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CROPS AND SOILS RESEARCH PAPER

Genetic dissection of flour whiteness by unconditional and conditional quantitative trait locus mapping in wheat

Z. Y. DENG[1], W. J. LI[1], F. CHEN[1], W. Q. FANG[1], G. F. CHEN[2], C. L. SUN[1], Y. X. ZHANG[1], S. Y. WANG[1] AND J. C. TIAN[1]*

[1] *State Key Laboratory of Crop Biology, Key Laboratory of Crop Biology of Shandong Province, Cooperation Innovation Centre of Efficient Production with High Annual Yield of Wheat and Corn, Group of Wheat Quality Breeding, Agronomy College, Shandong Agricultural University, Taian, Shandong, 271018, People's Republic of China*
[2] *College of Ecology and Garden Architecture, Dezhou University, Dezhou, 253023, China*

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SUMMARY

Flour whiteness (FW) is an important factor in assessing flour quality and determining the end product quality. It is an integrated sensory indicator reflecting flour colour and is negatively correlated with protein content. In order to dissect the genetic relationship between FW and its five related traits at the quantitative trait locus (QTL)/gene level, a recombinant inbred line population was evaluated under three environments. Quantitative trait loci for FW were analysed by unconditional and conditional QTL mapping. Four unconditional additive QTLs and 16 conditional additive QTLs were detected across the three environments. Of these QTLs, only one major additive QTL (*Qfw1D1-1*) was consistently identified using both unconditional and conditional QTL analysis. This QTL was independent of flour colour a* (a function of red-green with a positive a* for redness and negative for greenness) and b* (a green-blue value with positive value for yellowness and negative for blueness) and was only slightly affected by flour protein content. A minor additive QTL (*Qfw4A-4*) was also detected using these two QTL mapping methods, being independent of flour colour a* and b*. Five unconditional and ten conditional epistatic minor QTLs were detected, from which only one pair (*Qfw3A-10*/*Qfw6B-6*) was identified by both unconditional and conditional QTL mapping, also independent of flour colour a* and b*. The major QTL (*Qfw1D1-1*) identified in the current study for the first time can be used for improving wheat FW in marker-assisted breeding.

INTRODUCTION

Flour colour is one of the most important factors in assessing flour quality and determining end product quality. For most consumers, white flour lacking major pigmentation is preferred, motivating the practice of bleaching flour with additives such as benzoyl peroxide in order to increase flour whiteness (FW), despite the potential health hazards (Kruger & Reed 1988; Lamsal & Faubion 2009). In fact, FW is an integrated sensory indicator with holistic reflection of flour colour. Different final products may require different FW. For example, flour with high levels of yellow pigmentation is preferred for Chinese and Japanese alkaline noodles, while higher FW is desirable for Chinese steamed and baked bread (He *et al*. 2004). Moreover, FW may be affected by grain and flour quality traits (Zhang & Tian 2008), such as grain protein content and grain hardness. Negative correlations have been observed between FW and protein content (Li *et al*. 2011, 2012): FW is higher from wheat with low rather than high protein content at the same flour milling extraction ratio. The most widely used colorimetric measurement assay for FW is based on L* (brightness), a* (greenness or red-green chromaticity) and b* (yellowness). For a pure white flour, L* should be 100, and a* and b* should be 0 (Sun *et al*. 2002). Flour with low L* and high a* value is undesirable because it has a dull grey colour.

* To whom all correspondence should be addressed. Email: jctian9666@163.com

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For wheat breeders, phenotypic measurements and selection of FW and colour are laborious because they require many grain samples to be milled, and it is very difficult to practice selection in early wheat breeding generations. Therefore, studies have been conducted on the genetic basis of FW and colour in order to help breeders select at early stages of breeding. Previous work on flour colour, FW, yellow pigment content, polyphenol oxidase (PPO), etc. has been focused on unconditional quantitative trait locus (QTL) mapping on common wheat and durum wheat using different populations. Quantitative trait loci controlling flour colour and its related traits have been found on 16 chromosomes: 1A (Patil *et al*. 2008; Zhang *et al*. 2009*b*; Tsilo *et al*. 2011), 1B (Zhang *et al*. 2009*b*; Tsilo *et al*. 2011), 2A (Blanco *et al*. 2011; Li *et al*. 2011), 2B (Sadeque & Turner 2010; Roncallo *et al*. 2012) , 2D (He *et al*. 2007), 3A (Parker *et al*. 1998; Mares & Campbell 2001; Sadeque & Turner 2010; Blanco *et al*. 2011; Li *et al*. 2011; Roncallo *et al*. 2012; Crawford & Francki 2013), 3B (Li *et al*. 2012), 4A (Li *et al*. 2011; Roncallo *et al*. 2012), 4D (Zhang *et al*. 2006), 5A (Hessler *et al*. 2002; Blanco *et al*. 2011; Tsilo *et al*. 2011), 5B (Patil *et al*. 2008; Sadeque & Turner 2010; Tsilo *et al*. 2011; Li *et al*. 2012), 5D (Li *et al*. 2011; Tsilo *et al*. 2011), 6A (Mares & Campbell 2001; Zhang *et al*. 2006; Roncallo *et al*. 2012), 6B (Pozniak *et al*. 2007), 7A (Parker *et al*. 1998; Mares & Campbell 2001; He *et al*. 2008; Patil *et al*. 2008; Zhang *et al*. 2009*a*; Blanco *et al*. 2011; Roncallo *et al*. 2012) and 7B (Pozniak *et al*. 2007; Zhang & Dubcovsky 2008; Tsilo *et al*. 2011; Roncallo *et al*. 2012). Ficco *et al*. (2014) reviewed the literature for colours of durum wheat and summarized the important markers. Up to now, some functional markers, especially those controlling yellow pigment, have been used for wheat flour colour (Crawford & Francki 2013; Ravel *et al*. 2013).

