

Chapter 2

Conditional QTL Mapping of Major Quality Traits

Abstract Till now many gene/QTL for wheat grain protein content have been previously identified, but the effects of these QTLs belonged to the cumulative effects of mature. So it couldn't explain the dynamic expression of QTL during the development of protein synthesis.

Keywords Quality traits • Protein dynamic accumulation • Protein-fraction • Total starch content • Starch components • Protein and starch interaction • Sedimentation values • Four whiteness • Conditional QTL mapping

2.1 Conditional QTL Mapping for Protein Dynamic Accumulation Pattern in Wheat Grain

Till now many gene/QTL for wheat grain protein content have been previously identified, but the effects of these QTLs belonged to the cumulative effects of mature. So it couldn't explain the dynamic expression of QTL during the development of protein synthesis. Therefore the dynamic accumulation of grain protein content during grain filling stage was studied using one DH population, to clarify the temporal and spatial expression of QTL by analyzing the position and effect of QTL which identified the dynamic accumulation of protein, to reveal the molecular genetic mechanism of grain protein synthesis.

2.1.1 *Materials and Methods*

2.1.1.1 Materials

A population of 168 DH wheat lines derived from a cross of Huapei3/Yumai57 was used for the construction of a linkage map. Huapei3 and Yumai57 were approved by Henan Province in 2006 and by the state (China) in 2003, respectively. Both

were cultivated over a large area in the Huang-Huai Wheat Region in China. They were different in several agronomically important traits as well as in baking quality parameters.

2.1.1.2 Growth Conditions

A total of 168 lines and their parents were grown in six environments at Tai'an (36° 57'N, 116° 36'E), Shandong Province, China, in 2008 and 2009. The soil was brown earth, in which the available N, P and K contents were 40.2, 51.3 and 70.8 mg/kg in the top 20 cm. Additionally, 37,500 kg/hectare (ha) of farmyard manure or barnyard manure (nitrogen content, 0.05–0.1 %), 225 kg/ha of urea, 225 kg/ha of phosphorus diamine fertilizer, 225 kg/ha of potassium chloride, and 15 kg/ha of zinc sulphate were added as fertilizers before sowing. The plant materials were managed under three environments in 2008 and in 2009. The experiments of 2008 were E1, E2, and E3, and in 2009 they were E4, E5, and E6. E1 (2008) and E4 (2009) were watered at each stage of pre-overwintering, jointing, flowering, and grain filling, and were top-dressed with 225 and 112.5 kg/ha urea at jointing and flowering stages. E2 and E5 were fertilized as same as E1 and E4, but there was no irrigation applied during the whole wheat growth period. E3 and E6 were same in irrigation and fertilizing applying as E1 and E4, but there was no topdressing applied during the entire growing period.

The experimental field consisted of a randomized block with two replications, all lines and parental lines were grown in 2 m long by four-row plots (25 cm apart). The number of foundation seedlings was 2,250,000/ha, seeding on October 4, 2007 and October 6, 2008.

Sample with strain as the unit was chosen from the main stem spike that was marked in the same day of flowering, and was measured initially at the stage of 12 day after anthesis (DAF). After that, the measurement was conducted every 5 days from the flowering stage to mature. A total of five different time sampling points were taken for measuring, which was designated as 12 DAF (S1), 17 DAF (S2), 22 DAF (S3), 27 DAF (S4) and 32 DAF (S5), respectively. Samples were treated at 105 °C for 30 min and then dried at 65 °C until reaching constant dry weight. The grain protein content was determined by the Kjeldahl method. The protein content is the total nitrogen multiplied by 5.7.

2.1.1.3 Statistical Analysis

Analysis of variance (ANOVA) was carried out using the SPSS version 13.0 (SPSS, Chicago, USA) program. Both unconditional QTL and conditional QTL were detected with the mixed linear model using the software of QTLNetwork 2.0 (Yang and Zhu 2005). A QTL was declared if the phenotype was associated with a marker locus at $P < 0.005$. Conditional QTLs were predicted by the software QGASStation 1.0 (Zhu 1995) with the cumulative effects of QTLs from time $t-1$ to

time t . Unconditional QTLs indicate the cumulative effects of QTLs from the initial time to time t .

QTLs were named for protein content by the first two letters with the relevant chromosomal number (McCouch et al. 1997). If there were more than one QTL on a chromosome, then a serial number was added after the chromosomal number, such as “*QGsc5D-1*, *QGsc5D-2*”.

2.1.2 Result and Analysis

2.1.2.1 Phenotypic Data

The phenotypic values of wheat grain protein content for the DH population and their parents in six environments at five developmental stages are shown in Table 2.1. The same dynamic change from high to low to high trend was seen in the DH population and two parents in different environments. The lowest protein content value was seen at 22 DAF. The grain protein content of Yumai57 showed significantly higher than that of Huapei 3 at 12 DAF, but the opposite phenomena was seen at 32 DAF. In different stages, there were wide variations in the DH population, indicating significantly transgressive segregation. Segregation continuously among the DH population suggests that the trait approximately followed normal distributions. Most values of skewness and kurtosis were less than 1.0, indicating typical inheritance of quantitative traits and suitability of the data for QTL analysis.

2.1.2.2 Dynamic QTL of Grain Protein Content

2.1.2.2.1 Unconditional QTL During the Development of Grain Protein Content

Nine unconditional QTL were identified during the grain filling stages significantly affected the protein content (Table 2.2), which distributed on 1D, 2B, 3A, 4A, 4B, 5A, 5D and 7D chromosomes. Among them, the QTL *Gpc3A* can be detected in five stages with the negative additive effect indicating the additive allele from Yumai57. The additive effect varied from -0.45 to -0.21 % in different stages, and its maximum PVE was 14.79 % at 32 DAF, while the lowest was 7.71 % at 22 DAF. While *QGpc1D* was identified in three filling stages, and *QGpc4A-1* and *QGpc4A-2* could be detected in two stages, but the residue individual QTL only was found in one stage. The number of QTLs detected in different stages showed some differences. There were four unconditional QTLs at 12 DAF and 27 DAF, while three QTLs were detected in each other three stages. The number was more than that identified at mature, which indicated that the expression of gene/QTL was different in different filling stages.

Table 2.1 Phenotypic value of the DH population and parents for GPC in different environments (%)

Environment	Period	Parent		DH population			Minimum	Standard deviation	Skewness	Kurtosis
		Huapei3	Yumai57	Mean	Maximum					
E 1	12 days after anthesis	11.29	13.86	12.52	15.02		10.43	0.96	0.34	-0.10
	17 days after anthesis	10.44	10.23	11.41	14.39		9.58	0.95	0.73	1.10
	22 days after anthesis	10.22	9.48	10.90	14.22		9.00	0.83	0.67	1.10
	27 days after anthesis	12.04	11.25	12.55	16.38		9.33	1.24	0.68	0.81
	32 days after anthesis	13.07	12.89	13.86	17.49		10.63	1.30	0.29	0.29
E 2	12 days after anthesis	11.70	12.58	12.32	15.28		10.13	1.18	0.42	0.44
	17 days after anthesis	10.01	11.35	10.93	13.90		9.08	0.86	0.51	0.73
	22 days after anthesis	9.56	9.11	9.97	13.49		8.45	0.83	0.75	0.63
	27 days after anthesis	11.36	10.88	11.40	15.32		9.30	1.10	1.00	1.20
	32 days after anthesis	12.47	11.59	12.61	16.51		10.32	1.24	0.77	0.47
E 3	12 days after anthesis	10.91	13.45	13.06	16.2		10.03	1.14	0.12	-0.70
	17 days after anthesis	10.72	10.19	11.44	14.33		9.24	0.96	0.19	-0.30
	22 days after anthesis	9.54	9.67	10.62	13.56		8.53	0.94	0.47	0.39
	27 days after anthesis	10.91	10.87	11.94	16.17		9.56	1.23	0.72	0.37
	32 days after anthesis	12.67	11.53	13.18	16.27		10.43	1.36	0.56	0.21
E 4	12 days after anthesis	11.77	12.40	12.98	16.16		10.72	0.95	0.38	0.48
	17 days after anthesis	11.49	11.26	11.94	15.64		9.90	0.93	0.70	0.29
	22 days after anthesis	10.96	10.98	10.86	13.24		8.49	0.92	0.01	0.16
	27 days after anthesis	11.31	11.02	11.82	15.84		9.43	0.99	0.81	0.69
	32 days after anthesis	12.59	11.98	12.89	16.52		10.02	1.26	0.70	0.58

(continued)

Table 2.1 (continued)

Environment	Period	Parent		DH population			Standard deviation	Skewness	Kurtosis
		Huapei3	Yumai57	Mean	Maximum	Minimum			
E 5	12 days after anthesis	11.88	14.32	12.77	16.23	10.42	1.24	0.69	0.01
	17 days after anthesis	10.37	10.70	10.96	14.24	9.47	0.82	1.01	0.02
	22 days after anthesis	9.96	9.94	9.96	12.98	8.26	0.73	0.72	0.71
	27 days after anthesis	10.48	10.46	10.83	14.31	9.19	0.82	0.90	0.15
	32 days after anthesis	12.73	11.45	11.86	16.73	10.02	1.14	0.28	0.94
E 6	12 days after anthesis	12.40	13.24	12.90	16.62	10.61	1.09	0.70	0.83
	17 days after anthesis	11.31	12.25	11.59	14.13	9.72	0.84	0.44	0.19
	22 days after anthesis	9.96	10.39	10.49	13.17	8.74	0.75	0.54	0.89
	27 days after anthesis	10.85	11.15	11.34	14.42	9.38	0.83	0.53	0.58
	32 days after anthesis	11.63	11.45	12.35	16.38	10.30	1.21	0.65	0.43

2.1.2.2.2 Conditional QTL in Grain Development

Total ten conditional QTLs were identified in five stages, which located on 1D, 2B, 3A, 4A, 4B, 4D, 5B, 5D and 7A (Table 2.2 and Fig. 2.1). Among them, only *QGpc3A* and *QGpc1D* were found at two stages, no one QTL can be found in five stages.

By comparing the unconditional and conditional QTL, the gene expression controlling the grain protein showed activity at 12 DAF, and four QTL had the higher expression content. The unconditional and conditional QTL explained 42.62 % of total phenotypic variance. With the development of grain filling, the *QGpc5D-1* and *QGpc2B* originally detected were not found at 17 DAF, and then a new unconditional QTL on chromosome 5A and a new conditional QTL on chromosome 5D were identified. At 22 DAF, there was a tough for protein content because the QTL *QGpc4A-1* continually expressed at the first two stages was not detected. Although there were some new QTLs found on 1D and 7D chromosomes, their PVE showed lower. Under conditional QTL, only *QGpc3A* could be detected. But at 27 DAF, each new two unconditional QTLs and conditional QTLs were found on 4A, 4B and 4D chromosomes with total explaining 30.13 % of phenotypic variation, which indicated these new QTLs played an important role in protein synthesis. At 32 DAF, two new conditional QTLs were detected on 7A and 5B chromosomes with positive additive effect. At this stage, the PVE was total 35.11 %, and the protein content arrived at the maximum.

2.1.3 Comparison with Previous Researches

In this study, the unconditional QTL *QGpc3A* detected in five stages was located on the markers between *XBARC86* and *XWMC21*. Zhao et al. (2010) also found that this QTL could control the grain protein content and flour protein content at mature. But this QTL can be detected the net genetic effect at 12 DAF and 22 DAF, which indicated the expression of this QTL was dynamic change with minor expression, only accumulated at a degree and then it can be detected on unconditional mapping. In fact, this QTL is important for protein synthesis. Beside *QGpc3A*, the *QGpc4A-1* played an important role for protein accumulation at early stage. But at medium and later stages, *QGpc1D* and *QGpc4A-2* would be important for protein synthesis.

In addition, Four QTLs, *QGpc5D-2* (17 DAF), *QGpc4D* (27 DAF), *QGpc5B* (32 DAF) and *QGpc7A* (32 DAF), only were detected under conditional QTL mapping, which indicated they were covered because of the minor effect. So the conditional mapping can identify more minor genes, and further testify the selective expression in special time for protein development, which was similar to the previous researches (Yan et al. 1998; Sun et al. 2006; Liu et al. 2008).

Furthermore, in the medium of filling stages, the protein content showed the tough because the synthetic ratio of starch was faster than that of protein, but this couldn't testify the protein synthesis content was less. At the lowest stages, the PVE

Table 2.2 Additive effects of QTLs for GPC in different measurements

QTL	Marker interval	Additive effect	12 days after anthesis	17 days after anthesis	22 days after anthesis	27 days after anthesis	32 days after anthesis
<i>QGpc1D</i>	XGDM60-XWMC429	$a(t)$			0.15**	0.21**	0.29**
<i>QGpc2B</i>	XBARC373-XBARC1114	$a(4t - 1)$	-0.20**				0.10**
<i>QGpc3A</i>	XBARC86-XWMC21	$a(4t - 1)$	-0.20**				
<i>QGpc4A-1</i>	XWMC776-XBARC362	$a(t)$	-0.26**	-0.21**	-0.27**	-0.36**	-0.45**
<i>QGpc4A-2</i>	XWMC497-XWMC219	$a(4t - 1)$	-0.26**	0.25**	-0.08**		
<i>QGpc4B</i>	XWMC657-XWMC48	$a(t)$	0.14**			0.33**	0.20**
<i>QGpc4D</i>	XWMC331-XGWM194	$a(4t - 1)$	0.14**			-0.35**	
<i>QGpc5A</i>	XBARC358.2-XGWM186	$a(t)$		0.20**		-0.25**	
<i>QGpc5B</i>	XBARC1125-XGWM213	$a(4t - 1)$				-0.10**	
<i>QGpc5D-1</i>	XBARC1097-XCFD8	$a(t)$	-0.24**				0.11**
<i>QGpc5D-2</i>	XBARC320-XWMC215	$a(4t - 1)$	-0.24**				
		$a(4t - 1)$		-0.20**			

(continued)

Table 2.2 (continued)

QTL	Marker interval	Additive effect	12 days after anthesis	17 days after anthesis	22 days after anthesis	27 days after anthesis	32 days after anthesis
<i>QGpc7A</i>	XWMC593-XBARC157.2	$a(t)$					
		$a(q t - 1)$					0.19**
<i>QGpc7D</i>	XGDM67-XWMC634	$a(t)$			-0.13**		
		$a(q t - 1)$					
QTLs contribution rate for phenotypic variation (%)		$R^2(t)$	22.82	21.95	13.49	29.95	24.34
		$R^2(q t - 1)$	19.80	7.71	3.94	11.53	10.77
Total			42.62	29.66	17.43	41.48	35.11

** Significant at the 0.1 % probability level; $a(t)$ is the cumulative additive effects at time t ; $a(q|t - 1)$ is the conditional additive effects from time $(t - 1)$ to t ; $R^2(t)$ and $R^2(q|t - 1)$ indicate the contribution rate for phenotypic variation explained by unconditional QTL detected and conditional QTL detected, respectively

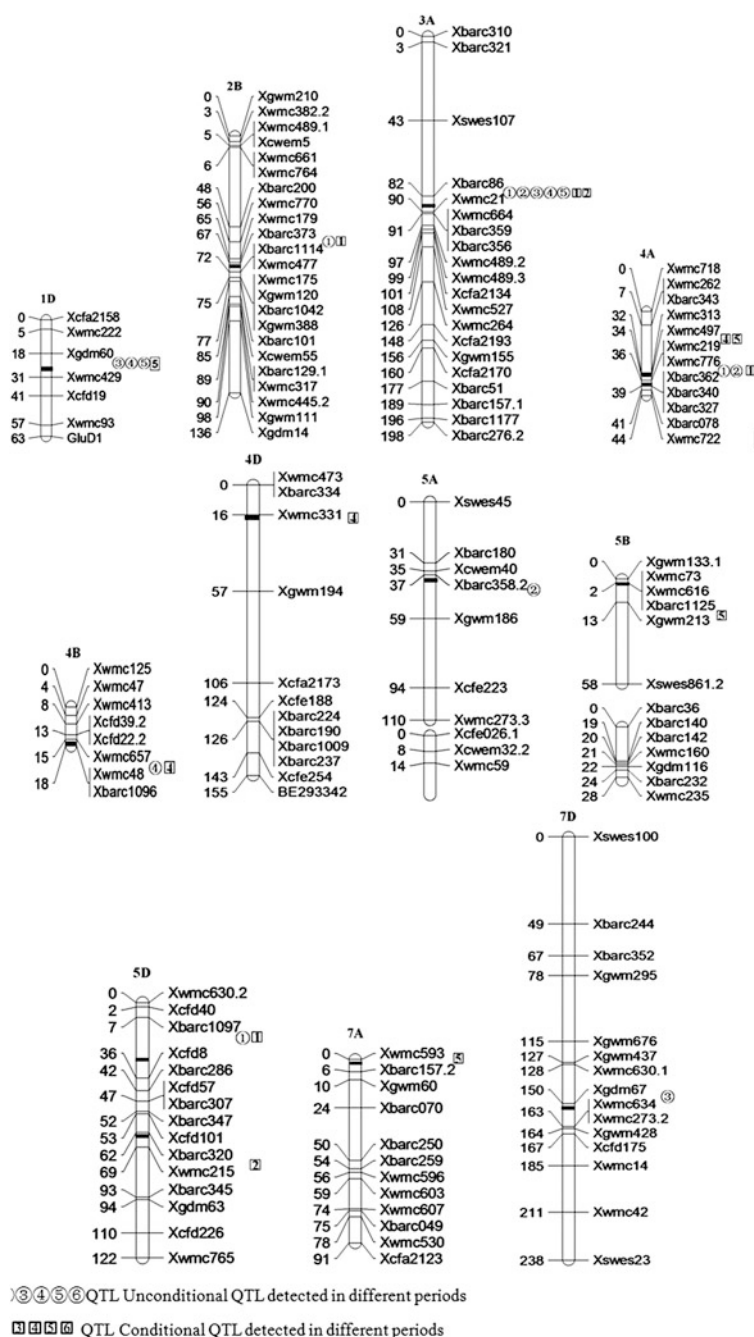


Fig. 2.1 Dynamic identification of QTLs controlling GPC at different periods

of QTLs identified was so smaller, which perhaps was caused by three reasons. (1) The trait studied was grain protein content, but not protein production (protein content multiplied by grain weight); (2) At this stage, the protein synthesis competed the same substrate with the starch, but the synthesis capability of starch showed be stronger than that of protein, so some genes controlling the protein perhaps covered by starch. (3) Beside the additive effect, there was epistatic effect found at this stage.

2.2 Conditional QTL Mapping for Developmental Behavior of Total Starch and Its Components Content in Wheat Grain

Starch in wheat (*Triticum aestivum* L.) is a major component of grain yield, accounting for 65–75 % of the wheat kernel's weight (Hurkman et al. 2003), and plays a critical role in the processing quality of wheat. Many studies have indicated that starch properties, synthesis of starch and its functions in food processing affect the appearance and nutritional quality of foods. A better understanding of the genetics affecting on accumulation and characteristics of starch components could be widely used for the control of wheat quality (Wang and Wang 2004). Many QTLs for important wheat quality traits have been detected was the unconditional (Kuche et al. 2006; Groos et al. 2007; Zhao et al. 2010). QTL mapping, from which can be estimated the accumulated effect of a QTL from the beginning of ontogeny to each observation time. The expression dynamics and direction of individual QTLs at different developmental stages is unable to be inferred. Moreover, there is no report on the conditional QTL mapping of starch about QTL/gene dynamic expression. So this study is to identify dynamic QTL mapping for TSC, AMS, and AMP in contents using a DH population derived from two Chinese winter wheat varieties under six environments in two years by unconditional and conditional mapping. The results would be helpful to explore the developmental genetic mechanism for regulating starch component syntheses in grains, and thus have important significance in increasing or decreasing starch component amounts in wheat grains by genetic engineering.

2.2.1 Materials and Methods

2.2.1.1 Experimental Material

Materials were same as ones of 2.1.1.1 in this chapter.

2.2.1.2 Growth Conditions

Growth conditions were same as ones of 2.1.1.2 in this chapter.

2.2.1.3 Measurement of Starch Components

Sample with strain as the unit was chosen from the main stem spike that was marked in the same day of flowering, and was measured initially at the stage of 12 DAF. After that, the measurement was conducted every 5 days from the flowering stage to mature. A total of five different time sampling points were taken for measuring, which was designated as 12 DAF (S1), 17 DAF (S2), 22 DAF (S3), 27 DAF (S4) and 32 DAF(S5), respectively. Samples were treated at 105 °C for 30 min and further dried at 65 °C until reaching constant dry weight. The wheat grains were ground into whole wheat flour. AMS and AMP were determined by the double wave method, and the TSC were the sums the AMS and AMP contents.

