidence for the presence of epistatic effects on dough rheological properties in a wheat DH population. In the present study, 24 pairs of QTLs with epistatic effects and/or AAE effects were detected for flour color in three environments (Table 4, Fig. 2), ranging from four to ten QTLs for each trait. Epistatic effects could explain 7.10%, 31.88%, and 21.36% of the phenotypic variance of  $a^*$ ,  $b^*$ , and  $L^*$ , respectively. Furthermore, epistatic effects had a significant effect on  $b^*$ , which

could account for 31.88% of the phenotypic variance. So epistasis is an important genetic basis of flour color in wheat. The results indicate the complexity of the genetics of wheat flour color.

## Relationship between additive effects and epistatic effects of flour color in wheat

It would be interesting to study the relationships between additive QTLs and epistatic QTLs identified. The majority (92.59%) of loci involved in the epistatic interactions did not appear to have significant additive effects on flour color in wheat. For example, ten pairs of epistatic effects were identified for  $L^*$ ; 15 single loci involved in epistatic effects had no additive effects. Similarly, Ma et al. (2007) observed that 37% of the main-effect QTLs were involved in the epistatic interactions in maize grain yield and its components. This indicated that many loci in epistatic effects might not have significant effects for flour color alone but might affect its expression by epistatic effects with the other loci. The results also suggest that some of the additive QTLs might be detected with effects confounded by epistatic effects, if the epistatic effects were ignored in QTL mapping. Thus, breeders must take into account such complexity and test for the effects of individual loci in the targeted genetic background in order to obtain the expected phenotypes of the interested genes.

## Comparison of the present study with previous researches

In previous studies (Knott 1984; Parker et al. 1998; Mares and Campbell 2001; Zhang et al. 2006), QTL studies for wheat flour color were conducted based on the additive-dominant model and usually assumed absence of epistasis among QTLs. However, epistasis between nonallelic QTLs for some complicated quantitative traits may exist. In the present study, both additive and epistatic effects of QTLs were obtained based on the method of mixed linear models. Furthermore, epistasis was an important component for  $b^*$ , accounting for 31.88% of the phenotype variation (Table 4).

Knott (1984) reported a gene for  $b^*$  linked to *Lr19* in 'Agatha' wheat. One SSR and SRAP marker closely linked to *Lr19* resistance trait were obtained, and were named *wms44* and *M73* on chromosome 7D

with genetic distances 0.9 and 2.6 cM, respectively (Li et al. 2005; Liu et al. 2007a). At a similar location, additive QTLs associated with  $a^*$  and  $L^*$  were also detected in this study. Near this location, one epistatic QTL was detected for  $b^*$ . Parker et al. (1998) identified ten RFLP marker loci that showed significant associations with QTLs for flour color, located on chromosomes 3A and 7A, by using a SSD population of 150 lines. Zhang et al. (2006) identified eight QTLs for  $b^*$ , located on chromosomes 1DS, 2DL, 3A, 4D, 5D, 6AL, 6D, and 7AL. Mares and Campbell (2001) reported that xanthophyll content was very strongly associated with QTLs located on chromosomes 3B and 7A in 163 DH lines derived from Sunco  $\times$  Tasman. In this study, three epistatic QTLs were also detected for  $b^*$  on chromosomes 3A, 5B, and 7A. Four QTLs with additive effects were resolved for  $b^*$  on chromosomes 2B, 3D, 4D, and 5A, and ten pairs of epistatic effects were detected for  $b^*$  on chromosomes 3D–5B, 1A–1B, 2A–3D, 2B–7A, 3A–5B, 3D–4D, and 6B–7D in the present study.

These differences might be attributed to following possibilities. First, differences in mapping populations and experimental environments were studied in various QTLs mapping. Second, different QTL mapping approaches were used in various studies. Third, some of the genes involved in the wheat flour color pathway did not show any allelic variation between the two parents (Huapei 3 and Yumai 57). Consequently, several loci could not be identified on some chromosomes. Fourth, the coverage of the genome was not complete in the present map; some QTLs associated with  $b^*$  could not be detected, which might possibly affect the QTLs in the DH population. For complete analysis, a detailed map with total genome coverage is required.

To date, very few studies reported the QTLs which are associated with  $a^*$  and  $L^*$ . A significantly negative correlation was detected between  $L^*$  and  $b^*$  ( $r = -0.559$ ) and  $a^*$  and  $b^*$  ( $r = -0.494$ ). So it was very important to study the genetic basis of  $a^*$  and  $L^*$  so as to achieve ideal flour color in wheat. In this study, six additive QTLs and four pairs of epistatic QTLs were detected for  $a^*$ . One major QTL, *qa1B*, was closely linked to *barc372* 0.1 cM and could account for 25.64% of the phenotypic variance of  $a^*$  without any environmental influence. So, *qa1B* can be used for wheat MAS. This is a new and promising locus to improve our knowledge of wheat flour genetic

mechanism for  $a^*$ . To better understand it, a marker-assisted backcrossing procedure had been initiated in our group. Near-isogenic line development and fine mapping of *qa1B* are now ongoing following a strategy described by Saito et al. (2004). Closely linked flanking markers associated with major QTL could be further developed by converting them to STS markers for application in MAS. Eight additive QTLs and ten pairs of epistatic QTLs were identified for  $L^*$ .

The present investigation might be the first report of epistatic QTLs associated with flour color using molecular markers. The results indicated that epistasis was an important genetic basis of flour color in wheat. Therefore, genetic models for QTL mapping assuming no epistasis could lead to a biased estimation of QTL parameters. The results showed that both additive and epistatic effects were important genetic bases of flour color. In addition, additive effects and epistatic interactions were also sometimes subject to environmental modifications.

In summary, a total of 18 additive QTLs and 24 pairs of epistatic QTLs were detected for flour color in 168 DH lines derived from a Huapei 3  $\times$  Yumai 57 cross, which were distributed on 19 of the 21 chromosomes. One major QTL, *qa1B*, was closely linked to *barc372* 0.1 cM and could account for 25.64% of the phenotypic variation of  $a^*$  without any influence from the environment. Therefore, *qa1B* could be used in MAS in wheat breeding programs. The results showed that both additive and epistatic effects were important in the genetic basis of flour color, and were also sometimes subject to environmental modifications.

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