In 1995, Zhu demonstrated that the conditional QTL mapping method could be used to exclude the contribution of a causal trait to the variation of the resultant trait (Zhu 1995). The remaining variation of the resultant trait is defined as conditional variation, or net variation, which indicates the net effects of genes that are independent of the causal trait (Liu *et al*. 2008). Recently, the dynamic behaviour of developmental traits and the genetic interactions between the conditional variations of the resultant trait and its related traits have been studied using this method in many crops, such as cotton, rice, wheat, maize and rapeseed (Zhu 1995; Jiang *et al*. 2008; Liu *et al*. 2008, 2010; Wang *et al*. 2010, 2012; Cui *et al*. 2011; Zhang *et al*. 2013). The genetic relationship among different related traits should be dissected at the QTL level. In wheat, conditional QTL mapping has been reported on different developmental stages for plant height, tiller number, grain protein content, starch content and other phenotypic characters (Liu *et al*. 2010; Wang *et al*. 2010; Cui *et al*. 2011; Tian *et al*. 2011; Zhu *et al*. 2011). Moreover, genetic interactions among related traits in wheat were also dissected by conditional QTL mapping at the individual QTL level, such as the interactions between grain protein and starch content (Deng *et al*. 2015), and between wheat kernel weight and kernel length and width (Zhang *et al*. 2014).

Although extensive QTL analyses for flour colour have been carried out, less information is available about the genetic inter-relationship between FW and flour protein content, flour colour, and flour ash content at QTL/gene level. Why does FW have a significant negative correlation with flour protein content and ash content? How can the genetic relationship between them be dissected? So far, no study has been carried out on the relationship at the QTL/gene level.

The objective of the current paper was to dissect the genetic relationship between FW and flour protein content, flour colour and flour ash content using unconditional and conditional QTL mapping analysis. By comparing unconditional and conditional QTLs, the genetic interdependencies between them can be identified at the individual QTL level. This comparison might provide valuable information for marker-assisted selection to improve FW without negative effects on flour protein content or with positive effects on flour L*.

MATERIALS AND METHODS

Plant materials

A recombinant inbred line (RIL) population of 256 lines was developed from a cross between two winter wheat cultivars Nuomai 1 (female, NM1) and Gaocheng 8901 (male, GC8901) by single-seed descent to the F_{10} generation. Cultivar NM1 (Jiangsubaihuomai/Guandong107), bred by China Agricultural University and released in 2005 in Beijing, has unique starch properties related to high-quality white salt noodles. Cultivar GC8901 (77546-2/Linzhang), with high gluten strength and good

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bread-making qualities, was bred by Gaocheng Agricultural Science Research Institute and was released in 1998 in Hebei Province.

Field trials

The RIL population along with the parents was grown in three different locations in the 2009/10, 2010/11 and 2011/12 growing seasons, respectively. E1 represents Tai'an, Shandong Province (36°12′N, 117°04′E, 800 m a.s.l.), China, in the 2009/10 growing season; E2 refers to Suzhou, Anhui Province (33°63′N, 116°97′E, 30 m a.s.l.), China, in 2010/11; E3 stands for Jiyuan, Henan Province (35°05′N, 112°36′E, 1711 m a.s.l.), China, in 2011/12.

These lines were sown in a randomized block design with two replicates at each location. Each replication in E1 was designed based on a six-row plot 2·3 m long and 26 cm between rows, whereas those in E2 were six-row plots 4 m × 25 cm and in E3 were three-row plots 2·6 m × 26 cm.

Fertilization was applied before planting and at jointing stages (Zadoks growth stage (GS) 31–32) (Zadoks *et al*. 1974) in all experimental locations (as described in Deng *et al*. 2015).

The crop was irrigated at stages of pre-overwintering (GS 27–29), jointing (GS 31–32) and grain filling (GS 71–74) in all experimental locations. All recommended local crop management practices were followed, and no damage attributable to lodging, disease or pests was observed during the growing seasons.

Phenotyping methods

Milling and flour colour measurements (L*, a* and b*) were carried out according to Zhang *et al*. (2009*b*).

Flour whiteness was determined by a WSB-IV intelligent whiteness determination meter (Dajiguangdian Instruments, Hangzhou, China).

Flour protein content was measured by near-infrared reflectance spectroscopy (NIRS) on a Perten DA-7200 (Perten Instruments, Huddinge, Sweden) and expressed on a 14% moisture basis using AACC approved method 39–25 (AACC 2004).

Flour ash content in E1 and E3 was also measured by NIRS on the Perten DA-7200.

Data analysis and quantitative trait loci mapping

Statistical analyses (e.g., normal distribution and correlation) were performed using the software SPSS 17·0 (SPSS, Chicago, USA) and Excel 2010. Trait heritability was calculated by applying ANOVA of SAS program (SAS Institute, NC, USA).