2.2.1.4 Construction of the Genetic Linkage Map

A previously constructed linkage map of the DH population with 323 markers was located on 21 chromosomes (Zhang et al. 2008), including 284 SSR loci, 37 EST loci, 1 inter-simple sequence repeat (ISSR) locus and 1 HMW-GS locus. This linkage map covered a total length of 2,485.7 cM with an average distance of 7.67 cM between adjacent markers. The linked markers formed 24 linkage groups at LOD 4.0. The map was suitable for genome-wide QTL scanning in this study, because the recommended map distance for genome wide QTL scanning was an interval length less than 10 cM (Doerge 2002).

2.2.1.5 Statistical Analysis

Analysis of variance (ANOVA) was carried out using the SPSS version13.0 (SPSS, Chicago, USA) program. Both unconditional QTL and conditional QTL were detected with the mixed linear model using the software of QTL Network2.0 (Yang and Zhu 2005). A QTL was declared if the phenotype was associated with a marker locus at $P < 0.005$. Conditional QTLs were predicted by the software QGASStation1.0 (Zhu 1995) with the cumulative effects of QTLs from time $t - 1$ to time t . Unconditional QTLs indicate the cumulative effects of QTLs from the initial time to time t .

QTLs were named for starch properties by the first two letters with the relevant chromosomal number (McCouch et al. 1997). If there were more than one QTL on a chromosome, then a serial number was added after the chromosomal number, such as “*QGsc-5D.1* and *QGsc-5D.2*”.

2.2.2 Result and Analysis

2.2.2.1 Phenotypic Variation

The phenotypic values of wheat starch and its components of the DH population and their parents in six environments at five developmental stages are shown in Tables 2.3, 2.4 and 2.5. The DH means for AMS, AMP and TSC showed differences across all measuring stages in all environments. Wheat starch contents showed a persistent increasing trend in the DH population during the period from stage 1 to stage 5. In the growth process of wheat starch, Huapei 3 had higher values than Yumai57 for 12 DAF in E2, E5, while in other environments; Yumai57 had higher values than Huapei3. Some of the AMS and AMP have the same phenomenon, which may have been influenced by environment. High phenotypic variability was observed in the population, for example wheat starch with contents ranging from 43.57 to 74.69 %, indicating significantly transgressive segregation. Segregation continuously among the DH population suggests that the trait approximately followed normal distributions. Most values of skewness and kurtosis were less than 1.0, indicating typical inheritance of quantitative traits and suitability of the data for QTL analysis.

2.2.2.2 Additive QTL

2.2.2.2.1 Unconditional QTL for TSC, AMS and AMP During the Grain Development

Unconditional QTLs detected at different stages were attributed to the cumulative gene expression from the initial time to the final stage (Table 2.6). Seven QTLs for grain starch with additive effects were mapped to chromosomes 2A, 3A, 3B, 4A, and 5D. Among them, the *QTsc-2A*, *QTsc-3A* and *QTsc-3B* were detected only at only one stage with a smaller effect. Of these, *QTsc-4A* was expressed steadily at all the five developing stages with 3.52 %, 9.67 %, 18.20 %, 19.20 %, and 15.81 % of the phenotypic variance, respectively. The *QTsc-4A* had the most significant effect on values at the different developing stages. The allele that increased starch content was contributed by Yumai57. Three additive QTLs for starch were located on 5D chromosomes, which were detected at the stages of 12 DAF, 17 DAF and 27 DAF, and these QTLs have different effects on starch content variation. The number of unconditional QTLs was also different. The three QTLs were found at stage 12 DAF, which explained 13.57 % of the phenotypic variance. There were two QTLs discovered at the stages 17 DAF, 22 DAF, 27 DAF and 32 DAF. These contributed 16.57, 21.96, 22.53 and 22.90 % of the phenotypic variation, respectively.

When the data of six environments were analyzed together, more QTLs significantly affecting amylose were detected, which included four additive QTLs (Table 2.6), identified in the *Xbarc356-Xwmc489.2* interval on chromosome 3A, in

Table 2.3 Phenotypic values of DH population and parents for TSC in different environments (%)

Environment	Period ^a	Parent		DH population				Standard deviation	Skewness	Kurtosis
		Huapei3	Yumai57	Mean	Maximum	Minimum				
E 1	S1	11.96	12.19	15.84	25.00	9.03		2.75	0.64	0.78
	S2	27.58	36.17	34.97	48.03	24.58		4.41	0.31	0.13
	S3	46.65	51.22	45.56	53.56	35.26		3.83	-0.15	-0.41
	S4	48.64	58.41	51.23	61.45	40.21		4.01	0.13	-0.02
	S5	56.74	64.40	56.89	66.55	48.18		4.16	0.28	-0.46
E 2	S1	12.99	11.44	17.80	29.39	9.20		2.97	0.43	1.08
	S2	24.78	33.82	33.52	46.44	24.09		3.63	0.53	1.31
	S3	29.88	37.78	42.02	48.98	29.43		3.29	-0.43	0.39
	S4	39.74	48.86	51.31	62.44	39.57		4.32	-0.08	0.45
	S5	54.35	55.79	51.68	64.96	41.24		3.88	-0.04	-0.09
E3	S1	10.50	17.73	16.27	26.38	8.02		3.11	0.32	0.35
	S2	27.24	35.98	33.06	40.05	25.25		2.93	0.06	-0.21
	S3	30.99	45.85	42.76	51.54	32.73		3.46	-0.27	0.35
	S4	43.92	53.32	48.87	58.72	38.03		3.19	-0.08	0.83
	S5	51.24	60.78	52.40	66.97	42.69		3.32	0.44	2.59
E4	S1	17.77	19.61	20.51	35.17	4.91		3.97	-0.06	2.05
	S2	27.61	37.43	34.31	45.29	26.64		2.93	0.15	1.01
	S3	39.07	47.98	44.32	53.14	33.37		3.17	-0.41	0.88
	S4	47.93	51.83	49.41	59.11	39.59		3.60	0.09	-0.08
	S5	54.66	59.25	54.19	62.08	44.58		3.38	-0.04	0.18

(continued)

Table 2.3 (continued)

Environment	Period ^a	Parent		DH population				Skewness	Kurtosis
		Hupei3	Yumai57	Mean	Maximum	Minimum	Standard deviation		
E5	S1	28.20	27.73	20.26	31.15	9.39	4.03	0.34	-0.07
	S2	38.73	46.15	36.44	44.40	24.28	3.03	-0.05	1.20
	S3	49.57	52.26	46.18	52.83	32.43	3.23	-0.33	1.07
	S4	51.47	57.71	51.39	63.02	39.86	3.53	-0.09	0.97
	S5	55.09	61.31	55.91	67.19	46.07	3.89	-0.01	-0.11
E6	S1	18.34	29.61	19.75	30.70	7.50	4.20	0.10	0.25
	S2	37.75	39.27	34.19	41.39	25.55	2.26	-0.28	1.3
	S3	46.04	46.48	44.09	51.52	36.90	2.87	0.18	0.03
	S4	49.99	54.53	50.46	60.40	41.53	3.68	0.19	-0.23
	S5	56.09	56.37	54.63	74.69	43.57	3.93	0.69	0.067

^aDifferent growing stages after flowering, S1: 12 days after flowering (DAF), S2: 17 days after flowering, S3: 22 days after flowering, S4: 27 days after flowering, S5: 32 days after flowering; The same as below

Table 2.4 Phenotypic values of DH population and parents for AMS in different environments (%)

Environment	Period ^a	Parent		DH population				Skewness	Kurtosis
		Huapei3	Yumai57	Mean	Maximum	Minimum	Standard deviation		
E1	S1	5.17	5.38	3.01	6.37	1.20	1.31	0.52	-0.22
	S2	7.91	14.92	10.34	21.08	3.82	3.32	0.46	-0.08
	S3	16.92	18.81	14.87	21.81	7.47	3.31	-0.18	-0.85
	S4	17.72	20.90	17.47	26.15	7.11	3.45	0.12	-0.19
	S5	21.25	25.11	20.29	27.34	11.80	3.21	-0.12	-0.27
E2	S1	3.33	4.19	4.01	9.69	1.67	1.21	1.35	1.17
	S2	7.93	10.69	8.58	16.13	2.74	2.34	0.81	1.22
	S3	12.17	13.22	11.97	19.47	4.84	2.69	-0.07	-0.23
	S4	12.79	19.45	16.38	29.61	7.52	3.71	-0.22	0.11
	S5	21.77	22.50	19.11	24.45	8.43	2.96	-0.17	0.04
E3	S1	3.11	4.48	2.49	5.77	1.03	1.11	0.74	0.29
	S2	4.85	9.08	7.66	13.64	3.01	2.23	0.61	-0.10
	S3	8.32	16.15	12.23	19.47	4.08	2.99	-0.30	-0.26
	S4	11.84	17.80	15.82	21.58	7.35	2.45	-0.33	0.33
	S5	15.94	23.99	17.47	23.24	10.64	2.23	-0.08	0.43
E4	S1	4.96	5.64	5.07	9.02	1.84	1.37	0.17	-0.01
	S2	7.18	10.88	7.71	14.65	3.80	1.93	0.83	1.26
	S3	10.76	14.23	12.5	18.62	4.91	2.46	-0.25	0.15
	S4	14.38	16.82	15.17	22.68	9.14	2.88	0.16	-0.27
	S5	15.99	19.97	17.94	23.36	11.35	2.26	-0.17	-0.24
E5	S1	6.50	4.43	4.94	12.55	1.38	1.80	0.65	0.90
	S2	8.24	10.03	8.74	15.19	3.32	2.14	0.34	0.16
	S3	14.16	13.79	13.35	20.90	5.87	2.44	-0.08	0.19
	S4	15.44	18.70	15.85	22.69	6.67	2.54	-0.43	0.87
	S5	17.75	20.79	18.20	23.29	11.89	2.32	-0.19	-0.04

(continued)

Table 2.4 (continued)

Environment	Period ^a	Parent		DH population				Standard deviation	Skewness	Kurtosis
		Huapei3	Yumai57	Mean	Maximum	Minimum				
E6	S1	4.58	6.85	5.14	12.07	1.71		1.82	1.06	0.06
	S2	9.79	8.90	7.31	15.71	3.48		1.61	0.31	0.07
	S3	12.47	13.38	12.29	19.00	5.93		2.27	-0.06	-0.01
	S4	14.43	20.56	16.52	24.76	9.73		2.75	0.04	0.16
	S5	18.67	21.45	18.69	34.85	10.88		2.72	1.04	0.29

Table 2.5 Phenotypic values of DH population and parents for AMP in different environments (%)

Environment	Period ^a	Parent		DH population				Skewness	Kurtosis
		Huapei3	Yumai57	Mean	Maximum	Minimum	Standard deviation		
E1	S1	6.79	6.82	5.91	20.81	1.42	3.49	1.31	1.73
	S2	19.68	21.25	20.41	31.41	9.14	3.89	0.18	0.66
	S3	29.74	32.42	28.87	39.37	15.43	3.30	-0.56	2.01
	S4	30.92	37.50	33.19	40.73	20.87	2.68	-0.40	2.60
	S5	35.49	39.29	36.54	44.71	29.94	3.09	0.63	-0.03
E2	S1	9.67	7.25	9.93	21.70	2.36	3.86	0.51	0.38
	S2	16.85	23.14	20.51	27.88	9.84	3.29	-0.55	0.56
	S3	17.72	24.57	24.79	34.45	13.83	3.32	-0.41	0.81
	S4	26.95	29.41	28.56	36.62	17.59	3.61	-0.17	-0.11
	S5	32.58	33.29	33.32	50.32	22.37	4.09	0.53	0.58
E3	S1	7.39	13.25	9.17	21.10	7.02	4.45	0.45	-0.46
	S2	22.39	26.90	23.41	30.22	11.91	3.62	-0.77	0.73
	S3	22.67	29.70	27.39	34.05	17.52	2.80	-0.48	0.63
	S4	32.09	35.52	30.22	38.16	22.84	2.60	-0.04	-0.13
	S5	35.30	36.79	33.69	43.73	24.91	3.16	0.06	0.27
E4	S1	12.81	13.97	17.13	31.84	6.32	4.98	0.13	-0.68
	S2	20.43	26.56	21.48	39.79	12.56	4.56	-0.18	0.27
	S3	28.31	33.75	31.68	41.34	22.32	3.16	-0.06	0.21
	S4	33.55	35.01	35.20	43.21	28.60	2.91	0.14	-0.09
	S5	38.67	39.29	39.00	49.53	26.97	3.89	0.07	-0.16

(continued)

Table 2.5 (continued)

Environment	Period ^a	Parent		DH population				Standard deviation	Skewness	Kurtosis
		Huapei3	Yumai57	Mean	Maximum	Minimum				
E5	S1	21.71	23.30	19.10	36.07	3.73		4.67	0.25	-0.08
	S2	30.49	36.13	30.42	40.79	14.73		4.45	-0.32	1.05
	S3	35.42	38.48	36.13	43.60	26.75		3.02	-0.10	-0.11
	S4	36.04	39.01	39.26	46.03	32.49		2.55	-0.03	-0.09
	S5	37.35	40.52	42.18	52.56	28.37		3.35	0.06	1.49
E6	S1	13.77	22.77	18.66	24.97	6.33		4.44	-1.02	0.13
	S2	27.96	30.38	27.82	34.28	17.97		2.74	-1.00	1.41
	S3	33.57	33.10	32.60	38.32	24.38		2.09	-0.40	1.95
	S4	35.56	33.98	34.61	39.52	29.99		1.82	0.39	0.04
	S5	37.42	34.93	36.21	41.20	25.79		2.19	-0.87	1.31

Table 2.6 Additive effects of QTLs detected by unconditional mapping for components of starch at different growing stages

Trait	QTL	Marker interval	S1		S2		S3		S4		S5	
			A ^a	H ² (%) ^b	A	H ² (%)	A	H ² (%)	A	H ² (%)	A	H ² (%)
TSC	<i>QTsc-2A</i>	<i>Xgwm558-Xbarc015</i>									-0.76	7.09
	<i>QTsc-3A.1</i>	<i>Xbarc157.1-Xbarc1177</i>										
	<i>QTsc-3B.1</i>	<i>Xwmc1-Xgwm285</i>	1.17	4.09			0.87	3.76				
	<i>QTsc-4A</i>	<i>Xwmc262-Xbarc343</i>	-1.05	3.52	-1.61	9.67	-1.23	18.20	-1.21	19.20	-1.20	15.81
	<i>QTsc-5D.1</i>	<i>Xbarc320-Xwmc215</i>	-0.59	5.96								
	<i>QTsc-5D.2</i>	<i>Xbarc1097-Xcfd8</i>			-0.52	6.90						
	<i>QTsc-5D.3</i>	<i>Xcfd226-Xwmc765</i>							-1.06	3.33	0.41	7.22
	<i>QAns-3A.2</i>	<i>Xbarc356-Xwmc489.2</i>										
	<i>QAns-3B.1</i>	<i>Xwmc1-Xgwm285</i>	0.22	4.16								
	<i>QAns-4A</i>	<i>Xwmc262-Xbarc343</i>	-0.28	6.16	-0.89	27.89	-1.21	36.82	-1.33	35.39	-1.07	29.02
AMP	<i>QAns-5D.1</i>	<i>Xbarc320-Xwmc215</i>	-0.19	3.78								
	<i>QAnp-3A.1</i>	<i>Xbarc157.1-Xbarc1177</i>			0.33	4.52						
	<i>QAnp-3B.2</i>	<i>Xgwm566-Xcfe009</i>	0.81	7.55								
	<i>QAnp-5D.1</i>	<i>Xbarc320-Xwmc215</i>	-0.91	4.08	-0.38	5.99						

^aAdditive effects, positive value indicates that allele from Huapei 3 enhances the TSC, negative value indicates that allele from Yumai 57 enhances TSC;
^bPercentage of phenotypic variation explained by QTL with additive effect. The same as below

the *Xwmc1-Xgwm285* interval on chromosome 3B, in the *Xwmc262-Xbarc343* interval on chromosome 4A, and in the *Xbarc320-Xwmc215* interval on chromosome 5D, respectively.

As described above, it is clear that the *QAms-4A* was detected by integrated analysis using data of all stages, which contributed 6.16, 27.89, 36.82, 35.39, and 29.02 % of the phenotypic variation, respectively. Four additive QTLs on four chromosomes (3A, 3B, 4A, and 5D) for cohesiveness explained phenotypic variances ranging from 3.78 to 36.82 %. The *QAms-4A.1* had the highest contribution and explained 36.82 % of the phenotypic variance. Two QTLs (*QAms-3A.2*, *QAms-3B.1*) had positive effects and were contributed by Huapei3 alleles. The other two QTLs (*QAms-4A*, *QAms-5D*) had negative effects and came from Yumai57. This suggested that the alleles were dispersed between two parents to increase cohesiveness, according to the adhesiveness in phenotype variation between the parents and transgressive segregation among the DH population.

Three main-effect QTLs were identified for amylopectin (Table 2.6). These QTLs accounted for phenotypic variances ranging from 4.08 to 7.55 %. The total contribution of the main-effect QTLs explained 22.14 % of the phenotypic variance. Two alleles (*QAmp-3A.1*, *QAmp-3B.2*) came from Huapei3, and *QAmp-5D.1* was from Yumai57, indicating that QTLs detected at one specific stage did not entirely represent these effect at another stage, and that genes determining the amylopectin content might be selectively expressed during the growth process of the seed.

2.2.2.2.2 Conditional QTL for TSC, AMS and AMP During the Grain Development

Three additive QTLs (*QTsc-3B*, *QTsc-4A*, *QTsc-5D.1*) in 12 DAF (S1|S0) were identified by conditional mapping, accounting for 4.09, 3.52, and 5.96 % of the phenotypic variance, respectively (Table 2.7). The *QTsc-4A* was detected for grain starch explaining phenotypic variances of 14.20 %, which was expressed after flowering 12–17 days (S2|S1), and played an important role in the accumulation of starch content. The *QTsc-6A* was expressed after flowering 17–22 days (S3|S2) and was a new site with phenotypic variances of 3.11 %. The *QTsc-4A* continued to express after flowering 27–32 days (S4|S5), which explained 4.08 % of the phenotypic variance. There were no conditional QTL sites detected after flowering 22–27 days (S4|S3).

A total of six conditional A-QTLs (*QAms-2A*, *QAms-3A.2*, *QAms-3A.3*, *QAms-3B.1*, *QAms-4A*, and *QAms-5D.1*) for amylose were identified with significant effects in periods 1–5 (Table 2.7). Most of the favorable alleles came from Yumai57. Of these, three QTLs (*QAms-3B.1*, *QAms-4A*, and *QAms-5D.1*) were detected at 12 DAF (S1|S0). Two additive QTLs detected only at one stage were *QAms-3A.3* and *QAms-4A* at stage after flowering 12–17 days (S2|S1), and *QAms-3A.2*, and *QAms-4A* at stage after flowering 17–22 days (S3|S2), respectively. The *QAms-2A* was continuously expressed after flowering 27–32 days (S5|S4), with 6.34 % of the phenotypic variance.

Table 2.7 Additive effects of QTLs detected by conditional mapping for components of starch at different growing stages

Trait	QTL	Marker interval	S1 S0 ^a		S2 S1		S3 S2		S4 S3		S5 S4	
			A	H ² (%)	A	H ² (%)	A	H ² (%)	A	H ² (%)	A	H ² (%)
TSC	<i>QTsc-3B.1</i>	<i>Xwmc1-Xgwm285</i>	1.17	4.09								
	<i>QTsc-4A</i>	<i>Xwmc262-Xbarc343</i>	-1.06	3.52	-0.79	14.20					-0.72	4.08
	<i>QTsc-5D.1</i>	<i>Xbarc320-Xwmc215</i>	-0.59	5.96								
	<i>QTsc-6A</i>	<i>Xwmc553-Xgwm732</i>					-0.64	3.11				
	<i>QAns-2A</i>	<i>Xgwm558-Xbarc015</i>									-0.38	6.34
AMS	<i>QAns-3A.2</i>	<i>Xbarc356-Xwmc489.2</i>					0.35	4.71				
	<i>QAns-3A.3</i>	<i>Xcfa2193-Xgwm155</i>			0.12	1.20						
	<i>QAns-3B.1</i>	<i>XWMC1-XGWM285</i>	0.22	4.16								
	<i>QAns-4A</i>	<i>Xwmc262-Xbarc343</i>	-0.28	6.16	-0.63	18.47	-0.52	9.07				
	<i>QAns-5D.1</i>	<i>Xbarc320-Xwmc215</i>	-0.19	3.78								
AMP	<i>Qamp-1B</i>	<i>Xwmc57-Xcwm6.1</i>					0.61	4.96				
	<i>Qamp-3B.2</i>	<i>Xgwm566-Xcfe009</i>	0.81	7.55								
	<i>Qamp-5D.1</i>	<i>Xbarc320-Xwmc215</i>	-0.91	4.08					-0.74	5.31		
	<i>Qamp-5D.4</i>	<i>Xbarc347-Xcfd101</i>			0.71	4.44						

^aThe conditional additive effects from time ($t - 1$) to t , S1|S0: the time interval from the initial time of flowering to stage S1, S2|S1 the time interval from stage S1 to stage S2, and so on

A total of four conditional A-QTLs (*QAmp-1B*, *QAmp-3B.2*, *QAmp-5D.1*, and *QAmp-5D.4*) for amylopectin were identified with significant effects in periods 1–5 (Table 2.7). Two additive QTLs (*QAmp-3B.2*, *QAmp-5D.1*) 12 DAF (S1|S0) were identified by conditional mapping, accounting for 7.55 and 4.08 % of the phenotypic variance, respectively. The *QAmp-5D.4* and *QAmp1B* were detected only at one stage, after flowering 12–17 days (S2|S1), and 7–22 days (S3|S2), respectively. The *QAmp.5D.1* continuously expressed at period after flowering 22–27 days (S4|S3) with 5.31 % of the phenotypic variance.