Nine hundred and sixteen diversity arrays technology (DArT) markers developed by Triticarte Pty. Ltd. (http://www.triticarte.com.au, accessed 24 May 2016) in Australia and 183 simple sequence repeat (SSR) markers from Grain Gene 2·0 were used to test this population. The molecular genetic map was constructed by MAPMAKER/EXP ver. 3·0b (Lincoln *et al*. 1993). The commands 'group', 'sequence' and 'map' were used to develop the linkage groups and the position of markers on each chromosome, and the command 'try' and 'compare' were located on the unlinked markers on the chromosomes. The Kosambi (1994) mapping function was used to convert recombination fraction into cM values as map distances. Finally, the linkage map was drawn by Mapchart ver. 2·1 (Voorrips 2002).

Conditional genetic analysis was conducted based on the phenotypic values of FW conditioned on flour protein content, flour colour (L*, a* and b*) and flour ash content, which were obtained by the mixed-model approach (Zhu 1995; Wen & Zhu 2005). Conditional phenotypic values $y_{(T1|T2)}$ were obtained by the mixed model approach for the conditional analysis of quantitative traits described by Zhu (1995), where T1|T2 means trait 1 conditioned on trait 2 (for example, FW|Pr = flour whiteness conditioned on flour protein content). The software QGAStation 1·0 (http://ibi.zju.edu.cn/software/qga/, accessed 24 May 2016) was used to calculate the conditional phenotypic values $y_{(T1|T2)}$, which were subsequently used as input data for conditional QTL mapping.

Unconditional and conditional QTL mappings were performed firstly using the composite interval mapping method in ICIMapping 4·0 (Wang *et al*. 2014; http://www.isbreeding.net, accessed 24 May 2016) in individual environments, and then using the QTLNetwork version 2.0 (http://ibi.zju.edu.cn/software/qtlnetwork/, accessed 24 May 2016) based on the mixed linear model to analyse the interactions between QTL and environment. For ICIMapping 4·0, a walking speed of 1·0 cM and a logarithm of the odds (LOD) score of 2·5 (based on 1000 permutations) were used for detecting and declaring the presence of a putative QTL. For QTLNetwork 2·0, 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 a

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window size set at 10 cM. Significant thresholds for QTL detection were calculated for each data set using 1000 permutations and a genome-wide error rate of $P < 0{\cdot}10$ (suggestive) and $P < 0{\cdot}05$ (significant). The final genetic model incorporated significant additive and epistatic effects as well as their environmental interactions. A QTL was declared if the trait was associated with a marker locus at $P < 0{\cdot}05$.

To clarify the designations of the examined QTLs, the following rules were adopted: 'Q' denotes 'QTL'; the letter following 'Q' is an abbreviation of its corresponding trait; whereas a numerical number followed by a upper case letter, 'A', 'B' or 'D', is an indication of the chromosome number present in a given wheat genome where the corresponding QTL was detected; and if there is more than one QTL on a chromosome, a serial number behind a hyphen is added (e.g., *Qfw6A-2* stands for the second QTL for FW on chromosome 6A).

Quantitative trait loci detected through unconditional and conditional QTL mapping methods are known as unconditional and conditional QTLs, respectively.

RESULTS

Genetic map

A total of 501 markers were mapped including 479 DArT, 17 SSR, 2 HMW-GS, and 3 Wx protein markers. Compared with the previous map used by Zheng *et al*. (2013), three SSR markers were added on 1D. It covered 4213·2 cM with an average distance of 8·4 cM, producing 25 linkage maps. These markers were identified on 21 chromosomes.

Phenotypic data and correlations analysis

Continuous variation was found for FW in each environment (Table 1 and Fig. 1). Analysis of variance (ANOVA) showed that FW was significantly affected by genotype, environment and their interaction (Table 2). Its distribution was determined to be continuous (Fig. 1), indicating that it was a quantitative trait. The broad-sense heritability was 89·17% (Table 3). Transgressive segregation was observed on both the high and low sides of FW (Table 1 and Fig. 1), indicating that the alleles with positive effects were contributed by both parents.

Significant negative correlation was observed between FW and flour protein under the three environments, and also between FW and flour ash (Table 4). While significant positive correlation coefficients between FW and flour L* in all three environments were very high, with r values ranging from 0·93 to 0·94, the negative correlation between FW and flour b* were also very high, with r values ranging from −0·73 to −0·97. No significant correlation was found between FW and a*.

Additive effects of unconditional and conditional QTLs mapping of FW

Three unconditional additive QTLs were found in different environments (Table 5). Of these QTLs, *Qfw1D1-1*, with 33·60–44·84% phenotypic variation explained (PVE), was detected in all the environments, which indicated a stable major QTL affected little by environment. Another two unconditional additive QTL (*Qfw4A-4* and *Qfw6A2*) were detected but only in a single environment with PVE smaller than 10% (Table 5).

By removing the influence of flour protein, two conditional additive QTLs were found (Table 5) with PVE of 3·91–36·40%. Of these QTLs, the major conditional QTL *Qfw1D1-1* was detected in all environments, and its additive effects remained similar as those in unconditioned QTL, indicating that it was independent of flour protein content. The appearance of one conditional QTL, *Qfw4A-2*, indicated that this QTL was very likely to be repressed by flour protein content. In addition, the disappearance of two unconditional QTLs, *Qfw4A-4* and *Qfw6A2*, suggested that the expression of these two loci was associated with flour protein content.