2.2.2.3 Epistatic QTLs

2.2.2.3.1 Epistatic QTLs by Unconditional QTL Mapping

Independent analysis on the data coming from six different environments indicated that eight pairs of epistatic QTLs (*QTsc-3B.1-QTsc-5D.1*, *QTsc-3D.2-QTsc-6D*, *QTsc-4A-QTsc-7B*, *QAms-2B.3-QAms-3D*, *QAms-3B.2-QAms-4D*, *QAms-2B.2-QAms-5D.2*, *QAms-2B.1-QAms-3A*, and *QAmp-3D.1-QAmp-6D*) were detected at all the five developing stages by unconditional QTL mapping (Table 2.8). Of these, three pairs of epistatic QTLs for grain starch were detected only at 12 DAF and 32 DAF, and the contribution rate varied from 1.62 % to 5.60 %. They were located in 3B-5D (interval *Xwmc1-Xgwm285* and *Xbarc320-Xwmc215*), 3D-6D (interval *Xgdm8-Xwmc492* and *Xcfa2129-Xbarc080*), 4A-7B (interval *Xwmc497-Xwmc219*, and *Xwmc273.1-Xcfd22.1*) chromosomes, respectively.

Four pairs of epistatic QTLs for amylose were found only at one stage. They were *QAms-2B.3-QAms-3D* and *QAms-3B.2-QAms-4D* at the stage 22 DAF, *QAms-2B.2-QAms-5D.2* at 22 DAF, and *QAms-2B.1-QAms-3A* at 22 DAF. The contribution rate of epistatic effects of the paired QTLs varied from 2.50 % to 4.80 %.

A pair of epistatic QTLs for amylopectin, *QAmp-3D.1-QAmp-6D*, was found only 12 DAF, which explained 3.74 % of the total phenotypic variation.

As described above, it is clear that eight pairs of epistatic QTLs detected by integrated analysis using data of all six environments were interactions between non-main effect and QTL effects. The phenotypic contribution was too small to found by separate analysis when they existed alone, indicating that only two QTLs play a role together at the same time, and thus significantly controlled changes of the phenotype.

2.2.2.3.2 Epistatic QTLs by Conditional QTL Mapping

When the data in the six environments were analyzed together by conditional QTL mapping, a total of nine pairs of epistatic QTLs (Table 2.9) were found. Among them, four, four, and one pairs of epistatic QTLs were detected for TSC, AMS, and AMP, respectively (Table 2.9).

Table 2.8 Epistatic effects of QTLs detected by unconditional mapping for components of starch at different growing stages

Trait	QTL	Marker interval	QTL	Marker interval	S1		S2		S3		S4		S5	
					AA ^a	H^2 (%) ^b	AA	H^2 (%)	AA	H^2 (%)	AA	H^2 (%)	AA	H^2 (%)
TSC	<i>QTsc-3B.1</i>	<i>Xwmc1-Xgwm285</i>	<i>QTsc-5D.1</i>	<i>Xbarc320-Xwmc215</i>	0.66	1.62								
	<i>QTsc-3D.2</i>	<i>Xgdm8-Xwmc492</i>	<i>QTsc-6D</i>	<i>Xcfa2129-Xbarc080</i>	0.85	3.08								
	<i>QTsc-4A</i>	<i>Xwmc497-Xwmc219</i>	<i>QTsc-7B</i>	<i>Xwmc273.1-Xcfa22.1</i>										
	<i>QAms-2B.3</i>	<i>Xbarc200-Xwmc770</i>	<i>QAms-3D</i>	<i>Xcfa4-Xgwm52</i>					-0.41	2.50			-0.87	5.60
AMS	<i>QAms-3B.2</i>	<i>Xbarc1111-Xwmc307</i>	<i>QAms-4D</i>	<i>Xbarc334-Xwmc331</i>					0.35	2.53				
	<i>QAms-2B.2</i>	<i>Xcwem5-Xwmc661</i>	<i>QAms-5D.2</i>	<i>Xwmc215-Xbarc345</i>							-0.61	4.80		
	<i>QAms-2B.1</i>	<i>Xwmc382.2-Xwmc489.1</i>	<i>QAms-3A</i>	<i>Xwmc489.3-Xcfa2134</i>									-0.38	3.51
	<i>QAmp-3D.1</i>	<i>Xgwm52-Xgdm8</i>	<i>QGsc-6D</i>	<i>Xcfa2129-Xbarc080</i>	0.89	3.74								

^aEpistatic effects, positive value represents parent type effect is bigger than recombinant type effect, negative value represents the opposite

^bPercentage of variation explained by epistatic QTL. The same as below

Four pairs of epistatic QTLs for TSC, *QTsc-3B.1-QTsc-5D.1* and *QTsc-3D.2-QTsc-6D.2* were detected at the period after flowering of 12 d, while *QTsc-1B-QTsc-3B.2* and *QTsc-1B-QTsc-3B.3* were found at the period after flowering of 17–22 days. The contribution rates of four pairs of epistatic QTLs for grain starch varied from 1.62 to 5.64 %.

Four pairs of epistatic QTLs for AMS were located on the 2B-7B, 4B-4D, 4D-6D, and 1D-4D chromosomes respectively. The *QAms-2B.4-QAms-7B* was detected at the period after flowering 12–17 days. The *QAms-4B-QAms-4D.1* and *QAms-4D.1-QAms-6D.1* were detected at the period after flowering 17–22 days. And the *QAms-1D-QAms-4D.2* was detected at the period after flowering 27–32 days. Their phenotypic contributions were 5.35, 6.21, 4.08, and 5.11 %, respectively.

A pair of epistatic QTLs for amylopectin was found only at 12 DAF, the same as the unconditional QTL mapping, *QAmp-3D.1-QAmp-6D*, explained phenotypic variation 3.74 % of the total phenotype.

2.2.3 Comparson of the Results with Ones of Previous Studies

Relevant to starch quality traits, Fang et al. (2003) reported that starch content was influenced by additive and no-additive effects at the same time, and amylose and amylopectin content is mainly controlled by additive effects. Udall et al. (1999) mapped quantitative genetic variation for starch paste viscosity of wheat using the recombinant inbred (RIL) population of 78 lines. The population which involved the 2B, 1A, 2A, 2D, and 3B chromosomes, was planted in five environments. Araki (1999), using 98 single-chromosome recombinant substitution lines for amylose content, discovered that most of the genetic variation was explained by the allelic difference at the *Wx-B1* locus of chromosome 4A. An additional QTL of minor effect was mapped in the *Xbcd1738/Xcdo1387* interval on the short arm. Batey (2001) measured starch properties on the doubled haploid progeny of 2 crosses, consistent with the fact that Halberd is null for the *Wx-B1* and Cranbrook is a wheat line carrying the normal 3 *Wx* loci. Starch gelatinisation, peak temperature, and peak viscosity indicated a QTL on chromosome 7A, starch gelatinisation onset temperature indicated a significant QTL on chromosomes 2B and 7A, Heat of gelatinisation (H) indicated a suggestive QTL on chromosome 4A, and the A: B granule ratio analysis indicated a significant QTL on chromosome 4B. Igrejas (2002) located significant QTL for starch granule size on chromosome 7A with a contribution of 27 %, using the RIL population. In our research, the detected QTLs were located on the 2A, 3A, 3B, 4A, 5D, and 6A chromosomes. The *QTsc-4A* and *QAms-4A* was found in the *Xwmc262-Xbarc343* interval on the 4A chromosome, which frequent expresses and has the same genetic direction effect with a larger contribution in the whole growth period. The *QTsc-4A* and *QAms-4A* were common

Table 2.9 Epistatic effects of QTLs detected by conditional mapping for components of starch at different growing stages

Trait	QTL	Marker-interval	QTL	Marker interval	S1 S0 AA ^a	S2 S1		S3 S2		S4 S3		S5 S4	
						AA	H ² (%) ^b	AA	H ² (%)	AA	H ² (%)	AA	H ² (%)
TSC	<i>QTsc-3B.1</i>	<i>Xwmc1-Xgwm285</i>	<i>QTsc-5D.1</i>	<i>Xbarc320-Xwmc215</i>	0.66		1.62						
	<i>QTsc-3D.2</i>	<i>Xgdm8-Xwmc492</i>	<i>QTsc-6D.2</i>	<i>Xcfa2129-Xbarc080</i>	0.85		3.08						
	<i>QTsc-1B</i>	<i>Xgwm218-Xgwm582</i>	<i>QTsc-3B.2</i>	<i>Xbarc1111-Xwmc307</i>									
	<i>QTsc-1B</i>	<i>Xgwm218-Xgwm582</i>	<i>QTsc-3B.3</i>	<i>Xgwm566-Xcfe009</i>				-0.63	5.64				
	<i>QAmis-2B.4</i>	<i>Xwmc179- Xbarc373</i>	<i>QAmis-7B</i>	<i>Xgwm611-Xwmc581</i>				-0.59	5.32				
AMS	<i>QAmis-4B</i>	<i>Xwmc48- Xbarc1096</i>	<i>QAmis-4D.1</i>	<i>Xbarc334-Xwmc331</i>		0.35		0.41	6.21				
	<i>QAmis-4D.1</i>	<i>Xbarc334-Xwmc331</i>	<i>QAmis-6D.1</i>	<i>Xubc808- Xswes679.1</i>				-0.31	4.08				
	<i>QAmis-1D</i>	<i>Xwmc222-Xgdm60</i>	<i>QAmis-4D.2</i>	<i>Xbarc190-Xbarc1009</i>									
	<i>QAmis-3D.1</i>	<i>Xgwm52-Xgdm8</i>	<i>QGsc-6D</i>	<i>Xcfa2129-Xbarc080</i>	0.89		3.74					0.41	5.11

results of unconditional and conditional analysis through the whole period of wheat development. Hence, the real gene expression in the early period could explain gene action at a later stage. Sun et al. (2009) discovered a QTL controlling starch on the *Xwmc262-Xwmc419* and *Xswes124-Xubc827* intervals of the 4A chromosomes. These QTL might be located in the same or a similar position. The numbers and types of *Wx* protein can cause changes in the amylose, amylopectin contents, thus causing changes in grain starch content. The *Wx* protein was controlled by genes located on the 4A, 7A, and 7D chromosomes. We can infer that an inevitable connection exists in genes controlling *Wx* protein and starch synthase, because one gene controls the *Wx* protein on the 4A chromosome.

2.3 Conditional QTL Mapping for Protein and Starch Interaction in Wheat Grain

Protein and starch are the principal components of wheat endosperm and are responsible for wheat end-use quality. Several studies on the relationship between protein and wheat processing quality have been reported. Although extensive QTL analysis for protein content and starch content had been conducted in recent years, less information is available about the genetic interrelationship between protein content and starch content at QTL/gene level, especially why they had the significant negative correlation, and how to dissect the genetic relationship between them. To date, however, no studies about their relationship from QTL/gene level have investigated.

Therefore our objective was to dissect the genetic relationship between GPC and GSC using unconditional and conditional QTL mapping analysis. By comparing unconditional and conditional QTLs, the genetic interdependencies between GPC and GSC can be identified at the level of the individual QTL. This comparison might provide valuable information for marker-assisted selection to improve GPC without negative effects on GSC or enhance GSC without negative effects on GPC.

2.3.1 Materials and Methods

2.3.1.1 Plant Materials

Data presented in this study were derived from one DH (doubled haploid) and two RIL (recombinant inbred lines) populations. The two RILs are designated as RIL 1 and RIL 2.

The DH mapping population of 168 lines were derived from the androgenic haploid of (Huapei 3/Yumai 57) F_1 followed by genome doubling. The two parental

cultivars were approved by the State Variety Evaluation Committee in 2003 and released in Henan Province, China in 2006.

RIL1 population of 256 lines was developed from a cross between the two winter wheat cultivars Nuomai 1 (female) (NM1) and Gaocheng 8901 (male) (GC8901). Briefly, the RIL population was developed by a single-seed descent to the F₁₀ generation. NM1 (Jiangsu Baihuomai/Guandong107) carrying HMW-GS or alleles of Ax-null, Bx7 + By8, and Dx2.2 + Dy12 at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively, which was bred by China Agricultural University and released in 2005 in Beijing. It has three null waxy alleles (*Wx-A1b*, *Wx-B1b*, and *Wx-D1b*), similar to red winter wheat. Moreover, this cultivar has unique starch properties that are related to high-quality white salt noodles. GC 8901 (77546-2/Linzhang) has normal waxy alleles, which was bred by Gaocheng Agricultural Science Research Institute and was released in 1998 in Hebei province. This cultivar carries HMW-GS or alleles of Ax1, Bx7 + By8, and Dx5 + Dy10 at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively. It exhibits high gluten strength and good bread-making qualities.

RIL2 population of 182 lines was derived from a cross between Shannong 01-35 (39-1/Hesheng 2) (SN01-35) and Gaocheng 9411 (77546/Linzhang) (GC9411) (SG population). This population was also developed by single-seed descent, to the F₈₋₉ generation. The grains of SN01-35 appeared larger than those of GC9411, but the quality of GC9411 is better than that of SN01-35. Thus, the population showed large variations in yield and quality traits.

2.3.1.2 Field Trials

The three genetic populations (DH, RIL1 and RIL2), along with their corresponding parents, were grown in three distinct locations for the 2010-2011 and 2011-2012 growing seasons. E1 and E3 represent Tai'an, Shandong Province (36°12'N, 117°04'E), China, in 2010-2011 and 2011-2012 growing season, respectively; E2 refers to Suzhou, Anhui Province (33°63'N, 116°97'E), China, in 2010-2011 growing season; E4 stands for Jiyuan, Henan Province (35°05'N, 112°36'E), China, in 2011-2012 growing season.

These lines were sown in a randomized block design with two replicates at each location. Each replication in E1 and E3 was designed based on a six-row plot with 2.3 m long and 26 cm row-to-row distance, whereas that in E2 was a six-row plot with 4 m long and 25 cm row-to-row distance and that in E4 was a three row plot with 2.6 m long and 26 cm row-to-row distance.

Before planting, 37,500 kg/ha of farmyard manure or barnyard manure (nitrogen content, 0.05-0.1 %), 225 kg/ha of urea, 225 kg/ha of phosphorus diamine fertilizer, 225 kg/ha of potassium chloride, and 15 kg/ha of zinc sulphate were added as fertilizers in each of three locations. And 225 kg/ha urea at jointing stages was top-dressed in all experimental locations.

The rainfalls during the growth cycles (from October of this year to June of next year) in Tai'an location were 234.7 mm in 2010-2011 growing season and

221.8 mm in 2011–2012, respectively; while there was 233.9 mm in Jiyuan location in 2011–2012 growing season, and was 310.5 mm in Suzhou location in 2010–2011 growing season. Water irrigation was carried out at each stage of pre-overwintering, jointing, and grain filling in all experimental locations.

All recommended local crop management practices were followed, and damages attributed to lodging, disease, or pests were not observed during the growing seasons.

2.3.1.3 Methods

GPC was measured by near-infrared reflectance spectroscopy (NIRS) on a Perten DA-7200 instrument (Perten Instruments, Huddinge, Sweden) and expressed on a 14 % moisture basis using the AACC approved method 39–25 (2004) after calibration by Kjeldahl instrument using 100 samples. The correlation coefficient between two methods was 0.952.

GSC was also detected by NIRS on a Perten DA-7200 instrument (Perten Instruments, Huddinge, Sweden) after calibration by Polarimetry method using 100 samples. The correlation coefficient between two methods was 0.954.

2.3.1.4 Data Analysis and QTL Mapping

Statistical analyses (e.g., normal distribution and correlation) were performed using the software SPSS 13.0 (SPSS, Chicago, USA) and Excel 2003.

Conditional genetic analysis was conducted based on the phenotypic values of GPC or GSC conditioned on GSC or GPC, which were obtained by the mixed-model approach (Zhu 1995; Wen and 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 $GPC|GSC$ = grain protein content conditioned on grain starch content). The software QGAStation 1.0 (<http://ibi.zju.edu.cn/software/qga/>) was used to determine the conditional phenotypic values $y(T1|T2)$ as input data for conditional QTL mapping, which used the composite interval mapping method.

Unconditional and conditional QTL mappings were performed using the software QTLNetwork2.0 (<http://ibi.zju.edu.cn/software/qtlnetwork/>) based on the mixed linear model using three constructed genetic maps (Shi 2012; Zhang et al. 2008; Zheng et al. 2013). 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 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 0.10 (suggestive) and 0.05 (significant). The final genetic model incorporated significant additive and epistatic effects as well as their

environmental interactions. A QTL was declared if the phenotype was associated with a marker locus at $P < 0.005$.

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 an 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 are more than one QTL on one chromosome, a serial number behind a hyphen is added (e.g., *Qgpc6A-2* stands for the second QTL for GPC was detected on chromosome 6A).

QTLs detected through unconditional and conditional QTL mapping methods are called unconditional and conditional QTLs, respectively, in the present paper.

2.3.2 Result and Analysis

2.3.2.1 Phenotypic Data and Correlations

All of the evaluated traits exhibited approximately continuous variation in each of the environments (Tables 2.10 and 2.11). Transgressive segregation was observed on both the high and low sides for GPC and GSC in the three populations, indicating that alleles with positive effects were contributed from both parents.

Table 2.10 Phenotypic data of GPC from the three populations and the parents in different environments in common wheat

Population	Env. ^a	Parent		Mean ^b	S.D	Min.	Max.	Skewness	Kurtosis
		Huapei 3	Yumai 57						
DH Population (n = 168)	E1	13.88	14.25	14.81	1.08	12.72	18.62	0.753	0.94
	E3	14.26	15.35	14.82	1.07	12.63	18.62	0.623	0.922
	E4	13.06	13.68	14.04	0.93	11.71	17.28	0.731	1.218
		Nuomai 1	Gaocheng 8901						
RIL1 Population (n = 256)	E1	17.45	15.65	15.64	1.18	11.84	19.89	0.613	1.023
	E2	16.53	14.92	15	0.99	11.58	17.88	−0.177	1.125
	E3	17.56	16.11	16.1	0.95	13.44	20.03	0.467	1.049
	E4	14.95	14.18	15.04	0.97	10.45	17.7	−0.32	1.537
		Shannong 01-35	Gaocheng 9411						
RIL2 Population (n = 182)	E1	16.36	14.84	16.09	1.34	11.17	18.91	−0.26	0.612
	E2	15.78	14.11	15.02	0.99	12.49	17.69	−0.034	−0.141
	E3	15.46	14.4	15.07	1.06	12.8	18.02	0.277	−0.395
	E4	15.55	14.39	15.07	0.93	12.52	17.84	0.203	−0.155

^aE1: 2010–2011 Tai’an; E2: 2010–2011 Suzhou; E3: 2011–2012 Tai’an; E4: 2011–2012 Jiyuan

^bMean value is the each population mean in different environment

Table 2.11 Phenotypic data of GSC from the three populations and the parents in different environments in common wheat

Population	Env. ^a	Parent		Mean ^b	S.D	Min.	Max.	Skewness	Kurtosis
		Huapei3	Yumai57						
DH Population (n = 168)	E1	80.5	79.96	79.56	0.81	77.11	81.56	−0.622	0.273
	E3	80	79.56	79.71	0.93	76.94	82.16	−0.44	0.676
	E4	80.82	80.51	80.03	0.8	76.93	81.78	−1.065	1.879
		Nuomai1	Gaocheng8901						
RIL1 Population (n = 256)	E1	78.24	76.88	77.04	1.02	73.94	79.77	−0.112	−0.064
	E2	78.8	76.5	77	0.9	74.66	79.36	0.129	−0.228
	E3	78.53	77.67	78.31	0.86	75.65	80.44	−0.151	−0.105
	E4	80.82	78.12	78.94	0.8	76.82	80.91	−0.037	−0.307
		Shannong01-35	Gaocheng9411						
RIL1 Population (n = 182)	E1	78.22	78.87	78.35	0.86	76.36	80.28	−0.272	−0.637
	E2	78.72	78.73	79.1	0.76	77.16	80.91	−0.008	−0.214
	E3	78.73	78.42	78.56	0.74	76.78	80.36	0.014	−0.578
	E4	78.86	79.29	78.92	0.76	76.7	81.08	−0.207	0.266

^a and ^b are the same as for Table 2.10

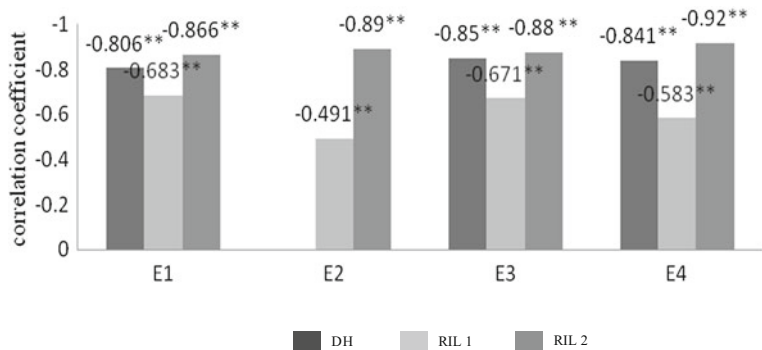


Fig. 2.2 Correlation analysis between grain protein content and starch content in different environments in common wheat. *E1* 2010–2011 Tai’an; *E2* 2010–2011 Suzhou; *E3* 2011–2012 Tai’an; *E4* 2011–2012 Jiyuan

Significant negative correlation coefficients were observed between GPC and GSC in the three populations under four environments (Fig. 2.2).

2.3.2.2 QTL Mapping of GPC and GSC in the Three Populations

A total of 13 unconditional additive QTLs were detected for GPC in the three populations (Table 2.12). They were mainly distributed on 11 chromosomes (2A,

3A, 7A, 1B, 2B, 3B, 4B, 5B, 2D, 1D and 5D). Of these QTLs, there was one additive QTL of PVE (Percentage of phenotypic variance explanation) (10.51 %) that appeared at higher than 10 %. There were also 13 conditional additive QTLs being distributed on 10 wheat chromosomes (3A, 5A, 7A, 1B, 3B, 4B, 5B, 1D, 2D and 5D), but their effects were minor.

For GSC, 7 unconditional additive QTLs (on 1B, 1D, 2B, 4A, 5B, 7B and 7D chromosomes) and 9 conditional QTLs (on 1A, 1B, 1D, 3A, 3D, 4A, 7A, 7B and 7D chromosomes) in the three populations were detected, respectively. Of these QTLs, there were two major unconditional additive QTLs and two major conditional additive QTLs.

2.3.2.2.1 Unconditional and Conditional Additive QTLs of GPC

In the DH population, two unconditional QTLs, *Qgpc2A-12* and *Qgpc6D-3*, were identified explaining 10.51 and 1.95 % of the phenotypic variance, respectively (Table 2.12). The two positive loci were derived from Huapei 3.

By removing the influence of GSC, we were able to identify three QTLs (Table 2.12). Of these QTLs, one QTL, *Qgpc5D-12*, would not be detected with the unconditional analysis, indicating that this QTL was very likely repressed by GSC. The two unconditional QTLs, *Qgpc2A-12* and *Qgpc6D-3*, were also detected when GPC were conditioned on GSC, and their additive effects were very similar, suggesting that these two QTLs were independent of conditional GSC.

In RIL1 population (Table 2.12), unconditional mapping analyses resulted in identifications of five additive QTLs for GPC, four of which expressed positive additive effects resulting from Nuomai 1, whereas the remaining one locus showed negative additive effects contributed by the other parent, Gaocheng 8901.

Only one unconditional additive locus, *Qgpc1D-15*, could be detected when GPC was conditioned on GSC, but its additive effect as conditional QTL was less than that of unconditional QTL (Table 2.12). The other four unconditional QTLs were not identified when the GSC's role was excluded, indicating these loci were fully dependent of GSC. Additionally, other four new conditional QTLs were discovered after excluding the influence of GSC, suggesting that these four QTLs were likely suppressed by GSC.

In RIL2 population, the use of unconditional mapping identified six QTLs, and most of these positive loci were derived from Shannong 01-35 (Table 2.12). Only one locus, *Qpc3A-38*, was derived from Gaocheng 9411.

By removing the influence of GSC, we were able to identify one QTL, named *Qgpc1D-13*, which would not be detected with the unconditional analysis, indicating this locus was repressed by GSC. The four unconditional QTLs, *Qgpc2D-23*, *Qgpc2D-31*, *Qgpc4B-7* and *Qgpc5B-19*, could also be detected when GPC were conditioned on GSC (Table 2.12). The additive effect of unconditional *Qgpc4B-7* was larger than that of conditional *Qgpc4B-7*, indicating the locus was partially affected by GSC. The other three loci showed very likely similar additive effect under both unconditional and conditional QTL analysis, suggesting that the

expression of these three QTLs were completely associated with GPC. Therefore, the three QTLs were considered to be independent of GSC. While the unconditional QTLs, named *Qgpc5B-38* and *Qgpc7A-14*, were not found when conditioned on GSC, which indicated the expression of these two QTLs were completely associated with GSC.

Table 2.12 Additive effects of unconditional and conditional QTLs for GPC and GSC in common wheat

Population	Trait ^a	QTL	Chromosome	Marker Interval	position	A	h^2 (a)%
DH Population (n = 168)	Unconditional QTL						
	GPC	<i>Qgpc6D-3</i>	2A	<i>Xbarc353-Xbarc296</i>	69.6	0.35	10.51
			6D	<i>Xcfd42-Xcfd13</i>	35	0.15	1.95
	GSC	<i>Qgsc3A-4</i>					
			3A	<i>Xbarc86-Xwmc21</i>	86.5	0.32	11.36
	Conditional QTL						
	GPC GSC	<i>Qgpc2A-12</i>	2A	<i>Xbarc353-Xbarc296</i>	69.6	0.33	9.52
		<i>Qgpc5D-12</i>	5D	<i>Xbarc345-Xgdm63</i>	92.7	-0.14	1.59
		<i>Qgpc6D-3</i>	6D	<i>Xcfd42-Xcfd13</i>	35	0.15	1.97
	Unconditional QTL						
RIL1 Population (n = 256)	GPC	<i>Qgpc1B-5</i>	1B	<i>wPt4515- wPt3465</i>	108.2	0.13	1.37
		<i>Qgpc1D-15</i>	1D	<i>wPt666414- wPt3738</i>	186.7	-0.30	7.18
		<i>Qgpc2A-1</i>	2A	<i>wPt0408- wPt2838</i>	0	0.16	2.06
		<i>Qgpc2B-7</i>	2B	<i>wPt0694- wPt0473</i>	32.8	0.52	0.84
		<i>Qgpc3B-1</i>	3B	<i>Xgpcw7148-wPt1940</i>	0	0.14	1.58
	GSC	<i>Qgsc1B-5</i>	1B	<i>wPt4515-wPt3465</i>	125.2	-0.25	5.88
		<i>Qgsc1D-1</i>	1D	<i>Xcfg183-wPt729773</i>	0	0.21	4.45
		<i>Qgsc4A-9</i>	4A	<i>wPt664948-wPt0105</i>	102.5	0.14	1.86
		<i>Qgsc5B-8</i>	5B	<i>wPt3457-wPt6105</i>	121	-0.10	0.9
		<i>Qgsc7B-15</i>	7B	<i>wPt5463-wPt669158</i>	159.8	0.42	20.79
		<i>Qgsc7D1-2</i>	7D1	<i>wPt731416-wPt667560</i>	1	-0.11	1.24
	Conditional QTL						
	GPC GSC	<i>Qgpc1D-1</i>	1D	<i>Xcfg183-wPt729773</i>	13	0.27	8.78
		<i>Qgpc1D-15</i>	1D	<i>wPt666414-wPt3738</i>	180.7	-0.19	4.24
		<i>Qgpc3B-4</i>	3B	<i>wPt6047-wPt1625</i>	33	0.10	1.3
		<i>Qgpc4A-13</i>	4A	<i>wPt7280-wPt671707</i>	106.3	0.17	3.5
		<i>Qgpc7A-6</i>	7A	<i>wPt5590-wPt2780</i>	132.5	0.11	1.43
	GSC GPC	<i>Qgsc1A-31</i>	1A	<i>wPt4916-wPt731357</i>	237.2	0.14	2.87
		<i>Qgsc1D-1</i>	1D	<i>Xcfg183-wPt729773</i>	4	0.29	11.62
		<i>Qgsc4A-9</i>	4A	<i>wPt664948-wPt0105</i>	99.5	0.22	6.78
		<i>Qgsc7A-4</i>	7A	<i>wPt6872-wPt0744</i>	107.4	0.11	1.7
		<i>Qgsc7B-15</i>	7B	<i>wPt5463-wPt669158</i>	151.8	0.41	21.15
		<i>Qgsc7D1-2</i>	7D1	<i>wPt731416-wPt667560</i>	1	-0.13	2.37

(continued)

Table 2.12 (continued)

Population	Trait ^a	QTL	Chromosome	Marker Interval	position	A	h^2 (a)%
RIL2 Population (<i>n</i> = 182)	Unconditional						
	GPC	<i>Qgpc2D-23</i>	2D	<i>wPt4413-wPt667294</i>	102.7	0.19	1.7
		<i>Qgpc2D-31</i>	2D	<i>wPt2644-wPt664520</i>	183.4	0.18	4.17
		<i>Qgpc4B-7</i>	4B	<i>wPt7569-wPt3908</i>	95.1	0.44	7.86
		<i>Qgpc5B-19</i>	5B	<i>wPt3503-Xcfe186</i>	117.3	0.28	8.98
		<i>Qgpc5B-38</i>	5B	<i>wPt664746-wPt1370</i>	158.9	-0.16	0.89
		<i>Qgpc7A-14</i>	7A	<i>wPt3883-wPt7785</i>	188.4	0.20	1.63
	Conditional						
	GPC GSC	<i>Qgpc1D-13</i>	1D	<i>wPt6963-wPt667287</i>	98.9	-0.20	3.39
		<i>Qgpc2D-23</i>	2D	<i>wPt4413-wPt667294</i>	103.7	0.25	2.45
		<i>Qgpc2D-31</i>	2D	<i>wPt2644-wPt664520</i>	183.4	0.13	4.28
		<i>Qgpc4B-7</i>	4B	<i>wPt7569-wPt3908</i>	96.1	0.30	6.09
		<i>Qgpc5B-19</i>	5B	<i>wPt3503-Xcfe186</i>	117.3	0.23	5.85
	GSC GPC	<i>Qgsc1B-9</i>	1B	<i>Xgpcw2067-Xcfe063</i>	67.5	-1.05	0.68
		<i>Qgsc3A-36</i>	3A	<i>wPt729826-wPt666853</i>	214.3	1.11	1.14
		<i>Qgsc3D-4</i>	3D	<i>wPt5313-Xgpcw7646</i>	52.3	-1.63	0.44

^aGPC grain protein content; GSC grain starch content; GPC|GSC, GPC conditioned on GSC; GSC|GPC, GSC conditioned on GPC

2.3.2.2.2 Unconditional and Conditional Additive QTLs of GSC

In the DH population (Table 2.12), only one unconditional QTL, *Qgsc3A-4*, was detected explaining 11.36 % of the phenotypic variance. No QTL was detected when GSC was conditioned on GPC.

In RIL1 population, six additive QTLs were identified for GSC, distributing on 1B, 1D, 2A, 5B and 7D1 chromosomes (Table 2.12). Of these QTLs, one major QTL, designated *Qgsc7B-15*, was found explaining 20.75 % of the phenotypic variance. Three loci, named *Qgsc1D-1*, *Qgsc4A-9* and *Qgsc7B-15*, were derived from Nuomai 1, while the residual three loci were derived from Gaocheng 8901.

When conditioned on GPC, six conditional additive QTLs were detected (Table 2.12). Of which, two major QTLs, *Qgsc1D-1* and *Qgsc7B-15*, were found explaining 11.62 and 21.51 % of the phenotypic variance, respectively. There were four QTLs detected both in unconditional and conditional QTL mapping. By comparing the difference in the effects of unconditional QTLs and conditional QTLs, there were two QTLs, named *Qgsc7B-15* and *Qgsc7D1-2*, to be independent from GPC because these two QTLs had the very similar effects. While the other two QTLs, *Qgsc1D-1* and *Qgsc4A-9* were partly affected by GPC because of large variation in additive effects. In addition, two new conditional QTLs, *Qgsc1A-31* and *Qgsc7A-4*, were found by removing the effect of GPC, indicating these two QTLs were suppressed by GPC. However, the unconditional QTL, *Qgsc5B-8*, was not detected when conditioned on GPC, indicating this QTL was completely contributed by GPC.

Table 2.13 Phenotypic values of two parents and the DH population averaged across three environments

Trait	Parent		The DH population ($n = 168$)					
	Huapei3	Yumai57	Min.	Max.	Mean	S.D.	skewness	kurtosis
SD	22.83	33.17	16.75	53	30	6.75	0.53	-0.08
Protein	10.89	10.39	9.76	13.16	11.27	0.64	0.42	0.06
Starch	80.88	82.05	75.82	82.31	79.53	1.39	-0.45	-0.23
Fat	1.13	1.16	0.82	1.36	1.099	0.09	0.03	0.32
FN	303.3	417.3	192	555.67	78.18	392.6	-0.08	-0.37
GC	37.9	30.4	27.3	46.7	36.71	2.93	-0.02	0.25
DC	12.58	10.0	12.5	40.2	24.03	4.61	0.49	0.30
GI	47.5	83.6	37	100	65.34	10.74	0.22	-0.04

SD Sedimentation volume (ml); *Protein* Protein content (%); *Starch* starch content (%); *Fat* Fat content (%); *FN* Falling number (s); *GC* Wet gluten content (%); *DC* Dry gluten content (%); *GI* Gluten index; *Min.* minimum, *Max.* maximum; *S.D.* standard deviation

Table 2.14 Correlation analyses between SD and the other seven quality traits using the average data of three environments

Trait	Correlations						
	Protein	Starch	Fat	FN	GC	DC	GI
Starch	-0.810**						
Fat	-0.032	0.038					
FN	-0.080	0.043	0.141				
GC	0.905**	-0.728**	0.053	-0.132			
DC	0.570**	-0.479**	0.067	-0.147	0.575**		
GI	0.217**	-0.195*	0.074	-0.103	0.181**	0.897**	
SD	0.378**	-0.408**	0.170**	0.295**	0.252**	0.047	-0.065

FN, GC, DC, GI and SD and are as shown in Table 2.13; * and ** Correlations are significant at the 0.05 and 0.01 level, respectively

In RIL2 population, there was no QTL identified by unconditional mapping. When conditioned on GPC, three new QTLs distributing on 1B, 3A and 3D were found, indicating these QTLs were suppressed by GPC, that is, only removing the effect of GPC, they would be detected (Table 2.12).

2.3.3 Comparison of the Results with Ones of Previous Studies

Compared with previous reports, several chromosomes were the same for GPC. But for starch content, the QTLs were located on 1A, 1D, 2A, 2D, 7A, 7B, and 7D chromosomes (Sun et al. 2008; McCartney et al. 2006), while we detected QTLs for

grain starch content on 1B, 1D, 3A, 3D, 4A, 5B, 7A, 7B, and 7D chromosomes by unconditional and conditional QTL mapping. By comparing these chromosomes, there were some important critical loci controlling starch amylase synthesis on 4A, 7A and 7D chromosomes detected in the present research. Seven QTLs were identified for GPC, that is, *Qgpc2A-12*, *Qgpc6D-3*, *Qgpc1D-15*, *Qgpc2D-23*, *Qgpc2D-31*, *Qgpc5B-19*, and *Qgpc4B-7*, which did not have much effect on grain starch content. Although most of these QTLs have minor additive effects, GPC can be possibly increased by pyramiding these QTLs through marker-assisted breeding. Four QTLs were found for grain starch content, named *Qgsc1D-1*, *Qgsc4A-9*, *Qgsc7B-15*, and *Qgsc7D1-2*, which did not have much influence on grain protein content. The major QTL *Qgsc7B-15* can directly be used for improving the grain starch content in molecular marker-assisted breeding. QTLs detected in the present study could help in simultaneous improvement of grain protein and starch content in wheat.

2.4 Conditional QTL Mapping for Sedimentation Values on Seven Quality Traits in Common Wheat

Zeleny sedimentation values has been useful not only in detecting the gluten strength of the flour but also in estimating the quality of the wheat as food and an ingredient in cooking (Mesdag 1964; Dexter and Matsuo 1980; Dick and Quick 1983; He et al. 2004; Ozturk et al. 2008). In addition, the sedimentation value was found to be a good predictor of the viscoelasticity of cooked pasta discs (Kovacs et al. 1995). However, sedimentation volume (SD) is a quantitative trait.

In wheat research, a few studies have analyzed QTL for SD (Blanco et al. 1998; Kunert et al. 2007; Sun et al. 2008; Patil et al. 2009; Zhao et al. 2009). Two major QTL for SD were mapped on the long arms of chromosome 1A and 1D, respectively, around *Glu-A1* and *Glu-D1*. Four QTLs and three QTLs for SD were identified in two advanced backcross populations (BC₂F₃) (Kunert et al. 2007) and in a RIL population derived from Chuan 35050 and Shannong 483 cross (Sun et al. 2008), respectively.

Although the gluten strength and protein content of flour are important determinants of SD, other components of flour, such as starch, fat, and the interactions between them, also affect the SD. The previously identified QTLs belonged to the class of unconditional QTLs. These loci could not reflect interactions among flour components that influenced SD. Therefore, our objective was to evaluate the genetic influence of variations in protein, starch, fat, falling number (FN), gluten content (GC), dry gluten content (DC), and gluten index (GI) on SD. SD may be genetically correlated with other secondary traits. If, for example, the secondary trait is protein content, the conditioning of SD on protein content would allow for the analysis of SD independently of variations in protein content. The same approach would apply to other secondary traits. By comparing unconditional and

conditional QTLs for SD, the genetic interdependencies between SD and the other seven related traits listed above can be identified at the level of the individual QTL. This comparison might provide valuable information for marker-assisted selection to improve SD without negative effects on protein, starch, fat, FN, GC, DC, or GI.

2.4.1 *Materials and Methods*

2.4.1.1 Plant Materials and Genetic Map

Materials were same as ones of 2.1.1.1 in this chapter.

The genetic linkage map used for mapping contained 368 markers, which were 44 markers more mapped than the previous map (Zhao et al. 2009; Zhao et al. 2010). The map covered a total length of 3074.1 cM with an average distance of 8.35 cM between adjacent markers. These linked markers formed 24 linkage groups over 21 chromosomes (Li et al. 2012a).

2.4.1.2 Field Trials and Trait Evaluation

The field trials were conducted at Tai'an, Shandong province, China, in 2005 (E1) and 2006 (E2) and at Suzhou, Anhui Province, China, in 2006 (E3). The details of these three environments were described by Zhao et al. (2010).

Data were collected on seven quality traits. The SD was determined using AACC method 56-61A.

The flour protein content was determined by near-infrared reflectance (NIR) using AACC approved method 39-25 (2004). The crude starch content of the flour was detected with an automatic polarimeter (WZZ-2B) according to Chinese method GB 5006-85. The crude fat content was measured with a semi-automatic fat detector (Foss Tector Soxtec Avanti 2055) using the AACC 30-10 method. FN was determined with a fungal-type falling instrument (FN1500 type) according to Chinese method GB/T10361. GC, DC and GI were tested with a gluten instrument (Perten 2200 type) according to AACC38-11.

2.4.1.3 Data Analysis and QTL Mapping

Analysis of variance (ANOVA) was performed using SPSS version 13.0 software (SPSS, Chicago, USA). Conditional phenotypic values $y_{hk(T1|T2)}$ were obtained with 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 $SD|protein$ = SD conditioned on flour protein content). The phenotypic variances were calculated from the trait means over three environments. Both unconditional QTL and conditional QTL were detected with the mixed linear model

using QTL Network 2.0 software (Yang and Zhu 2005). A QTL was considered to have been detected if the phenotype was associated with a marker locus at $P < 0.005$. Data on conditional phenotypic values were obtained by first using QGA Station 1.0 software (Zhu 1995) and then by conducting QTL mapping with QTL Network 2.0.

QTL were designated according to recommended international nomenclature for QTL in wheat (McIntosh et al. 1994).