When the influence of flour L* was removed, *Qfw1D1-1* remained significant in only one environment with reduced PVE (Table 5), indicating that the locus was strongly affected by flour L*. Five other conditional QTLs emerged on chromosomes 1A, 2A, 3B and 4A (Table 5), indicating these loci were repressed by flour L*.

When the influence of flour a* was removed, the conditional QTL *Qfw1D1-1* detected in all environments was the same as that in unconditional QTL with a similar additive effect (Table 5), suggesting that this locus was independent of flour a*. In E3, *Qfw6A2* was also identified using conditional QTL mapping, whose additive effects remained similar as found in unconditioned QTL, indicating that it was independent of flour a*.

By removing the influence of flour b*, *Qfw1D1-1* remained the same as those in unconditional QTL in

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Table 1. *Phenotypic data of flour whiteness for the recombinant inbred line (RIL) population and the parents in different environments*

	Environment*	E1	E2	E3	Across environments
	N	231	244	241	716
Parents	GC8901	77·3	75·3	74	75·53
	NM1	82·2	80·4	80·3	80·97
RIL	Mean†	78·4	77·0	75·7	77·0
	S.E.	0·22	0·22	0·21	0·13
	S.D.	3·38	3·40	3·20	3·50
	Variance	11·45	11·57	10·26	12·25
	Skewness	0·00	0·03	0·07	0·07
	Kurtosis	−1·28	−1·39	−1·24	−1·11
	Minimum	71	70·95	69·6	69·6
	Maximum	85	83·5	82	84·85

* E1: 2009/10 Taian; E2: 2010/11 Suzhou; E3: 2011/12 Jiyuan.

† Mean value is RIL population in individual environments.

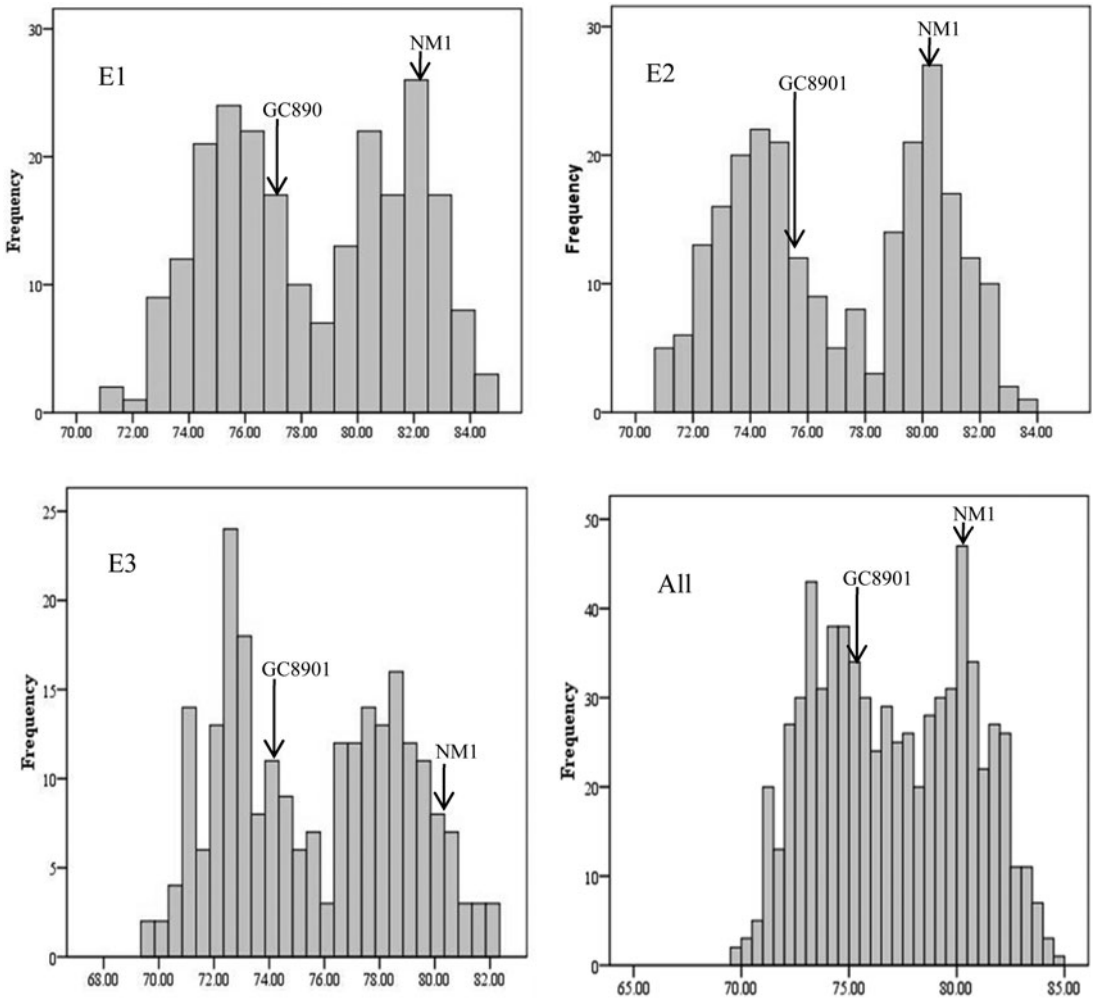


Fig. 1. Frequency distribution of flour whiteness (FW) in the recombinant inbred line (RIL) population derived from GC8901 and NM1 in three environments. E1:2009/10 Taian; E2: 2010/11 Suzhou; E3: 2011/12 Jiyuan; All: averaged data across three environments.