2.4.2 Result and Analysis

2.4.2.1 Evaluation of Quality Traits and Correlations with SD

The DH population was evaluated for SD, protein content, starch content, fat content, FN, GC, DC and GI. Mean, maximal and minimal values were calculated from the averages over three environments of these traits in the parents and the DH population (Table 2.13). ‘Yumai 57’ showed higher values than ‘Huapei 3’ for SD, starch content, fat content, FN and GI. Strong transgressive segregations towards the higher and lower sides were observed for all traits. These results indicated that both parents contributed to the trait values. The absolute values of skewness and kurtosis, both less than 1.0 for all the traits indicated that the DH population segregation values approximately followed normal distributions. The correlations between SD and the other five quality traits were shown to be highly significant (Table 2.14). SD was positively correlated with protein content, fat content, FN, GC and DC, but was negatively correlated with starch content and GI. Protein content showed a significantly negative correlation with starch content.

2.4.2.2 Unconditional and Conditional QTLs with Additive Effects

In all, ten additive QTLs affecting SD were detected on chromosomes 1B (2), 1D (1), 2B (2), 3A (1), 4B (1), 5A (1), and 5D (2) (Table 2.15 and Fig. 2.3). Unconditional mapping identified six QTLs for SD, with significant additive effects ranging in absolute size from 0.68 to 1.93 %. Together, these QTLs explained 55.42 % of the variance of the phenotypic trait. Three major QTLs, designated *QSD.sdau-1B.1*, *QSD.sdau-1B.2* and *QSD.sdau-1D*, were found explaining 16.98, 18.4 and 10.82 % of the phenotypic variance, respectively. The number of conditional QTLs controlling SD conditioned on the protein, starch, fat, FN, GC, DC and GI traits (seven quality traits), were 4 (1B, 1D and 5D), 4 (1B, 1D and 5D), 7 (1B, 1D, 2B, 3A, 5A and 5D), 6 (1B, 1D, 2B, 4B, 5A and 5D), 4 (1B, 1D and 5D), 6 (1B, 1D, 2B, 3A and 5D) and 4 (1B, 1D and 5D), respectively. By comparing the difference in the effects of unconditional QTLs and conditional QTLs (Table 2.15 and Fig. 2.3), one can determine whether the QTLs for SD were associated with the related quality traits. There were two QTLs, named *QSD.sdau-1B.1* and *QSD.sdau-1D*, to be independent

Table 2.15 Unconditional and conditional QTLs with significant additive effects for SD

Trait	Unconditional QTL	Flanking markers ^a	Position (cM)	Range (cM)	A ^b	P-value	$h^2(a)$ (%) ^c
SD	<i>QSD.sdau-1B.1</i>	<i>XBARC312-XCFE023.1</i>	36.1	34.9–36.1	−1.29 ^d	0	16.98
	<i>QSD.sdau-1B.2</i>	<i>XGWM582-XGPW7388</i>	49.7	45.7–53.7	−1.93	0	18.40
	<i>QSD.sduu-1D</i>	<i>XWMC93-GLUD1</i>	61.9	59.9–61.9	−1.92	0	10.82
	<i>QSD.sduu-3A.2</i>	<i>XWMC21-XWMC664</i>	121	115.4–121.5	−0.68	0.004	3.88
	<i>QSD.sduu-5A</i>	<i>XBARC358.2-XGWM186</i>	47.3	45.8–55.3	1.35 ^e	0	2.03
	<i>QSD.sduu-5D.1</i>	<i>XCFD101-XBARC320</i>	59.5	55.5–67.2	−1.45	0	3.31
	Total ^f						55.42
	Conditional QTL						
SD protein	<i>QSD.sdau-1B.1</i>	<i>XBARC312-XCFE023.1</i>	36.1	35.2–36.1	−1.36	0	18.00
	<i>QSD.sdau-1B.2</i>	<i>XGWM582-XGPW7388</i>	50.7	46.7–53.7	−1.38	0	20.38
	<i>QSD.sduu-1D</i>	<i>XWMC93-GLUD1</i>	61.9	59.9–61.9	−2.32	0	16.66
	<i>QSD.sduu-5D.1</i>	<i>XCFD101-XBARC320</i>	56.5	49.5–62.2	−0.60	0.016	0.54
	Total						55.58
SD starch	<i>QSD.sdau-1B.1</i>	<i>XBARC312-XCFE023.1</i>	36.1	35.2–36.1	−1.48	0	16.62
	<i>QSD.sdau-1B.2</i>	<i>XGWM582-XGPW7388</i>	50.7	46.7–54.7	−1.55	0	17.81
	<i>QSD.sduu-1D</i>	<i>XWMC93-GLUD1</i>	61.9	59.9–61.9	−2.06	0	13.22
	<i>QSD.sduu-5D.1</i>	<i>XCFD101-XBARC320</i>	59.5	54.5–68.2	−0.73	0.004	1.02
	Total						48.67
SD fat	<i>QSD.sdau-1B.1</i>	<i>XBARC312-XCFE023.1</i>	36.1	34.9–36.1	−1.67	0	19.38
	<i>QSD.sdau-1B.2</i>	<i>XGWM582-XGPW7388</i>	49.7	45.7–53.7	−1.58	0	20.55
	<i>QSD.sduu-1D</i>	<i>XWMC93-GLUD1</i>	61.9	59.9–61.9	−2.06	0	13.13
	<i>QSD.sduu-2B.2</i>	<i>XBARC373-XBARC1114</i>	108.1	104.2–111.1	0.55	0.035	1.91
	<i>QSD.sduu-3A.2</i>	<i>XWMC21-XWMC664</i>	121	115.4–121.5	−0.88	0	4.66
	<i>QSD.sduu-5A</i>	<i>XBARC358.2-XGWM186</i>	47.3	45.8–56.3	1.15	0	2.13
	<i>QSD.sduu-5D.1</i>	<i>XCFD101-XBARC320</i>	59.5	55.5–67.2	−1.61	0	3.79
	Total						65.55
SD FN	<i>QSD.sdau-1B.1</i>	<i>XWMC412.2-XCFE023.2</i>	36.1	36.1–37.0	−2.66	0	18.12
	<i>QSD.sduu-1D</i>	<i>XWMC93-GLUD1</i>	61.9	59.9–61.9	−2.19	0	13.08
	<i>QSD.sduu-2B.1</i>	<i>XGPW2228-XWMC179</i>	94.7	84.3–100.7	0.72	0.004	1.30
	<i>QSD.sduu-4B</i>	<i>XWMC125-XWMC47</i>	1	0.0–5.2	−1.23	0	4.70
	<i>QSD.sduu-5A</i>	<i>XBARC358.2-XGWM186</i>	50.3	45.8–59.3	0.79	0.002	1.80
	<i>QSD.sduu-5D.2</i>	<i>XBARC320-XWMC215</i>	64.2	57.5–68.2	−1.14	0	1.61
	Total						40.61
SD GC	<i>QSD.sdau-1B.1</i>	<i>XBARC312-XCFE023.1</i>	36.1	35.2–36.1	−1.36	0	19.63
	<i>QSD.sdau-1B.2</i>	<i>XGWM582-XGPW7388</i>	50.7	46.7–53.7	−1.80	0	22.04
	<i>QSD.sduu-1D</i>	<i>XWMC93-GLUD1</i>	61.9	59.9–61.9	−2.28	0	17.29
	<i>QSD.sduu-5D.2</i>	<i>XBARC320-XWMC215</i>	65.2	55.5–68.2	−1.63	0	1.50
	Total						60.46
SD DC	<i>QSD.sdau-1B.1</i>	<i>XBARC312-XCFE023.1</i>	36.1	34.9–36.1	−1.39	0	18.02
	<i>QSD.sdau-1B.2</i>	<i>XGWM582-XGPW7388</i>	49.7	45.7–53.7	−1.80	0	20.09
	<i>QSD.sduu-1D</i>	<i>XWMC93-GLUD1</i>	61.9	59.9–61.9	−2.02	0	13.08
	<i>QSD.sduu-2B.2</i>	<i>XBARC373-XBARC1114</i>	109.1	104.2–111.1	0.79	0.002	1.29
	<i>QSD.sduu-3A.2</i>	<i>XWMC21-XWMC664</i>	121	115.4–123.8	−1.05	0	3.99
	<i>QSD.sduu-5D.1</i>	<i>XCFD101-XBARC320</i>	59.5	55.5–68.2	−1.50	0	3.12
	Total						59.59
SD GI	<i>QSD.sdau-1B.1</i>	<i>XBARC312-XCFE023.1</i>	36.1	35.2–36.1	−1.41	0	15.59

(continued)

Table 2.15 (continued)

Trait	Unconditional QTL	Flanking markers ^a	Position (cM)	Range (cM)	A ^b	P-value	h ² (a)(%) ^c
	<i>QSD.sdau-1B.2</i>	<i>XGWM582-XGPW7388</i>	50.7	46.7–53.7	–2.19	0	17.43
	<i>QSD.sduu-1D</i>	<i>XWMC93-GLUD1</i>	61.9	59.9–61.9	–1.63	0	11.29
	<i>QSD.sduu-5D.1</i>	<i>XCfD101-XBARC320</i>	59.5	55.5–67.2	–1.92	0	4.58
	<i>Total</i>						48.89

^aFlanking marker, means the interval of QTL; ^bA additive effects; ^ch²(a)%, phenotypic variation explained (PVE) by a effects; ^dA negative value indicate that the allele from Yumai57; ^ea positive value indicates that the allele from Huapei3; SD, FN, GC, DC and GI are as shown in Table 2.13; SD|protein, SD|starch, SD|fat, SD|FN, SD|GC, SD|DC and SD|GI indicates SD conditioned on protein, starch, fat, FN, GC, DC and GI respectively

^fTotal phenotypic variance explained by the additive effects of the mapped QTL

** P < 0.01 and ***P < 0.001, respectively

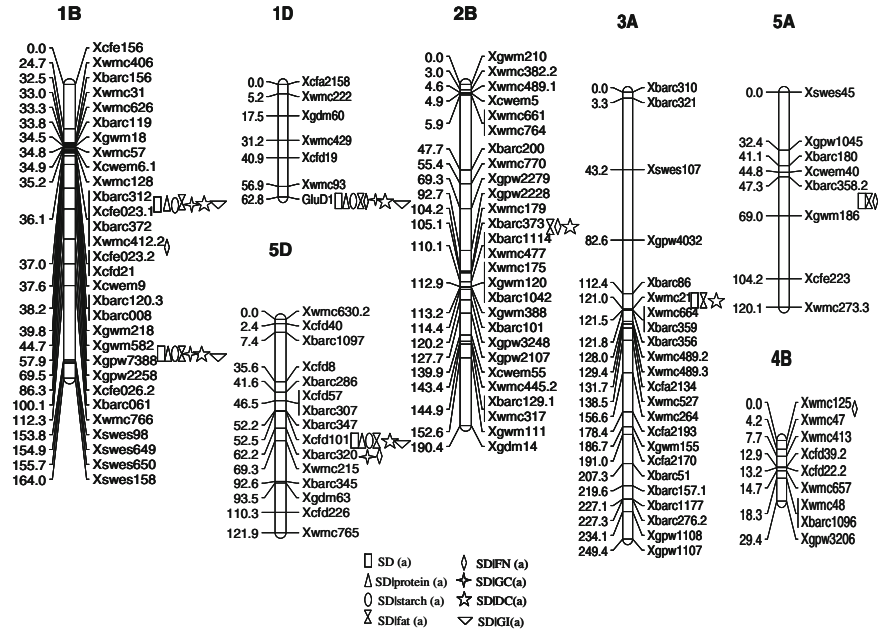


Fig. 2.3 Significant additive QTLs for SD mapped in the DH population based on unconditional and conditional composite interval mapping. a Additive effects; SD Sedimentation volume; FN Falling number; GC Wet Gluten content; DC Dry gluten content; GI Gluten index; SD|protein, SD|starch, SD|fat, SD|FN, SD|GC, SD|DC and SD|GI indicates SD conditioned on protein, starch, fat, FN, GC, DC and GI, respectively

from these seven traits because these two QTLs were detected under both unconditional and conditional QTL analysis with the similar effects. Therefore, these two QTLs were genetically very important for SD. *QSD.sdau-1B.2* was detected in unconditional and conditional QTL except for FN, indicating this QTL was completely contributed by FN to SD. *QSD.sdau-5D.1* was contributed by FN and GC,

while *QSD.sdau-5D.2* was suppressed by them. *QSD.sdau-5A* controlling SD was not detected if it was conditioned on the seven quality traits except for fat content and FN, and *QSD.sdau-3A.2* not detected except for fat content and DC. These results suggested that the expression of these two QTLs were completely associated with the variation of other five traits. Therefore, the two QTLs were considered to be independent of these three traits, i.e., they were mainly influenced by other five traits. In contrast, the QTL of *QSD.sdau-2B.2* was not unconditionally detected, but it appeared if it was conditioned on the fat content and DC. This finding indicated that the QTL was co-suppressed by the two traits. The two QTLs, *QSD.sdau-2B.1* and *QSD.sdau-4B* were detected when conditioned on FN trait, indicating they were suppressed by the FN. These results indicated their effects could only be revealed if the influence of the variation in these traits was removed.

When SD was conditioned on the seven traits, these QTLs explained 55.58, 48.67, 65.55, 40.61, 60.46, 59.59 and 48.89 % of the variance of the phenotypic traits, respectively. These values were similar to the 55.42 % detected for the unconditioned SD. Three QTLs, *QSD.sdau-1B.1*, *QSD.sdau-1B.2* and *QSD.sdau-1D*, considered major loci because of their ability to explain over 10 % of the phenotypic variance, were found in unconditional and conditional QTL. In addition, the interactions between the additive effects of QTLs and environments were detected by QTL mapping for SD, but they were all not significant.

2.4.2.3 Unconditional and Conditional QTLs with Epistatic Effects

The genetic relationship between SD and the seven quality traits was also detected by the mapping of locus pairs with additive $\times$ additive epistatic effects (Table 2.16). Fifteen loci pairs were identified in unconditional and conditional mapping. The three locus pairs, *QSD.sdau-1B.1/QSD.sdau-5A*, *QSD.sdau-2B.2/QSD.sdau-3A.1* and *QSD.sdau-5D.2/QSD.sdau-6B*, were only unconditionally detected, which indicated they were affected by the seven traits. The *QSD.sdau-1B.1/QSD.sdau-1B.2* locus pair was all detected when conditioning on protein, starch, fat, GC, and DC, but their effects showed different with the unconditional effect of SD indicating that the locus pair was partially affected by this five traits. The *QSD.sdau-1B.2/QSD.sdau-2B.2* locus pair was found if both SD were conditioned on fat content and DC. The same pair was also found with unconditional mapping. However, the epistatic effects were significantly different in the two cases. These results indicated that this locus pair partially contributed to fat content and DC. The *QSD.sdau-5D.1/QSD.sdau-7B* locus pair was unconditionally detected, and also found when conditioned on starch content. This indicated this locus pair was mainly influenced by other six traits for SD. There was one interesting locus pair, *QSD.sdau-1B.1/QSD.sdau-1B.2*, not detected unconditionally but found when conditioned on protein content, starch content, fat content, GC and DC. Their epistatic effects were similar, indicating it was co-suppressed by the five traits. The *QSD.sdau-1B.1/QSD.sdau-5D.4* locus pair was co-suppressed by the FN and GC. Only by removing them could the effects of the two pairs be expressed. In addition, there was still some new locus pairs found

Table 2.16 Unconditional and conditional QTLs with estimated additive × additive epistatic effects (aa) of locus pairs for SD

Trait	QTLi	Flanking markers	QTLj	Flanking markers	AA ^a	P-Value	<i>h</i> ² (aa) ^b (%)
SD	<i>QSD.sduu-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sduu-1B.2</i>	<i>XGWM582-XGPW7388</i>	-1.90 ^c	0	1.49
	<i>QSD.sduu-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sduu-5A</i>	<i>XBARC358.2-XGWM186</i>	-0.66	0.004588	0.09
	<i>QSD.sduu-1B.2</i>	<i>XGWM582-XGPW7388</i>	<i>QSD.sduu-2B.2</i>	<i>XBARC373-XBARC1114</i>	1.22 ^d	0.000016	0.84
	<i>QSD.sduu-2B.2</i>	<i>XBARC373-XBARC1114</i>	<i>QSD.sduu-3A.1</i>	<i>XBARC310-XBARC321</i>	0.93	0.00017	0.80
	<i>QSD.sduu-5D.1</i>	<i>XCFD101-XBARC320</i>	<i>QSD.sduu-7B</i>	<i>XGPW2260-XBARC72</i>	1.27	0.000001	1.05
	<i>QSD.sduu-5D.2</i>	<i>XBARC345-XGDM63</i>	<i>QSD.sduu-6B</i>	<i>XCFD48-XWMC415</i>	1.16	0.000001	1.07
	Total ^e						5.34
	Conditional QTL						
SD protein	<i>QSD.sduu-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sduu-1B.2</i>	<i>XGWM582-XGPW7388</i>	-1.64	0	2.19
	<i>QSD.sduu-1B.2</i>	<i>XGWM582-XGPW7388</i>	<i>QSD.sduu-2D</i>	<i>XWMC658.1-XGDM93</i>	1.50	0	4.09
	<i>QSD.sduu-5D.3</i>	<i>XWMC215-XBARC345</i>	<i>QSD.sduu-7B</i>	<i>XGPW2260-XBARC72</i>	-1.28	0.000026	2.99
SD starch							9.27
	<i>QSD.sduu-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sduu-1B.2</i>	<i>XGWM582-XGPW7388</i>	-1.52	0	2.16
	<i>QSD.sduu-3A.3</i>	<i>XGWM155-XCFA2170</i>	<i>QSD.sduu-4A</i>	<i>XBARC343-XWMC313</i>	1.15	0.000001	3.08
	<i>QSD.sduu-5D.1</i>	<i>XCFD101-XBARC320</i>	<i>QSD.sduu-7B</i>	<i>XGPW2260-XBARC72</i>	-0.94	0.00019	1.58
SD fat							6.82
	<i>QSD.sduu-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sduu-1B.2</i>	<i>XGWM582-XGPW7388</i>	-1.7236	0	1.34
	<i>QSD.sduu-1B.2</i>	<i>XGWM582-XGPW7388</i>	<i>QSD.sduu-2B.2</i>	<i>XBARC373-XBARC1114</i>	1.0742	0.000299	1.02
SD FN							2.36
	<i>QSD.sduu-1B.1</i>	<i>XWMC412.2-XCFE023.2</i>	<i>QSD.sduu-5D.4</i>	<i>XBARC320-XWMC215</i>	0.84	0.000659	0.92
	<i>QSD.sduu-1D</i>	<i>XWMC93-GLUD1</i>	<i>QSD.sduu-4B</i>	<i>XWMC125-XWMC47</i>	-0.81	0.000908	0.80
	<i>QSD.sduu-2D</i>	<i>XCFD53-XWMC18</i>	<i>QSD.sduu-3D</i>	<i>XBARC071-XGWM114</i>	-1.37	0	2.02
							3.74

(continued)

Table 2.16 (continued)

Trait	QTLi	Flanking markers	QTLj	Flanking markers	AA ^a	P-Value	$h^2(aa)^b$ (%)
SD GC	<i>QSD.sdaui-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sdaui-1B.2</i>	<i>XGWM582-XGPW7388</i>	-1.59	0	1.46
	<i>QSD.sdaui-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sdaui-5D.4</i>	<i>XBARC320-XWMC215</i>	0.69	0.009194	0.91 %
							2.37
SD DC	<i>QSD.sdaui-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sdaui-1B.2</i>	<i>XGWM582-XGPW7388</i>	-1.93	0	1.49
	<i>QSD.sdaui-1B.2</i>	<i>XGWM582-XGPW7388</i>	<i>QSD.sdaui-2B.2</i>	<i>XBARC373-XBARC1114</i>	0.83	0.003609	0.77
	<i>QSD.sdaui-2A.2</i>	<i>XGPW7218-XBARC264</i>	<i>QSD.sdaui-3A.4</i>	<i>XCFA2134-XWMC527</i>	-1.50	0	4.25
							6.51
							2.14
SD GI	<i>QSD.sdaui-2A.1</i>	<i>XBARC380-XGWM636</i>	<i>QSD.sdaui-4B</i>	<i>XWMC657-XWMC48</i>	-1.17	0.00003	2.14
	<i>QSD.sdaui-2B.3</i>	<i>XWMC477-XWMC175</i>	<i>QSD.sdaui-3A</i>	<i>XBARC310-XBARC321</i>	1.29	0	3.17
							5.31
Trait	QTLi	Flanking markers	QTLj	Flanking markers	AA ^a	P-Value	$h^2(aa)^b$ (%)
SD	<i>QSD.sdaui-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sdaui-1B.2</i>	<i>XGWM582-XGPW7388</i>	-1.90 ^c	0	1.49
	<i>QSD.sdaui-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sdaui-5A</i>	<i>XBARC358.2-XGWM186</i>	-0.66	0.004588	0.09
	<i>QSD.sdaui-1B.2</i>	<i>XGWM582-XGPW7388</i>	<i>QSD.sdaui-2B.2</i>	<i>XBARC373-XBARC1114</i>	1.22 ^d	0.000016	0.84
	<i>QSD.sdaui-2B.2</i>	<i>XBARC373-XBARC1114</i>	<i>QSD.sdaui-3A.1</i>	<i>XBARC310-XBARC321</i>	0.93	0.00017	0.80
	<i>QSD.sdaui-5D.1</i>	<i>XCFLD101-XBARC320</i>	<i>QSD.sdaui-7B</i>	<i>XGPW2260-XBARC72</i>	1.27	0.000001	1.05
	<i>QSD.sdaui-5D.2</i>	<i>XBARC345-XGDM63</i>	<i>QSD.sdaui-6B</i>	<i>XCFLD48-XWMC415</i>	1.16	0.000001	1.07
	Total ^e						5.34
	Conditional QTL						
SD protein	<i>QSD.sdaui-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sdaui-1B.2</i>	<i>XGWM582-XGPW7388</i>	-1.64	0	2.19
	<i>QSD.sdaui-1B.2</i>	<i>XGWM582-XGPW7388</i>	<i>QSD.sdaui-2D</i>	<i>XWMC658.1-XGDM93</i>	1.50	0	4.09
	<i>QSD.sdaui-5D.3</i>	<i>XWMC215-XBARC345</i>	<i>QSD.sdaui-7B</i>	<i>XGPW2260-XBARC72</i>	-1.28	0.000026	2.99
							9.27