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Table 2. *Analysis of variance (ANOVA) results of flour whiteness by using the general linear model (GLM) procedure*

Source	D.F.	Type III SS	Mean square	*F* value	*P*
Geno	255	15156·59192	59·43762	674·01	<0·001
Env	2	1475·4633	737·73165	8365·67	<0·001
Geno × Env	460	3146·1981	6·83956	77·56	<0·001
rep(Env)	3	0·09864	0·03288	0·37	NS

Geno, genotype; Env, environment.

Table 3. *Heritability of flour whiteness*

Source	Variance
Geno	9·39889
Env	1·58601
Geno × Env	3·38109
rep(Env)	0
Error	0·08819
h^2	0·8917

Geno, genotype; Env, environment.

E1 and E3, but their additive effects were different. Four new conditional QTLs on 2B and 3B chromosomes emerged (Table 5). Among them, there were three major conditional QTLs, *Qfw2B-1, Qfw2B-2* and *Qfw3B*.

When the influence of flour ash was removed, *Qfw1D1-1* remained the same as that in unconditioned QTL with markedly different additive effects (Table 5), indicating that this QTL was partially affected by flour ash. One unconditional QTL, *Qfw6A2*, disappeared and a conditional QTL, *Qfw1B*, emerged.

In order to check the accuracy of the results, the QTLNetwork2·0 was also used to analyse the data, and very similar results were obtained (Table 6). The main difference was an unconditional QTL, *Qfw6A2-1*, that was newly detected and four conditional QTLs, *Qfw4A-1, Qfw7B-18, Qfw6A2-1* and *Qfw4A-4* identified after removing the effect of flour protein, L*, a* and flour b*. In addition, no major interaction additive effect between QTL and environment (AE) was found in the current study.

In summary, four unconditional additive QTLs and 16 conditional additive QTLs were found. Among them, a stable major additive QTL, *Qfw1D1-1*, was identified (Fig. 2), which could improve FW without being affected by the other four factors at the QTL/gene level. Another major conditional additive QTL, *Qfw3B*, was co-suppressed by flour colour L* and b*. The minor additive QTL, *Qfw4A-4*, was independent of flour colour a* and b*, but was co-affected by the remaining three quality traits. The remaining minor conditional additive QTLs were regulated by different traits. In addition, several new QTLs were identified after removing the corresponding factors, evidencing the ability of conditional QTL mapping in revealing masked QTLs.

Epistatic effects of unconditional and conditional quantitative trait loci mapping of flour whiteness

Unconditional mapping detected five pairs of epistatic QTLs located on chromosomes 1A, 2A, 3A, 3B, 5A, 6B and 7D (Table 7). Contributions of these epistatic QTLs ranged from 1·89 to 4·01%. The epistatic effect of each QTL seemed to be minor, but the total PVE was 15·93%.

When the influence of flour protein was removed, these five pairs of unconditional epistatic QTLs disappeared, and five other pairs of epistatic QTLs emerged (Table 7). Total PVE of conditional epistasis was 15·75%.

By removing the influence of flour a*, five pairs of epistatic QTLs were identified. Of these, only the conditional epistatic QTLs *Qfw3A-10/Qfw6B-6* remained the same as that in unconditioned QTL with similar epistatic effect value (Table 7), whereas another set of four pairs of epistatic QTLs emerged.

After removing the influence of flour b*, four pairs of epistatic QTLs were detected (Table 7). The conditional epistatic QTLs *Qfw3A-10/Qfw6B-6* remained the same as that in unconditioned QTL but with largely different epistatic effect values, and other three pairs of epistatic QTLs emerged. However, there were no pairs of epistatic QTLs identified when the influences of flour L* and flour ash were removed.

Hence, only one pair of epistatic QTL (*Qfw3A-10/Qfw6B-6*) was identified in both unconditional and conditional QTL mapping. It was independent of

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Table 4. *Correlation analysis between flour whiteness and its related traits*

	Flour whiteness		
	E1	E2	E3
Protein	−0·33 ($P < 0·01$)	−0·32 ($P < 0·01$)	−0·27 ($P < 0·01$)
L*	0·94 ($P < 0·01$)	0·94 ($P < 0·01$)	0·93 ($P < 0·01$)
a*	0·09 NS	−0·03 NS	0·13 ($P < 0·05$)
b*	−0·73 ($P < 0·01$)	−0·97 ($P < 0·01$)	−0·91 ($P < 0·01$)
Ash	−0·25 ($P < 0·01$)	–	−0·29 ($P < 0·01$)

E1: 2009/10 Taian; E2: 2010/11 Suzhou; E3: 2011/12 Jiyuan; L*, brightness; a*, greenness or red-green chromaticity; b*, yellowness; NS, not significant.

Table 5. *Additive effects of unconditional and conditional quantitative trait loci (QTL) of flour whiteness (FW) in individual environments**

Trait	Env.†	QTL	Position (cM)	Marker interval	LOD	Add‡	PVE (%)
Unconditional QTL							
FW	E1	*Qfw1D1-1*	5	*cfd183-Xgwm337*	24·40	1·78	33·60
	E2	*Qfw1D1-1*	5	*cfd183-Xgwm337*	26·11	2·21	44·84
		Qfw4A-4	139	*wPt3349-wPt7821*	3·13	0·63	3·5
	E3	*Qfw1D1-1*	5	*cfd183-Xgwm337*	23·38	1·79	34·75
		Qfw6A2	75	*wPt4229-wPt665805*	3·16	0·56	3·36
Conditional QTL							
FW|Pr	E1	*Qfw1D1-1*	5	*cfd183-Xgwm337*	25·19	1·71	34·72
	E2	*Qfw1D1-1*	5	*cfd183-Xgwm337*	22·45	1·90	36·40
		Qfw4A-2	128	*wPt671707-wPt730913*	3·25	0·62	3·91
	E3	*Qfw1D1-1*	5	*cfd183-Xgwm337*	21·46	1·59	29·88
FW|L*	E1	*Qfw1D1-1*	5	*cfd183-Xgwm337*	5·63	0·30	8·48
		Qfw2A-3	311	*wPt9277-wPt3281*	3·25	−0·25	6·05
	E2	*Qfw1A1*	144	*wPt665613-wPt3904*	4·62	−0·29	6·93
		Qfw3B	162	*wPt666008-wPt5870*	3·78	1·67	13·80
		Qfw4A-3	152	*wPt2951-wPt672107*	3·43	0·25	5·02
	E3	*Qfw1A1-18*	138	*wPt6654-wPt2872*	4·15	−0·34	9·35
FW|a*	E1	*Qfw1D1-1*	5	*cfd183-Xgwm337*	23·40	1·83	35·39
	E2	*Qfw1D1-1*	5	*cfd183-Xgwm337*	25·96	2·20	44·54
	E3	*Qfw1D1-1*	5	*cfd183-Xgwm337*	23·96	1·83	36·85
		Qfw6A2	75	*wPt4229-wPt665805*	3·0	0·54	3·20
FW|b*	E1	*Qfw1D1-1*	5	*cfd183-Xgwm337*	24·24	1·84	24·24
	E2	*Qfw2B-1*	33	*wPt0694-wPt0473*	21·65	−0·58	45·01
		Qfw2B-2	36	*wPt1068-wPt7004*	30·05	0·69	62·84
		Qfw3B	162	*wPt666008-wPt5870*	12·36	3·65	27·83
	E3	*Qfw1D1-1*	5	*cfd183-Xgwm337*	4·53	0·31	5·97
		Qfw2B-3	0	*wPt6576-wPt4199*	3·36	0·27	4·34
		Qfw3B	162	*wPt666008-wPt5870*	3·18	−1·87	16·16
FW|Ash	E1	*Qfw1D1-1*	5	*cfd183-Xgwm337*	21·86	1·70	32·76
	E3	*Qfw1D1-1*	5	*cfd183-Xgwm337*	18·41	1·42	23·88
		Qfw1B	50	wPt8226-wPt0605	3·84	−0·93	10·07

LOD, logarithm of the odds; PVE, phenotypic variation explained.

* The results in this table were obtained with ICIMapping 4·0 software.

† E1: 2009/10 Taian; E2: 2010/11 Suzhou; E3: 2011/12 Jiyuan.

‡ Additive effect, positive and negative values indicate that high phenotypic values are contributed by NM1 and GC8901, respectively.

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Table 6. *Additive effects (A) and additive × environment (AE) interactions of unconditional and conditional quantitative trait loci (QTL) of flour whiteness (FW)**

Trait	QTL	Marker interval	Position (cM)	*A*†	*P*-value	H^2 (%)‡	AE effect§					
							AE1	*P*-value	H^2 (%)	AE3	*P*-value	H^2 (%)
Unconditional QTL												
FW	*Qfw1D1-1*	*cfd183-Xgwm337*	5	1·79	<0·001	36·76						
	Qfw4A-4	*wPt7821-wPt5212*	139·7	0·35	<0·001	1·30						
	Qfw6A2-1	*Xgpw4085-wPt667562*	34·0	1·2	0·00	1·89						
Conditional QTL												
FW|Pr	*Qfw1D1-1*	*cfd183-Xgwm337*	5	1·82	<0·001	32·64						
	Qfw4A-1	*Wx-B1-wPt0105*	108·8	0·49	<0·001	2·78						
FW|L*	*Qfw1A1-18*	*wPt6654-wPt2872*	137·8	−0·28	<0·001	5·42						
	Qfw1D1-1	*cfd183-Xgwm337*	5	0·065	NS	0·59	0·22	<0·01	1·93	−0·19	<0·01	1·59
	Qfw7B-1	*wPt665293-wPt4038*	0·0	−0·18	<0·001	2·99						
FW|a*	*Qfw1D1-1*	*cfd183-Xgwm337*	5	1·99	<0·001	36·64						
	Qfw4A-4	*wPt7821-wPt5212*	139·7	0·42	<0·001	1·32						
	Qfw6A2-1	*Xgpw4085-wPt667562*	34·0	0·86	<0·001	1·80						
	Qfw7B-18	*wPt0276-wPt6936*	235·8	0·46	<0·001	3·30						
FW|b*	*Qfw1D1-1*	*cfd183-Xgwm337*	5	1·78	<0·001	36·5						
	Qfw4A-4	*wPt7821-wPt5212*	139·7	0·43	<0·001	1·24						
	Qfw7B-18	*wPt0276-wPt6936*	234·8	0·38	<0·001	3·08						
FW|Ash	*Qfw1B-1*	*wPt8226-wPt0605*	47	−1·05	<0·001	2·90						
	Qfw1D1-1	*cfd183-Xgwm337*	5	1·83	<0·001	33·21						

NS, not significant.