(continued)

Table 2.16 (continued)

Trait	QTLi	Flanking markers	QTLj	Flanking markers	AA ^a	P-Value	$h^2(aa)^b$ (%)
SD starch	<i>QSD.sduu-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sduu-1B.2</i>	<i>XGWM582-XGPW7388</i>	-1.52	0	2.16
	<i>QSD.sduu-3A.3</i>	<i>XGWM155-XCFA2170</i>	<i>QSD.sduu-4A</i>	<i>XBARC343-XWMC313</i>	1.15	0.000001	3.08
	<i>QSD.sduu-5D.1</i>	<i>XCFA101-XBARC320</i>	<i>QSD.sduu-7B</i>	<i>XGPW2260-XBARC72</i>	-0.94	0.00019	1.58
							6.82
SD fat	<i>QSD.sduu-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sduu-1B.2</i>	<i>XGWM582-XGPW7388</i>	-1.7236	0	1.34
	<i>QSD.sduu-1B.2</i>	<i>XGWM582-XGPW7388</i>	<i>QSD.sduu-2B.2</i>	<i>XBARC373-XBARC1114</i>	1.0742	0.000299	1.02
							2.36
							0.92
SD FN	<i>QSD.sduu-1B.1</i>	<i>XWMC412.2-XCFE023.2</i>	<i>QSD.sduu-5D.4</i>	<i>XBARC320-XWMC215</i>	0.84	0.000659	0.92
	<i>QSD.sduu-1D</i>	<i>XWMC93-GLUD1</i>	<i>QSD.sduu-4B</i>	<i>XWMC125-XWMC47</i>	-0.81	0.000908	0.80
	<i>QSD.sduu-2D</i>	<i>XCFA53-XWMC18</i>	<i>QSD.sduu-3D</i>	<i>XBARC071-XGWM114</i>	-1.37	0	2.02
							3.74
SD GC	<i>QSD.sduu-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sduu-1B.2</i>	<i>XGWM582-XGPW7388</i>	-1.59	0	1.46
	<i>QSD.sduu-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sduu-5D.4</i>	<i>XBARC320-XWMC215</i>	0.69	0.009194	0.91 %
							2.37
							1.49 %
SD DC	<i>QSD.sduu-1B.1</i>	<i>XBARC312-XCFE023.1</i>	<i>QSD.sduu-1B.2</i>	<i>XGWM582-XGPW7388</i>	-1.93	0	1.49 %
	<i>QSD.sduu-1B.2</i>	<i>XGWM582-XGPW7388</i>	<i>QSD.sduu-2B.2</i>	<i>XBARC373-XBARC1114</i>	0.83	0.003609	0.77 %
	<i>QSD.sduu-2A.2</i>	<i>XGPW7218-XBARC264</i>	<i>QSD.sduu-3A.4</i>	<i>XCFA2134-XWMC527</i>	-1.50	0	4.25 %
							6.51
SD GI	<i>QSD.sduu-2A.1</i>	<i>XBARC380-XGWM636</i>	<i>QSD.sduu-4B</i>	<i>XWMC657-XWMC48</i>	-1.17	0.00003	2.14 %
	<i>QSD.sduu-2B.3</i>	<i>XWMC477-XWMC175</i>	<i>QSD.sduu-3A</i>	<i>XBARC310-XBARC321</i>	1.29	0	3.17 %
							5.31

^aaa effects, the epistatic effect; ^b $h^2(aa, \%)$ phenotypic variation explained (PVE) by epistatic effects QTL; ^ca negative value that recombinant allele combinations increase phenotypic values; ^da positive value of the epistatic effect indicates that parental allele combinations, SD, FN, SD|protein, SD|fat, SD|FN, SD|GC, SD|DC and SD|GI are as shown in Table 3; ^eTotal phenotypic variance explained by the epistatic effects of the mapped QTL

* $P < 0.05$ and *** $P < 0.001$, respectively

when conditioned on the seven traits besides formerly just mentioned. Although the interactions between QTL pairs and environments were detected, they were all not significant.

The fraction of the total variance in the traits explained by the epistatic effects of the conditional and the unconditional SD mappings of the locus pairs differed greatly, but they belonged to minor QTLs.

2.4.3 Comparison of the Results with the Previous Studies

In this research, two additive QTL loci close to the glutenin loci were found by both unconditional and conditional QTL mapping on 1B chromosome, which indicated this chromosome could be important for sedimentation volume. These results were similar to the previous research under unconditional QTL mapping (Patil et al. 2009; Li et al. 2012a, b). There were also two important chromosomes, 1D and 5D. By unconditional mapping, two QTLs (*QZsv.sdau-1D.1* and *QZsv.sdau-1D.2*) were mapped on 1D chromosome, which was close to the Glu-D1 locus. This result was the same as the reported (Sun et al. 2008; Li et al. 2009, 2012a, b). For sedimentation volume, the additive effect is more important than the epistatic effect. Three QTLs controlling sedimentation volume by both unconditional and conditional QTL mapping can be used in MAS.

2.5 Genetic Analysis of Wheat Four Whiteness Conducted Through Conditional and Unconditional QTL Mappings

Flour color is one of the most important factors in assessing the flour quality and determining the end products quality. For most of consumers, white flour lacking the major pigmentation is favored, and sometimes the flour is bleached with the additives such as bezoyl peroxide in order to increase the flour whiteness (FW). In fact, the FW is a sensory integrated indicator with comprehensive reflection of flour color. Different final products perhaps need different FW. For example, the Chinese and Japanese alkaline noodles prefer the flour with high levels of yellow pigmentation, but the higher FW is desirable for the Chinese steamed bread and baked bread (He et al. 2004). Moreover the FW may be affected by grain and flour quality traits (Zhang et al. 2008a), such as grain protein content, grain hardness, etc. The negative correlations have been observed between the FW and protein content (Li et al. 2012a, c). The FW from low protein content wheat is higher than that from high protein content wheat at the same flour milling extraction ratio. Because the FW is an important quality trait for many end-use products, besides its testing value, the most widely used assay for flour color measures the FW with colorimetric

measurements based on L^* (brightness), a^* (greenness or red-green chromaticity) and b^* (yellowness). For a pure white flour, the L^* should be one hundred, and a^* and b^* should be zero. The flour with lower L^* and higher a^* value is undesirable because it showed gray and dull color.

Although extensive QTL analysis for flour color had been researched, less information is available about the genetic interrelationship between FW and flour protein content, flour color, and flour ash content at QTL/gene level, especially why FW had the significant negative correlation with flour protein content and ash content, and how to dissect the genetic relationship between them. However, no studies have investigated about their relationship from QTL/gene level.

So our objective was to dissect the genetic relationship between FW and flour protein content, flour color 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^* .

2.5.1 Materials and Methods

2.5.1.1 Plant Materials

The RIL population of 256 lines was developed from a cross between the two winter wheat cultivars Nuomai 1 (female) (NM1) and Gaocheng8901 (male) (GC8901) by a single-seed descent to the F_{10} generation. NM1 (Jiangsu Baihuomai/Guandong107) with three null waxy alleles (Wx-A1b, Wx-B1b, and Wx-D1b), was bred by China Agricultural University and released in 2005 in Beijing. It is similar to red winter wheat. Moreover, this cultivar has unique starch properties that are related to high-quality white salt noodles. GC8901 (77546-2/Linzhang) with normal waxy alleles, was bred by Gaocheng Agricultural Science Research Institute and was released in 1998 in Hebei province. It has high gluten strength and good bread-making qualities.

2.5.1.2 Field Trials

The RIL genetic population along with their corresponding parents, were grown in three distinct locations for the 2009–2010, 2010–2011 and 2011–2012 growing seasons. E1 represents Tai'an, Shandong Province (36°12'N, 117°04'E), China, in 2009–2010 growing season; E2 refers to Suzhou, Anhui Province (33°63'N, 116° 97'E), China, in 2010–2011 growing season; E3 stands for Jiyuan, Henan Province (35°05'N, 112°36'E), China, in 2011–2012 growing season.

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 with 2.3 m long and 26 cm row-to-row distance, whereas that in E2 was a six-row plot with 4 m long and 25 cm row-to-row distance and that in E3 was a three row plot with 2.6 m long and 26 cm row-to-row distance.

Fertilization was performed before planting and at jointing stages in all experimental locations (described in Deng et al. 2015).

Water irrigation was carried out at each stage of pre-overwintering, jointing, and grain filling in all experimental locations. All recommended local crop management practices were followed, and damages attributed to lodging, disease, or pests were not observed during the growing seasons.

2.5.1.3 Methods

Flour milled and flour color measurements (L^* , a^* and b^*) were carried out according to the Zhang et al.'s description (2009).

FW was determined by WSB-IV intelligent whiteness instrument (Dajiguangdian Instruments, Hangzhou, China).

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

Flour ash content in E1 and E3 was also detected by NIRS on a Perten DA-7200 instrument (Perten Instruments, Huddinge, Sweden).

2.5.1.4 Data Analysis and QTL Mapping

Statistical analyses (e.g., normal distribution and correlation) were performed using the software SPSS 17.0 (SPSS, Chicago, USA) and Excel 2010.

Conditional genetic analysis was conducted based on the phenotypic values of flour whiteness conditioned on flour protein content, flour color (L^* , a^* and b^*) and flour ash content, which were obtained by the mixed-model approach (Zhu 1995; Wen and 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 QGASStation 1.0 (<http://ibi.zju.edu.cn/software/qga/>) was used to determine the conditional phenotypic values $y_{(T1|T2)}$ as input data for conditional QTL mapping, which used the composite interval mapping method.

Unconditional and conditional QTL mappings were applied firstly using the software ICIMapping 4.0 (Wang et al. 2014; <http://www.isbreeding.net>) for the data in each environment, and then using the QTLNetwork version 2.0 (<http://ibi.zju.edu.cn/software/qtlnetwork/>) based on the mixed linear model to analyze the

interaction between QTL and environments for the data in three environments by the constructed genetic maps (Zheng et al. 2013). When using the ICIMapping 4.0 software, the walking speed for all of the QTL was 1.0 cM; an LOD score of 2.5 was used for declaring the presence of a putative QTL, and the threshold LOD scores for detection of a QTL were calculated based on 1000 permutations. While for QTL Network 2.0 software, 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 window size set at 10 cM. Significant thresholds for QTL detection were calculated for each data set using 1,000 permutations and a genome-wide error rate of 0.10 (suggestive) and 0.05 (significant). The final genetic model incorporated significant additive and epistatic effects as well as their environmental interactions. A QTL was declared if the phenotype was associated with a marker locus at $P < 0.005$.

2.5.2 Result and Analysis

2.5.2.1 Phenotypic Data and Correlations Analysis

Approximately continuous variation was exhibited for the flour whiteness (FW) in each of the environments (Table 2.17). Transgressive segregation was observed on both the high and low sides for FW in the RIL population, indicating that the alleles with positive effects were contributed from both parents.

Table 2.17 Phenotypic data of flour whiteness from the RIL population and the parents in different environments

Population	Env. ^a		E1	E2	E3	Three environments
	N	Valid	231	244	241	716
		Missing	25	12	15	52
Parents	GC8901		77.3	75.3	74	75.53
	NM1		82.2	80.4	80.3	80.97
RIL	Mean ^b		78.37	76.99	75.68	76.99
	SE		0.223	0.218	0.206	0.131
	SD		3.384	3.401	3.202	3.500
	Variance		11.448	11.569	10.255	12.253
	Skewness		0.002	0.025	0.074	0.066
	Kurtosis		−1.284	−1.39	−1.239	−1.113
	Range		14	12.55	12.4	15.25
	Minimum		71	70.95	69.6	69.6
	Maximum		85	83.5	82	84.85

^aE1: 2009–2010 Tai'an; E2: 2010–2011 Suzhou; E3: 2011–2012 Jiyuan

^bMean value is the RIL population mean in different environment

Table 2.18 Correlation analysis between flour whiteness and its related traits

CC ^b	Flour whiteness		
	^a E1	E2	E3
Pr	−0.326**	−0.315**	−0.272**
<i>L</i> *	0.936**	0.941**	0.930**
<i>a</i> *	0.088	−0.025	0.130*
<i>b</i> *	−0.73**	−0.965**	−0.912**
Ash	−0.245**		−0.286**

^aE1: 2009–2010 Tai'an; E2: 2010–2011 Suzhou; E3: 2011–2012 Jiyuan

^bCC correlation coefficients

Significant negative correlation was observed between FW and flour protein in the RIL population under the three environments (Table 2.18). So did between FW and flour ash. While there was significant positive correlation coefficients between FW and flour *L** in all three environments, but in E2 and E3, significant negative correlation coefficients were observed between FW and flour *b**.

2.5.2.2 Additive Effects of Unconditional and Conditional QTL Mapping of Flour Whiteness

Total seven unconditional additive QTLs were found in each and all three environments (Tables 2.19 and 2.20). Of which, the unconditional additive QTL *Qfw1D-1* with flanking markers cfd183 and wPt729773 was detected in E1, E2 and E3 with explaining from 61.78 to 71.22 % of the phenotypic variance, which indicated this major QTL was a stable QTL and almost not affected by environments. By analyzing the interaction between QTL and environments (Table 2.20), this additive QTL was also identified explaining 35.79 % of the phenotypic variance with no interaction with the environments. Each other six unconditional additive QTLs was detected only in the single environment with the minor PVE smaller than 10 %. In addition, the *Qfw4A-4* found in E2 (Table 2.19) perhaps was the same as the *Qfw4A-23* (Table 2.20) because they had the same flanking marker wPt7821.

By removing the influence of flour protein, total five conditional additive QTLs were found in E1, E2 and E3 (Table 2.19) with explaining the 3.13–58.97 % of the phenotypic variation, and there were two conditional QTLs (Table 2.20) in the three environments with the explanation of 2.77 and 32.54 %, respectively. Of these QTLs, the conditional major QTL *Qfw1D-1* was detected in all environments. Compared with the unconditional QTL *Qfw1D-1*, their additive effects were very similar, suggesting that this major QTL was independent of conditional flour protein content. So did the QTL *Qfw2B-4* in E2. The other three conditional QTLs, *Qfw4A-1*, *Qfw4A-2* and *Qfw1B*, would not be detected with the unconditional analysis, indicating that this QTL was very likely repressed by flour protein content. In addition, the two unconditional QTLs, *Qfw4A-23* and *Qfw6A2-1* (Table 2.20),

Table 2.19 Additive effects of unconditional and conditional QTLs of flour whiteness in each environment

Trait	Env. ^a	QTL	Position (cM)	Marker interval	LOD	Add ^b	PVE (%)
<i>Unconditional QTL</i>							
FW	E1	<i>Qfw1D-1</i>	7	cf1183-wPt729773	29.10	2.60	65.08
	E2	<i>Qfw1D-1</i>	7	cf1183-wPt729773	35.16	2.80	71.22
		<i>Qfw2B-4</i>	87	wPt0047-wPt1454	2.62	-0.59	2.97
		<i>Qfw4A-4</i>	139	wPt3349-wPt7821	3.03	0.62	3.49
	E3	<i>Qfw1D-1</i>	7	cf1183-wPt729773	32.71	2.44	61.78
		<i>Qfw2A-1</i>	46	wPt2838-wPt668181	2.74	0.65	4.26
		<i>Qfw6A2</i>	67	wPt8124-wPt731120	3.23	0.57	3.40
<i>Conditional QTL</i>							
FW Pr	E1	<i>Qfw1D-1</i>	7	cf1183-wPt729773	25.61	2.01	47.76
		<i>Qfw4A-1</i>	109	Wx-B1-wPt0105	2.70	0.58	4.05
	E2	<i>Qfw1D-1</i>	7	cf1183-wPt729773	27.46	2.41	58.97
		<i>Qfw2B-4</i>	87	wPt0047-wPt1454	2.81	-0.59	3.38
		<i>Qfw4A-2</i>	128	wPt671707-wPt730913	3.57	0.66	4.35
	E3	<i>Qfw1B</i>	226	wPt7273-wPt3566	2.51	0.51	3.13
		<i>Qfw1D-1</i>	7	cf1183-wPt729773	21.84	2.12	53.11
	E1	<i>Qfw1D-1</i>	7	cf1183-wPt729773	5.55	0.29	8.51
		<i>Qfw2A-3</i>	311	wPt9277-wPt3281	3.04	-0.25	5.92
FW L*	E2	<i>Qfw1A1</i>	144	wPt665613-wPt3904	4.81	-0.30	7.19
		<i>Qfw3B</i>	162	wPt666008-wPt5870	3.34	1.67	13.27
		<i>Qfw4A-3</i>	152	wPt2951-wPt672107	3.03	0.23	4.43
	E3	<i>Qfw1A1-18</i>	138	wPt6654-wPt2872	3.20	-0.32	8.30

(continued)

Table 2.19 (continued)

Trait	Env. ^a	QTL	Position (cM)	Marker interval	LOD	Add ^b	PVE (%)
FW a*	E1	<i>Qfw1D-1</i>	7	cf1183-wPt729773	24.92	2.13	47.10
	E2	<i>Qfw1D-1</i>	7	cf1183-wPt729773	34.49	2.73	68.47
	E3	<i>Qfw1D-1</i>	7	cf1183-wPt729773	27.98	2.44	65.63
FW b*	E1	<i>Qfw1D-1</i>	7	cf1183-wPt729773	25.14	2.15	48.54
	E2	<i>Qfw2B-1</i>	33	wPt0694-wPt0473	20.57	-0.57	43.01
		<i>Qfw2B-2</i>	36	wPt1068-wPt7004	29.28	0.69	61.15
		<i>Qfw3B</i>	162	wPt666008-wPt5870	12.36	3.65	27.83
	E3	<i>Qfw1A2</i>	256	wPt4916-wPt731357	2.68	0.25	3.99
		<i>Qfw1D-1</i>	7	cf1183-wPt729773	4.65	0.38	9.29
		<i>Qfw2A-1</i>	46	wPt2838-wPt668181	2.77	0.26	4.10
		<i>Qfw2B-3</i>	0	wPt6576-wPt4199	3.67	0.30	5.38
		<i>Qfw3B</i>	162	wPt666008-wPt5870	3.18	-1.87	16.16
		<i>Qfw7D1</i>	3	wPt667098-wPt664056	2.62	-0.25	4.02
FW Ash	E1	<i>Qfw1B-1</i>	47	wPt8226-wPt0605	2.72	-0.81	7.40
		<i>Qfw1D-1</i>	7	cf1183-wPt729773	22.45	1.84	38.73
		<i>Qfw2A-2</i>	253	wPt2273-wPt2994	2.73	0.78	2.93
	E3	<i>Qfw1B-1</i>	47	wPt8226-wPt0605	2.71	-0.92	9.91
		<i>Qfw1D-1</i>	7	cf1183-wPt729773	19.69	2.12	52.92

^aE1: 2009-2010 Tai'an; E2: 2010-2011 Suzhou; E3: 2011-2012 Jiyuan
^bAdditive effect, positive values indicate that positive effect alleles are derived from NMI, whereas negative values indicate that the negative effect alleles are contributed by GC8901

Table 2.20 Additive effects (A) and additive × environment (AE) interactions of unconditional and conditional QTLs of flour whiteness

Trait	QTL	Marker interval	Position (cM)	A ^a	P-Value	H ² (%) ^b	AE effect ^c		AE3	P-value	H2(%)
Unconditional QTL											
FW	<i>Qfw1D-1</i>	cfd183-wPr729773	7.0	1.98****	0.00	35.79					
	<i>Qfw4A-23</i>	wPr7821-wPr5212	139.7	0.38****	0.00	1.40					
	<i>Qfw6A2-1</i>	Xgpn4085-wPr667562	34.0	1.16****	0.00	1.91					
Conditional QTL											
FW Pr	<i>Qfw1D-1</i>	cfd183-wPr729773	7.0	1.82****	0.00	32.54					
	<i>Qfw4A-1</i>	Wx-B1-wPr0105	109	0.50****	0.00	2.77					
	<i>Qfw1A1-18</i>	wPr6654-wPr2872	138	-0.28****	0.00	5.42					
FW L *	<i>Qfw1D-1</i>	cfd183-wPr729773	7.0	0.09	0.10	0.64	0.28***	0.00	-0.25*	0.00	1.57
	<i>Qfw7B-1</i>	wPr665293-wPr4038	0.0	-0.18****	0.00	2.99					
	<i>Qfw1D-1</i>	cfd183-wPr729773	7.0	2.02****	0.00	36.66					
FW a*	<i>Qfw4A-23</i>	wPr7821-wPr5212	139.7	0.44****	0.00	1.32					
	<i>Qfw6A2-1</i>	Xgpn4085-wPr667562	34.0	0.85****	0.00	1.80					
	<i>Qfw7B-18</i>	wPr0276-wPr6936	235.8	0.40****	0.00	3.30					
FW b*	<i>Qfw1D-1</i>	cfd183-wPr729773	7.0	2.05****	0.00	36.42					
	<i>Qfw4A-23</i>	wPr7821-wPr5212	139.7	0.43****	0.00	1.24					
	<i>Qfw7B-18</i>	wPr0276-wPr6936	234.8	0.38****	0.00	3.08					
FW Ash	<i>Qfw1B-1</i>	wPr8226-wPr0605	47	-1.05****	0.00	2.90					
	<i>Qfw1D-1</i>	cfd183-wPr729773	7.0	1.83****	0.00	33.21					

^aadditive effects, positive values indicate that positive effect alleles are derived from NM1, whereas negative values indicate that the negative effect alleles are contributed by GC8901; ^bcontribution explained by filtrated putative additive QTL; ^cthe interaction additive effect between QTL and environment; * significance at $P < 0.05$; *** significance at $P < 0.00$; **** significance at $P < 0.00$

were not identified when conditioned on flour protein, suggesting that the expression of these two loci was associated with flour protein content.