* The results in this table were obtained with QTLNetwork 2·0 software.

† Additive effects, positive and negative values indicate that high phenotypic values are contributed by NM1 and GC8901, respectively.

‡ Contribution explained by filtrated putative additive QTL.

§ The interaction additive effect between QTL and environment.

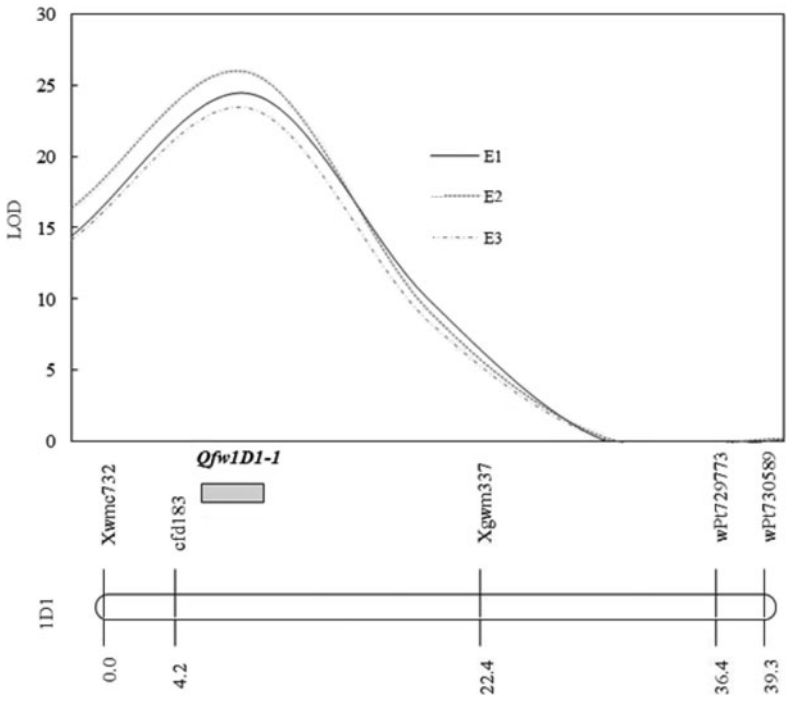


Fig. 2. Linkage map of chromosome 1D with logarithm of odds (LOD) curves obtained from inclusive composite interval mapping (ICIM) of flour whiteness (FW) in three environments. The LOD threshold of 3·0 determined by permutations is indicated by the horizontal dashed-dot line.

flour colour a* but partly affected by flour colour b* with greatly increased conditional epistatic effect.

DISCUSSION

In previous studies (Parker *et al*. 1998; Mares & Campbell 2001; He *et al*. 2008; Patil *et al*. 2008; Zhang *et al*. 2009*a*; Li *et al*. 2011, 2012; Tsilo *et al*. 2011), QTL mapping of FW and flour colour were carried out using the unconditional mapping method, and most were conducted based on the additive-dominant model, usually ignoring the epistasis between QTLs. However, in the current research, the conditional QTL mapping method was used to analyse the genetic interactions between FW and its effectors at the QTL level by additive and epistasis models including the interactions between QTL and environment. The results provided more information than those of unconditional mapping. Using unconditional mapping, Li *et al*. (2011) reported three QTLs for FW distributed on 3A, 4A and 5D chromosomes with PVE less than 10%. Li *et al*. (2012) found four major QTLs on 1D, 3B, 5B and 7D, of which the QTL on 1D with 11·90% of PVE was only detected under one environment using WY population. In the current work, only one major QTL was found, *Qfw1D1-1*, with flanking markers *cfd183-Xgwm337* ranging from 4·2 to 22·4 cM on 1D chromosome, and the peak position of this QTL was very close to the maker *cfd183* with a genetic distance of 0·8 cM. Meanwhile, this QTL was stable in all environments and therefore can be used in marker-assisted breeding. If it is transferred to other genotypes with low FW, then FW is expected to improve by 1·78–2·21. In the same marker interval, Zheng *et al*. (2013) also found a major QTL for mixograph peak value using unconditional QTL mapping. In addition, QTLs on 2B, 4A, 2A and 6A with minor effects were identified in the current study. Previous studies also reported some QTLs on these loci not only for FW (Li *et al*. 2011, 2012) but also for flour colour L*, a* and b* (Zhang *et al*. 2009*a*; Sadeque & Turner 2010; Blanco *et al*. 2011; Roncallo *et al*. 2012). Five pairs of epistatic QTLs were found on chromosomes 1A-5A, 1A-7D, 2A-3B and 3A-6B. Although Li *et al*. (2011) also found one epistatic QTL on 1A-7D chromosome, its locus was different from the current study.