When FW was conditioned on flour L^* , we could also detect the unconditional QTL *Qfw1D-1* (Tables 2.19 and 2.20), but there was significant difference between the conditional additive effects and unconditional additive effects, indicating the locus was partially affected by flour L^* . There were the residual six conditional QTLs on 1A, 2A, 3B, 4A and 7B chromosomes, which would not be detected with the unconditional analysis, indicating these loci were repressed by flour L^* .

When the influence of flour a^* on flour whiteness was considered, the conditional QTL *Qfw1D-1* was detected in E1, E2, E3 and all three environments (Tables 2.19 and 2.20), which was also identified with unconditional analysis. Their additive effects of unconditional and conditional QTLs were very similar, suggesting that this loci was independent of conditional flour a^* . In Table 2.20, the QTL *Qfw4A-23* was also independent of conditional flour a^* , but the conditional QTL *Qfw6A2-1* was partially affected by flour a^* because of the significant difference of additive effects under unconditional and conditional mapping. The QTL, *Qfw7B-18*, would not be detected with the unconditional analysis, indicating that this QTL was very likely repressed by flour a^* .

The unconditional QTLs *Qfw1D-1* and *Qfw4A-23* could also be detected when flour whiteness were conditioned on flour b^* (Table 2.20). The additive effects of unconditional *Qfw1D-1* and *Qfw4A-23* were very similar to that of conditional *Qfw1D-1* and *Qfw4A-23*, suggesting that the expression of these two QTLs were completely associated with flour whiteness. Therefore, the two QTLs were considered to be independent of flour b^* . The conditional QTL *Qfw7B-18* was not detected with unconditional analysis, suggesting that the expression of this locus was repressed by flour b^* .

By excluding the influence of flour ash, three QTLs were able to be identified (Table 2.19 and Table 2.20). Of which, one QTL, *Qfw1D-1*, was also detected with the unconditional analysis, but their additive effects showed largely different, indicating that this QTL was partially affected by flour ash. The two unconditional QTLs, *Qfw4A-23* and *Qfw6A2-1*, were not identified when the flour ash's role was excluded, suggesting that the expression of these two loci was associated with flour ash. The conditional QTL *Qfw1B-1* was not found under the unconditional analysis, indicating that this QTL was very likely suppressed by flour ash content.

2.6 Epistatic Effects of Unconditional and Conditional QTL Mapping of Flour Whiteness

Unconditional mapping detected five pairs of epistatic QTLs that were located on chromosomes 1A, 2A, 3A, 3B, 5A, 6B and 7D (Table 2.21). Contributions of these epistatic QTLs ranged from 1.89 to 4.01 %. The epistatic effect of each QTL

Table 2.21 Epistasis effects (AA) and epistasis $\times$ environment (AAE) interactions of unconditional and conditional QTLs of flour whiteness

Trait	QTL _u _j	Marker interval _u _j	Position _u _j (cM)	QTL _c _j	Marker interval _c _j	Position _c _j (cM)	AA ^a	P-value	H ² (%) ^b
<i>Unconditional QTL</i>									
FW	<i>Qfw1A2-4</i>	wPt5167-wPt667814	4.2	<i>Qfw5A-1</i>	wPt3334-wPt1903	0.0	0.38	0.00	1.89
	<i>Qfw1A2-30</i>	wPt7001-wPt4916	205.3	<i>Qfw7D2-13</i>	wPt664252-wPt663849	40.9	-0.80	0.00	3.55
	<i>Qfw2A-5</i>	wPt1657-wPt664251	135.2	<i>Qfw3B-11</i>	wPt0544-wPt9432	40.6	-0.59	0.00	3.87
	<i>Qfw3A-10</i>	wPt7341-wPt8855	66.2	<i>Qfw6B-8</i>	wPt3733-wPt2218	54.0	0.56	0.00	4.01
	<i>Qfw3A-10</i>	wPt7341-wPt8855	66.2	<i>Qfw6B-6</i>	wPt3060-wPt664174	33.5	0.36	0.00	2.61
<i>Conditional QTL</i>									
FW Pr	<i>Qfw1A2-8</i>	wPt2847-wPt4709	17.8	<i>Qfw5A-2</i>	wPt1903-wPt3069	0.0	0.66	0.00	2.34
	<i>Qfw1D-5</i>	Glu-D1-wPt3743	109.4	<i>Qfw5A-3</i>	wPt3069-wPt3462	51.2	0.83	0.00	3.45
	<i>Qfw2A-22</i>	wPt2273-wPt2994	253.9	<i>Qfw3A-15</i>	wPt8876-wPt730156	178.5	0.61	0.00	4.04
	<i>Qfw3A-11</i>	wPt8855-wPt2748	70.0	<i>Qfw6B-8</i>	wPt3733-wPt2218	55.0	0.65	0.00	3.48
	<i>Qfw3D-4</i>	wPt671773-wPt666814	107.1	<i>Qfw6A1-12</i>	wPt7027-wPt3524	59.3	-0.39	0.00	2.25
	<i>Qfw1A2-4</i>	wPt5167-wPt667814	4.2	<i>Qfw5A-1</i>	wPt3334-wPt1903	0.0	0.41	0.00	1.86
FW a*	<i>Qfw1D-8</i>	wPt667287-wPt8854	116.0	<i>Qfw5A-3</i>	wPt3069-wPt3462	47.2	0.85	0.00	2.82
	<i>Qfw2A-4</i>	wPt1853-wPt1657	115.9	<i>Qfw3B-10</i>	wPt0446-wPt0544	40.6	-0.99	0.00	2.77
	<i>Qfw3A-10</i>	wPt7341-wPt8855	66.2	<i>Qfw6B-10</i>	wPt9594-wPt7576	65.9	0.56	0.00	3.24
	<i>Qfw3A-10</i>	wPt7341-wPt8855	66.2	<i>Qfw6B-6</i>	wPt3060-wPt664174	33.5	0.32	0.00	2.60
	<i>Qfw1A2-4</i>	wPt5167-wPt667814	4.2	<i>Qfw5A-1</i>	wPt3334-wPt1903	0.0	0.49	0.00	2.00
	<i>Qfw1D-8</i>	wPt667287-wPt8854	115.0	<i>Qfw5A-3</i>	wPt3069-wPt3462	48.2	0.80	0.00	2.71
FW b*	<i>Qfw2A-4</i>	wPt1853-wPt1657	115.9	<i>Qfw3B-10</i>	wPt0446-wPt0544	40.6	-1.04	0.00	3.04
	<i>Qfw3A-10</i>	wPt7341-wPt8855	66.2	<i>Qfw6B-6</i>	wPt3060-wPt664174	33.5	0.74	0.00	3.42

^aEpistasis effects; ^bcontribution explained by filtrated putative epistasis QTL; *, significance at $P < 0.05$; ***, significance at $P < 0.001$; ****, significance at $P < 0.0001$

seemed to be minor, but the total contribution of unconditional epistasis was 15.93 %.

When flour whiteness was conditioned on flour protein, these five pairs of unconditional epistatic QTLs could not be identified, indicating that these epistatic QTLs were entirely contributed by flour protein (Table 2.21). Meanwhile, the new five pairs of epistatic QTLs were detected only by conditional mapping, suggesting that these epistatic loci were inhibited due to the effect of flour protein (Table 2.21). Total contribution of conditional epistasis was 15.56 %.

There were five pairs of epistatic QTLs identified after the influence of flour a* was excluded. Of which, only the conditional epistatic QTLs *Qfw3A-10/Qfw6B-6* could also be observed by unconditional mapping (Table 2.21), its positive effect was very similar compared to the unconditional epistatic effect, indicating that this epistatic QTL was not affected by flour a*. The other four pairs epistatic QTLs were only found by conditional mapping, indicating these loci were repressed by the flour a*. Total contribution of conditional epistasis was 13.29 %.

After removing the influence of flour b*, four pairs of epistatic QTLs were detected (Table 2.21). Although the conditional epistatic QTLs *Qfw3A-10/Qfw6B-6* could also be observed by unconditional mapping, its positive effect was greatly increased compared to the unconditional epistatic effect, indicating that this epistatic QTL was partially contributed by flour b*. The other three pairs epistatic QTLs were only identified by conditional mapping, suggesting they were suppressed by the flour b*. Total contribution of conditional epistasis was 11.17 %.

However, there were no pairs of epistasis QTLs identified when flour whiteness was conditioned on flour L* and flour ash, respectively.

2.6.1 Comparison of the Results with the Previous Studies

In previous studies (Parker et al. 1998; Mares and Campbell 2001; Zhang et al. 2009; Tsilo et al. 2011; Li et al. 2012a, b, c), QTLs mapping of flour whiteness and color were carried out by unconditional mapping method, and most of them were conducted based on the additive-dominant model and usually ignored the epistasis between QTLs. However in this present study, we used the conditional QTL mapping method to analyze the genetic interactions between flour whiteness and its effectors at the single QTL level by additive and epistasis model including the interactions between QTL and environments. The results gave us more information than that of unconditional mapping. By using unconditional mapping, Li et al. (2012a) reported the three QTLs for flour whiteness distributing on 3A, 4A and 5D chromosomes, but their PVE were less than 10 %; while Li et al. (2012c) found four major QTLs distributing on 1D, 3B, 5B and 7D chromosomes, but the QTL on 1D was only detected under one environment. However, in our study, we found only one major QTL on 1D chromosome and it was a stable QTL detected in all environments, which was first time reported and can be used in marker-assisted selection breeding. In the same marker interval, Zheng et al. (2013) also found one

major QTL for mixograph peak value using unconditional QTL mapping. In addition, there were some minor QTLs identified on 2B, 4A, 2A and 6A chromosomes in this research, previous researchers also found some QTLs on them for not only flour whiteness (Li et al. 2012a, c) but also flour color L^* , a^* and b^* (Zhang et al. 2009; Sadeque and Truner 2010; Roncallo et al. 2012). Five pairs epistatic QTLs were found on 1A-5A, 1A-7D, 2A-3B and 3A-6B chromosomes. Of which, there were two epistatic QTLs on 3A-6B chromosome with one same QTL *Qfw3A-10* on 3A chromosome. Li et al. (2012a) also found one epistatic QTL on 1A-7D chromosome, but there was no same result with Zhang et al. (2009) research.

By conditional QTL mapping, several QTLs were revealed with independent, partial and completely associated with flour whiteness. The one major additive QTL, *Qfw1D-1*, was found to be independent from flour protein content and flour color a^* and b^* , and its expression was slightly affected by flour ash content. So this QTL was important for improving the flour whiteness. It was interesting that in the same marker interval (cfd183-wPt729773) 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 the *Qgpc1D-1* for grain protein content was also found in the same marker interval only by conditional mapping, indicating its expression was covered by grain starch content (Deng et al. 2015). These indicated that some important genes with pleiotropic effects not controlling the flour whiteness but also grain starch and protein content would be present in this chromosome segment of the marker interval. In addition, some important additive QTLs for sedimentation volume on 1D chromosome were found by comparing unconditional and conditional QTLs using DH population (Deng et al. 2013).

Moreover there was one minor additive QTL, *Qfw4A-23*, identified under both unconditional and conditional QTL mapping, which was independent from flour color a^* and b^* , but was co-affected by the residual three quality traits. There was one stable minor conditional additive QTL, *Qfw1B-1*, found in each and all environments, which was completely repressed by flour ash content. Another major conditional additive QTL, *Qfw3B*, was identified in E2 and E3 when conditioned on flour color b^* , and it was also detected in E2 by removing the effect of flour color L^* , which indicated that this QTL was co-suppressed by flour color L^* and b^* . Two major conditional additive QTLs on 2B chromosome were found to be repressed by flour color b^* in E2 environment. The remaining minor conditional additive QTLs were regulated by different traits. Perhaps these additive QTLs were trait-specific and might only be suitable in this population.

Only one pair epistatic QTL (*Qfw3A-10/Qfw6B-6*) was identified by both unconditional and conditional QTL mapping. It was independent from flour color a^* , but was partly affected by flour color b^* with greatly increasing the conditional epistatic effect.

Although the interaction between QTL and environment was detected, most of them were not sensitive to environmental factors. Only one additive QTL was sensitive to E1 and E3 environments.

2.7 Conditional QTL Mapping for Wheat Seed Protein-Fraction Contents at Different Developmental Stages

Proteins are the most important wheat grain components governing end-use quality (Weegels et al. 1996). On the basis of their solubility properties, wheat grain proteins are traditionally classified into albumins, globulins, gliadins, and glutenins (Osborne 1907). To improve wheat grain quality in a sustainable manner, and selections of wheat cultivars with enhanced protein-fraction contents are therefore being developed. In the past decade years, QTL analysis has been successfully used to study the levels of various protein-fractions in wheat (Charmet et al. 2005; Zhang et al. 2011). In the cited QTL studies, phenotypic values of protein-fraction contents have usually been measured post-harvest. As a consequence, the detected QTLs could not account for net genetic effects during specific time intervals of plant development, which is an essential component of quantitative trait analysis (Yan et al. 1998; Sun et al. 2006).

In wheat, dynamic QTL analysis has been used to dissect the genetic control of quantitative traits such as plant height (Liu et al. 2011), protein content (Zhu et al. 2011), and antioxidant enzyme activity and malondialdehyde content (Jiang et al. 2013). However, conditional QTL mapping has not been used for the dynamic analysis of QTLs for protein-fraction contents during the wheat grain-filling stage. Therefore, the purpose of this research was to investigate the dynamic diversification of protein-fraction contents during grain development in wheat; to identify the dynamic expression of QTLs associated with protein-fraction contents using conditional and unconditional mapping methods; and to dissect the genetic basis of the dynamic accumulation of protein-fractions in wheat. Our results are a good starting point for understanding the genetic basis of selective expression of QTLs during different grain-filling stages. The detected QTLs may have important contributions to grain quality in wheat.

2.7.1 Materials and Methods

2.7.1.1 Plant Materials

Materials were same as ones of 2.1.1.1 in this chapter.

2.7.1.2 Experimental Design

The 168 DH lines and their parents were sown in 2011 and 2012 (for harvest in 2012 and 2013, respectively) in Baoding (38.85°N, 115.5°E), Xingtai (37.13°N, 114.68°E), and Cangzhou (38.58°N, 116.82°E), China. The experimental design

followed a randomized complete block design with three replications. Plants were grown under normal field conditions in three-row plots (3-m long with between-row spacing of 25 cm), with 100 seeds sown in each row. The date of anthesis was recorded for each material. Six plants of each experimental line, chosen from the middle of the inner row, were harvested at 15 (stage 1), 20 (stage 2), 25 (stage 3), 30 (stage 4) and 35 (stage 5) days after flowering. Ears of the harvested plants were dried for 30 min in an oven at 105°C and then continuously dried at 80°C until the seed mass was stable. The seeds were extracted from the dry ears by hand, milled into wholemeal flour using a Model 3100 hammer mill (Perten Instruments, Hägersten, Sweden), and passed through an 80-mesh sieve.

2.7.1.3 Protein-Fraction Extraction and Content Analysis

Wheat protein-fractions were prepared from wholemeal flour as follows: The milled wheat flour (0.1 g) was sequentially extracted with 50 mM phosphate buffer (pH 6.8) for albumin, the same buffer containing 0.1 M NaCl for globulin, 70 % aqueous ethanol for gliadin, and 0.05 M NaOH for glutenin. The sample was stirred with 10 ml of solvent at room temperature for 30 min. Extracts were separated from residues by centrifugation (4000 rpm for 10 min). The procedure was repeated three times. The extracts were stored in a freezer for further analysis. The protein content of extracts was measured by the semi-micro-Kjeldahl method (Chinese Bureau of Standardization 1982) using a Kjeltec 2100 Auto-analyser (Foss AB, Hägersten, Sweden). A nitrogen conversion factor of 5.7 was used to compute the protein value.

2.7.1.4 Data and QTL Analyses

Basic statistical analysis was carried out using SPSS version 17.0 (SPSS, Chicago, IL, USA). The genetic linkage map used for mapping contained 368 markers, an increase of 45 markers compared with previous maps (Zhao et al. 2010). The map covered a total length of 3074.1 cM, with an average distance of 8.35 cM between adjacent markers. Genomic locations of marker loci/linkage groups were determined based on the wheat consensus map constructed by Somers et al. (2004). Based on the recommendation of Doerge (2002), we used a maximum map distance of 10 cM for genome-wide QTL scanning.

Both unconditional and conditional QTLs were detected under a mixed linear model using IciMapping v3.2 (<http://www.isbreeding.net/>). Composite interval analysis was performed by forward-backward stepwise multiple linear regression, with a probability into and out of the model of 0.05 and a window size of 10 cM. For each data set, significant thresholds for QTL detection were calculated using 1000 permutations and genome-wide error rates of 0.10 (suggestive) and 0.05 (significant). A LOD score of 2.5 was set as a threshold for declaring the presence of a QTL.

For QTL mapping, unconditional and conditional phenotypic values were arranged in the same data file. Unconditional phenotypic values corresponded to the data measured at different stages. Conditional phenotypic values at time t given phenotypic values at time $t - 1$ were predicted by the software program QGA Station v1.0 (<http://ibi.zju.edu.cn/software/qga/>). Unconditional QTLs represent the cumulative effects of QTLs from the initial time to time t , while conditional QTLs correspond to the cumulative effects of QTLs from time $t - 1$ to time t . QTLs were designated using the format “QTL + trait + chromosome” in accordance with international nomenclatural recommendations for QTLs in wheat and related species (McIntosh et al. 1994).

2.7.2 Result and Analysis

2.7.2.1 Phenotypic Variation

Phenotypic values of the content of wheat grain protein-fractions at different developmental periods across six environments are shown in Tables 2.22, 2.23, 2.24 and 2.25, respectively. Although the exact changes in content were slightly different among different years and environmental conditions, the general dynamic trend was identical. Grain albumin content showed a decreasing tendency during the filling process (Table 2.22), whereas globulin content exhibited a “high-low-high” dynamic trend and increased during the final grain-filling stage (Table 2.23). Gliadin and glutenin contents increased during the filling process (Tables 2.24 and 2.25).