By conditional QTL mapping, several QTLs were discovered, showing no, partial or complete association with FW. The major additive QTL, *Qfw1D1-1*, was found to be independent of flour protein content and flour colour a* and b*, and its expression was

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Table 7. *Epistasis effects (AA) of unconditional and quantitative trait loci (QTL) of flour whiteness (FW)**

Trait	QTL_i	Marker interval_i	Position_i (cM)	QTL_j	Marker interval_j	Position_j (cM)	AA†	*P* value	H^2 (%)‡
Unconditional QTL									
FW	*Qfw1A2-4*	*wPt5167-wPt667814*	4·2	*Qfw5A-1*	*wPt3334-wPt1903*	0·0	0·38	<0·001	1·89
	Qfw1A2-30	*wPt7001-wPt4916*	205·3	*Qfw7D2-13*	*wPt664252-wPt663849*	40·9	−0·80	<0·001	3·55
	Qfw2A-5	*wPt1657-wPt664251*	135·2	*Qfw3B-11*	*wPt0544-wPt9432*	40·6	−0·59	<0·001	3·87
	Qfw3A-10	*wPt7341-wPt8855*	66·2	*Qfw6B-8*	*wPt3733-wPt2218*	54·0	0·56	<0·001	4·01
	Qfw3A-10	*wPt7341-wPt8855*	66·2	*Qfw6B-6*	*wPt3060-wPt664174*	33·5	0·36	<0·001	2·61
Conditional QTL									
FW|Pr	*Qfw1A2-8*	*wPt2847-wPt4709*	14·8	*Qfw5A-2*	*wPt1903-wPt3069*	0·0	0·61	<0·001	2·18
	Qfw1D2-5	*Glu-D1-wPt3743*	12·9	*Qfw5A-3*	*wPt3069-wPt3462*	47·2·2	1·07	<0·001	3·81
	Qfw2A-22	*wPt2273-wPt2994*	253·9	*Qfw3A-15*	*wPt8876-wPt730156*	178·5	0·61	<0·001	4·04
	Qfw3A-11	*wPt8855-wPt2748*	70·0	*Qfw6B-8*	*wPt3733-wPt2218*	55·0	0·72	<0·001	3·47
	Qfw3D-4	*wPt671773-wPt666814*	107·1	*Qfw6A1-12*	*wPt7027-wPt3524*	59·3	−0·39	<0·001	2·25
FW|a*	*Qfw1A2-4*	*wPt5167-wPt667814*	4·2	*Qfw5A-1*	*wPt3334-wPt1903*	0·0	0·41	<0·001	1·86
	Qfw1D2-8	*wPt667287-wPt8854*	18·9	*Qfw5A-3*	*wPt3069-wPt3462*	43·2	1·05	<0·001	2·92
	Qfw2A-4	*wPt1853-wPt1657*	115·9	*Qfw3B-10*	*wPt0446-wPt0544*	40·6	−0·99	<0·001	2·77
	Qfw3A-10	*wPt7341-wPt8855*	66·2	*Qfw6B-10*	*wPt9594-wPt7576*	64·9	0·71	<0·001	3·72
	Qfw3A-10	*wPt7341-wPt8855*	66·2	*Qfw6B-6*	*wPt3060-wPt664174*	33·5	0·32	<0·001	2·60
FW|b*	*Qfw1A2-4*	*wPt5167-wPt667814*	4·2	*Qfw5A-1*	*wPt3334-wPt1903*	0·0	0·42	<0·001	2·00
	Qfw1D2-8	*wPt667287-wPt8854*	116·0	*Qfw5A-3*	*wPt3069-wPt3462*	47·2	0·80	<0·001	2·71
	Qfw2A-4	*wPt1853-wPt1657*	115·9	*Qfw3B-10*	*wPt0446-wPt0544*	40·6	−1·04	<0·001	3·04
	Qfw3A-10	*wPt7341-wPt8855*	66·2	*Qfw6B-6*	*wPt3060-wPt664174*	33·5	0·75	<0·001	3·42

* The results in this table were obtained with QTLNetwork 2·0 software.

† AA: epistasis effects.

‡ H^2(%): contribution explained by filtrated putative epistasis QTL.

slightly affected by flour ash content. Hence this QTL was important for improving FW. It was noteworthy that in the same marker interval, the additive QTL *Qgsc1D-1* for grain starch content was also detected in both unconditional and conditional QTL mapping, which was partly affected by grain protein content (Deng *et al*. 2015). Meanwhile *Qgpc1D-1* for grain protein content was also found in the same marker interval but only by conditional mapping when the influence of grain starch content was removed (Deng *et al*. 2015). These results imply that an important gene with pleiotropic effects for FW, grain starch and protein content may be present in this chromosomal region. In addition, one important additive QTL for sedimentation volume on chromosome 1D with flanking markers *XWMC93* and *GLUD1* was found by comparing unconditional and conditional QTLs in a doubled haploid (DH) population (Deng *et al*. 2013).

In summary, four unconditional additive QTLs and 16 conditional additive QTLs for FW were detected across three environments, and five unconditional and ten conditional epistatic QTLs were identified. Although most of these QTLs have minor additive effects, FW might still be increased by pyramiding these QTLs, particularly *Qfw1D1-1*, through marker-assisted breeding.

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