With respect to contents during the different filling stages, the parents of the DH population, Huapei 3 and Yumai 57, were markedly different from one another. The DH population displayed a wide range of variation. Frequency distributions of protein-fraction contents showed continuous variation and significant transgressive segregation in both directions, indicating that these traits were under polygenic control and suitable for QTL analysis.

2.7.2.2 QTL Mapping

A total of 74 unconditional QTLs and 58 conditional QTLs for protein-fraction contents was detected on 20 chromosomes except for chromosome 5B (Tables 2.26 and 2.27). Individual QTLs in the six environments explained between 5.84 and 35.02 % of phenotypic variation.

Table 2.22 Statistical analysis of albumin content in parents Huapei 3 (HP3) and Yumai 57 (YM57) and the doubled-haploid (DH) population at different grain-filling stages for 2 years in three locations

E ^a	Stage	Parent		DH population					
		HP3	YM57	Mean	Maximum	Minimum	SD	Skew	Kurtosis
E1	T ₁	5.46	6.48	5.81	8.89	2.47	1.14	−0.19	0.26
	T ₂	3.77	4.01	4.06	6.89	2.04	0.89	0.25	0.12
	T ₃	3.18	4.15	3.72	5.91	1.72	0.72	0.24	0.31
	T ₄	3.74	2.91	2.61	5.43	1.35	0.72	0.58	0.40
	T ₅	2.47	1.74	2.27	4.46	1.15	0.76	0.25	0.13
E2	T ₁	5.01	7.23	5.72	8.50	2.46	1.11	−0.63	1.24
	T ₂	3.57	4.19	4.22	6.93	2.03	0.88	0.29	0.14
	T ₃	3.18	4.68	3.75	5.77	1.78	0.78	−0.23	0.18
	T ₄	3.75	2.92	2.67	5.30	1.38	0.74	0.23	−0.09
	T ₅	2.40	1.76	2.24	4.47	1.15	0.75	−0.63	0.39
E3	T ₁	5.19	6.90	5.43	8.49	2.34	1.16	−0.43	−0.02
	T ₂	3.72	4.06	4.10	6.88	2.00	0.87	0.28	0.42
	T ₃	3.19	4.16	3.45	5.85	1.69	0.75	−0.28	−0.05
	T ₄	3.77	2.97	2.50	5.49	1.37	0.73	0.20	−0.09
	T ₅	2.45	1.71	2.24	4.34	1.15	0.74	0.35	0.13
E4	T ₁	5.61	7.52	6.39	9.05	2.49	1.10	−0.89	0.74
	T ₂	3.71	4.27	4.14	6.93	1.73	1.05	0.43	0.91
	T ₃	3.55	4.68	3.78	6.24	2.25	0.78	0.15	0.09
	T ₄	3.98	3.06	3.04	5.81	1.42	0.80	0.00	0.34
	T ₅	2.87	2.30	2.23	5.01	1.21	1.21	0.63	0.73
E5	T ₁	6.28	7.59	6.64	9.15	2.57	1.06	−0.78	0.80
	T ₂	3.81	4.58	4.11	7.19	1.79	1.04	0.21	0.72
	T ₃	3.40	4.82	3.93	6.39	2.16	0.76	−0.16	0.81
	T ₄	4.04	3.16	3.18	5.98	1.43	0.74	0.01	−0.23
	T ₅	3.15	2.57	2.35	4.67	1.35	1.14	0.69	0.51
E6	T ₁	6.34	7.67	6.57	9.32	2.58	1.08	−0.58	0.46
	T ₂	3.59	4.37	3.89	6.98	1.65	0.98	−0.07	0.93
	T ₃	3.96	4.71	3.71	6.38	1.94	0.91	0.09	0.53
	T ₄	4.51	3.50	2.79	5.85	1.42	0.72	0.03	−0.14
	T ₅	3.25	2.52	2.25	4.84	1.25	0.93	0.48	0.89

^a Environments are as follows: E1: Baoding in 2011; E2: Xingtai in 2011; E3: Cangzhou in 2011; E4: Baoding in 2012; E5: Xingtai in 2012; and E6: Cangzhou in 2012

2.7.2.2.1 Unconditional and Conditional QTLs for Albumin Content

A total of 17 unconditional additive QTLs for albumin content was identified at five filling stages between 2 years at three locations (Table 2.26). These QTLs were mapped to chromosomes 1A, 2A, 2B, 3A, 6A, 7B, and 7D and accounted for 6.66–

Table 2.23 Statistical analysis of globulin content in parents Huapei 3 (HP3) and Yumai 57 (YM57) and the doubled-haploid (DH) population at different grain-filling stages for 2 years in three locations

E ^a	Stage	Parent		DH population					
		HP3	YM57	Mean	Maximum	Minimum	SD	Skew	Kurtosis
E1	T ₁	2.40	2.82	2.49	3.85	1.40	0.42	-0.27	0.83
	T ₂	1.19	1.55	1.18	1.57	0.78	0.20	-0.85	0.96
	T ₃	0.74	1.03	0.96	1.26	0.62	0.11	-0.20	0.58
	T ₄	1.17	1.01	1.07	1.37	0.75	0.10	0.37	-0.33
	T ₅	1.19	1.36	1.19	1.88	0.87	0.21	0.35	0.76
E2	T ₁	2.40	2.98	2.48	4.01	1.39	0.45	-0.27	0.82
	T ₂	1.25	1.57	1.20	1.73	0.77	0.18	0.18	0.00
	T ₃	0.79	1.02	0.98	1.26	0.62	0.11	0.04	0.37
	T ₄	1.22	1.07	1.10	1.33	0.74	0.10	0.11	-0.42
	T ₅	1.19	1.34	1.19	1.78	0.84	0.20	0.20	0.48
E3	T1	2.45	2.83	2.43	3.68	1.29	0.43	-0.44	0.65
	T2	1.23	1.56	1.18	1.57	0.78	0.18	-0.12	-0.29
	T3	0.70	0.99	0.95	1.27	0.61	0.13	-0.42	1.03
	T4	1.16	1.09	1.08	1.34	0.75	0.10	0.01	0.03
	T5	1.19	1.35	1.16	2.15	0.84	0.20	0.48	0.73
E4	T1	3.44	2.92	2.63	4.65	1.72	0.39	0.94	0.87
	T2	1.25	1.70	1.01	1.87	0.85	0.16	-0.34	0.73
	T3	0.74	0.99	0.81	1.25	0.70	0.12	0.34	0.74
	T4	0.98	0.95	0.94	1.49	0.75	0.19	0.24	0.53
	T5	1.26	1.47	1.33	1.81	0.84	0.18	0.15	0.10
E5	T1	3.71	2.91	2.71	4.66	1.68	0.38	0.81	0.81
	T2	1.21	1.67	1.03	1.87	0.86	0.16	-0.39	0.26
	T3	0.77	0.94	0.85	1.24	0.63	0.14	0.85	0.86
	T4	0.96	0.91	0.91	1.44	0.75	0.18	-0.43	1.07
	T5	1.22	1.43	1.37	2.21	0.84	0.22	0.52	0.84
E6	T1	3.83	2.99	2.72	4.50	1.71	0.38	0.46	0.81
	T2	1.23	1.68	1.03	1.87	0.85	0.16	-0.57	0.47
	T3	0.69	0.92	0.84	1.24	0.67	0.12	0.37	0.20
	T4	0.95	0.88	0.93	1.46	0.74	0.16	-0.19	0.46
	T5	1.28	1.50	1.34	1.90	0.86	0.21	-0.08	0.73

^a Environments are as follows: E1: Baoding in 2011; E2: Xingtai in 2011; E3: Cangzhou in 2011; E4: Baoding in 2012; E5: Xingtai in 2012; and E6: Cangzhou in 2012

21.67 % of the phenotypic variation. Among them, *QAlu1B-3* and *QAlu7B-1* were consistently detected at two filling stages. Eight major QTLs (*QAlu1B*, *QAlu1B-1*, *QAlu1B-3*, *QAlu1B-4*, *QAlu6A*, *QAlu6A-1*, *QAlu7B*, and *QAlu7B-1*) were detected and explained more than 10 % of the phenotypic variation, with *QAlu1B-4* having

Table 2.24 Statistical analysis of gliadin content in parents Huapei 3 (HP3) and Yumai 57 (YM57) and the doubled-haploid (DH) population at different grain-filling stages for 2 years in three locations

E ^a	Stage	Parent		DH population					
		HP3	YM57	Mean	Maximum	Minimum	SD	Skew	Kurtosis
E1	T ₁	0.25	0.27	0.23	0.57	0.10	0.07	−0.59	0.20
	T ₂	0.62	0.70	0.54	1.02	0.10	0.18	0.57	0.20
	T ₃	1.07	0.63	0.57	1.48	0.22	0.15	0.35	0.28
	T ₄	1.37	0.98	0.83	1.74	0.35	0.17	−0.01	0.09
	T ₅	3.29	2.14	2.64	7.18	1.51	1.06	0.59	0.83
E2	T ₁	0.28	0.30	0.25	0.51	0.10	0.07	−0.20	1.32
	T ₂	0.62	0.70	0.59	1.12	0.07	0.19	0.69	0.55
	T ₃	1.07	0.64	0.64	1.14	0.27	0.16	0.31	0.70
	T ₄	1.28	0.98	0.95	1.88	0.47	0.17	−0.24	−0.76
	T ₅	3.29	2.83	2.76	6.60	1.56	1.02	0.69	0.57
E3	T ₁	0.25	0.27	0.25	0.54	0.11	0.08	0.10	0.95
	T ₂	0.62	0.69	0.55	1.15	0.06	0.22	0.81	1.12
	T ₃	1.08	0.67	0.66	1.49	0.21	0.21	1.64	1.33
	T ₄	1.33	0.96	0.96	1.83	0.54	0.18	0.02	−0.83
	T ₅	3.28	2.70	2.78	8.97	1.52	1.04	1.27	0.56
E4	T ₁	0.18	0.16	0.21	0.68	0.11	0.06	0.17	−0.70
	T ₂	0.67	0.76	0.61	1.32	0.35	0.11	0.41	−0.08
	T ₃	1.17	0.77	0.75	1.64	0.46	0.11	0.11	−0.06
	T ₄	1.46	1.04	1.24	2.05	0.55	0.29	0.35	0.21
	T ₅	3.59	2.44	2.70	4.51	1.63	0.65	0.19	0.83
E5	T ₁	0.24	0.26	0.25	0.63	0.11	0.06	−0.09	−0.13
	T ₂	0.68	0.78	0.67	1.36	0.45	0.11	0.42	−0.18
	T ₃	1.27	0.84	0.80	1.66	0.36	0.10	−0.13	2.03
	T ₄	1.46	1.07	1.30	2.04	0.67	0.26	0.54	0.23
	T ₅	3.46	2.85	3.10	4.81	1.61	0.61	0.31	0.01
E6	T ₁	0.23	0.30	0.25	0.63	0.11	0.07	−0.06	−0.48
	T ₂	0.63	0.73	0.68	1.37	0.41	0.11	0.24	−0.37
	T ₃	1.27	0.81	0.80	1.67	0.49	0.10	0.14	0.42
	T ₄	1.43	1.02	1.31	2.05	0.80	0.28	0.56	−0.17
	T ₅	3.48	3.11	3.15	5.46	1.90	0.60	0.62	0.94

^a Environments are as follows: E1: Baoding in 2011; E2: Xingtai in 2011; E3: Cangzhou in 2011; E4: Baoding in 2012; E5: Xingtai in 2012; and E6: Cangzhou in 2012

the largest contribution to the phenotypic variation (21.67 %) at all five filling stages.

Eleven conditional additive QTLs for albumin content were identified during four filling stages (Table 2.27). These QTLs were mapped to chromosomes 1B, 2B, 3A, 6A, 7B, and 7D and accounted for 6.44–16.85 % of the phenotypic variation.

Table 2.25 Statistical analysis of glutenin content in parents Huapei 3 (HP3) and Yumai 57 (YM57) and the doubled-haploid (DH) population at different grain-filling stages for 2 years in three locations

E ^a	Stage	Parents		DH population					
		HP3	YM57	Mean	Maximum	Minimum	SD	Skew	Kurtosis
E1	T ₁	2.04	1.74	1.88	2.35	1.37	0.20	-0.85	0.53
	T ₂	2.59	2.19	2.23	2.84	1.54	0.25	-0.39	0.58
	T ₃	2.56	2.90	2.87	3.91	2.33	0.46	0.17	0.86
	T ₄	2.49	3.59	3.35	4.32	2.00	0.33	-0.15	0.59
	T ₅	3.59	3.82	4.13	5.59	3.24	0.48	-0.01	-0.03
E2	T ₁	2.10	1.76	1.86	2.16	1.38	0.16	-0.57	0.21
	T ₂	2.50	2.18	2.24	2.86	1.59	0.24	-0.01	0.17
	T ₃	2.56	3.00	2.85	3.83	2.45	0.42	0.84	0.70
	T ₄	2.68	3.54	3.36	4.43	1.96	0.35	-0.25	0.95
	T ₅	3.59	3.61	4.14	5.54	3.37	0.45	0.28	-0.07
E3	T ₁	2.02	1.78	1.81	2.13	1.33	0.16	-0.75	0.48
	T ₂	2.53	2.11	2.15	2.80	1.63	0.27	-0.10	0.63
	T ₃	2.63	2.78	2.78	3.86	2.35	0.37	-0.28	0.15
	T ₄	2.52	3.59	3.28	4.30	1.95	0.32	-0.68	0.97
	T ₅	3.39	3.20	4.00	5.58	3.45	0.53	0.17	0.91
E4	T ₁	1.93	1.70	1.73	2.37	1.35	0.17	0.46	0.16
	T ₂	2.32	2.13	2.14	3.03	1.60	0.25	0.65	0.90
	T ₃	2.45	2.79	2.73	3.81	2.05	0.24	0.01	0.03
	T ₄	2.68	3.76	3.42	4.26	2.25	0.38	0.65	0.36
	T ₅	3.61	4.09	4.55	5.86	3.37	0.44	0.26	0.59
E5	T ₁	1.92	1.67	1.75	2.16	1.34	0.15	0.27	0.11
	T ₂	2.38	2.06	2.16	3.09	1.57	0.23	0.90	0.70
	T ₃	2.45	2.70	2.69	3.96	2.15	0.22	-0.20	0.04
	T ₄	2.69	3.71	3.43	4.41	2.41	0.34	0.41	1.36
	T ₅	3.54	4.16	4.56	5.81	3.54	0.43	0.46	0.37
E6	T ₁	1.92	1.63	1.73	2.14	1.34	0.18	-0.82	0.69
	T ₂	2.36	1.93	2.11	3.07	1.58	0.25	0.98	0.84
	T ₃	2.31	2.66	2.64	3.82	2.24	0.24	0.12	0.11
	T ₄	2.45	3.56	3.36	4.56	2.32	0.29	0.39	0.24
	T ₅	3.44	4.07	4.45	5.88	3.31	0.43	0.25	0.21

^a Environments are as follows: E1: Baoding in 2011; E2: Xingtai in 2011; E3: Cangzhou in 2011; E4: Baoding in 2012; E5: Xingtai in 2012; and E6, Cangzhou in 2012

Among them, *QAlu1B-3* and *QAlu6A-1* were consistently detected at two filling stages. Seven major QTLs (*QAlu1B*, *QAlu1B*, *QAlu1B-3*, *QAlu6A-1*, *QAlu7B*, *QAlu7B-1*, and *QAlu7B-2*) were detected; the major QTL *QAlu1B-3* accounted for the largest amount of phenotypic variation (16.85 %) at the five filling stages.

Table 2.26 Unconditional QTLs for protein-fraction contents detected at different stages of measurement

Protein-fraction content	Stage	Locus	Marker interval	Environment ^a	LOD score	Additive ^b	PVE (%) ^c
Albumin content	T1	<i>QAlu-2A</i>	<i>Xbarc015-Xwmc401</i>	E1/E4	3.22/2.77	-0.32/-0.28	8.66/6.93
		<i>QAlu-6A</i>	<i>Xgpn3222-Xgpn7683</i>	E1/E3/E4/E6	3.72/2.64/3.01/3.82	-0.77/-0.63/-0.70/-0.80	10.97/7.39/8.89/11.38
		<i>QAlu-7D</i>	<i>Xbarc244-Xbarc352</i>	E5	2.76	0.62	7.38
		<i>QAlu-7D-2</i>	<i>Xgdm67-Xwmc634</i>	E1/E2/E6	3.05/2.54/3.12	-0.29/-0.28/-0.31	7.65/6.66/7.73
	T2	<i>QAlu-6A-1</i>	<i>Xcfe179.1-Xgpn3238</i>	E2/E6	4.19/3.74	0.34/0.36	12.46/11.86
		<i>QAlu-6A-2</i>	<i>Xcfe179.2-Xcfe179.1</i>	E3	3.75	0.33	9.79
		<i>QAlu-7B-1</i>	<i>Xgpn2224-Xgpn3256</i>	E1/E2/E3/E4/E5	2.80/2.84/3.10/3.35/3.49	-0.42/-0.41/-0.37/-0.35/-0.31	9.00/9.05/9.42/11.35/13.42
		<i>QAlu-1B</i>	<i>Xwmc406-Xbarc156</i>	E1/E2/E4	3.02/3.90/4.17	0.20/0.26/0.32	8.14/10.95/11.65
	T3	<i>QAlu-7B</i>	<i>Xgwm333-Xwmc10</i>	E2	2.71	0.21	10.7
		<i>QAlu-7B-1</i>	<i>Xgpn2224-Xgpn3256</i>	E1/E2/E4	2.55/2.85/2.95	-0.24/-0.21/-0.25	7.14/8.14/10.14
T4		<i>QAlu-1B-1</i>	<i>Xcwem9-Xbarc120.3</i>	E2/E3/E4/E5/E6	2.99/3.81/3.18/4.68/3.71	0.20/0.27/0.24/0.27/0.24	7.88/10.4/8.79/12.9/9.4
		<i>QAlu-1B-3</i>	<i>Xgpn7388-Xgpn2258</i>	E1/E2/E3/E4/E5/E6	5.32/5.51/4.92/5.41/5.34/5.01	0.34/0.31/0.28/0.34/0.34/0.31	17.41/17.07/13.41/15.47/16.41/15.07
		<i>QAlu-7D-1</i>	<i>Xgwm676-Xgwm437</i>	E6	3.25	-0.24	9.64
		<i>QAlu-1B-2</i>	<i>Xbarc120.3-Xbarc008</i>	E2	2.52	0.07	7.80
	T5	<i>QAlu-1B-3</i>	<i>Xgpn7388-Xgpn2258</i>	E1/E2/E3/E4/E5/E6	3.38/3.53.39/3.44/4.68/3.60	0.07/0.08/0.07/0.07/0.09/0.08	9.71/10.46/9.71/10.06/13.36/11.46
		<i>QAlu-1B-4</i>	<i>Xbarc061-Xwmc766</i>	E3	7.33	-0.54	21.67
		<i>QAlu-2B</i>	<i>Xbarc200-Xwmc770</i>	E2/E3/E4/E6	3.13/2.88/3.4/3.43	-0.33/-0.31/-0.28/-0.25	8.19/8.01/8.95/9.43
		<i>QAlu-3A</i>	<i>Xgwm155-Xcfa2170</i>	E1	2.63	-0.25	7.15
		<i>QAlu-6B</i>	<i>GluB-Xwms170.1</i>	E4	2.84	0.42	8.35
							(continued)

Table 2.26 (continued)

Protein-fraction content	Stage	Locus	Marker interval	Environment ^a	LOD score	Additive ^b	PVE (%) ^c
Globulin content	T1	<i>QGlo-1B-1</i>	<i>Xbarc156-Xwmc31</i>	E3	2.63	-0.1	6.97
		<i>QGlo-1B-3</i>	<i>Xgwm582-Xgpnw7388</i>	E1/E2/E4/E5	4.31/4.11/3.91/3.51	-0.16/-0.15/-0.14/-0.12	14.72/14.62/13.62/12.32
		<i>QGlo-2B</i>	<i>Xwmc661-Xwmc764</i>	E3	2.61	0.12	7.33
		<i>QGlo-7B</i>	<i>Xbarc1073-Xwmc273.1</i>	E2	2.64	0.38	7.42
	T2	<i>QGlo-1B-3</i>	<i>Xgwm582-Xgpnw7388</i>	E1/E2/E4/E5	2.61/3.45/2.53/3.05	-0.07/-0.15/-0.06/-0.14	8.77/13.47/8.18/10.47
		<i>QGlo-2D</i>	<i>Xbarc129.2-Xwmc658.2</i>	E6	2.76	-0.05	7.21
		<i>QGlo-6A</i>	<i>Xgpnw3238-Xwmc170.2</i>	E2/E3/E5/E6	2.73/2.91/2.68/2.91	0.05/0.05/0.04/0.05	8.36/8.87/8.18/9.07
		<i>QGlo-6D</i>	<i>Xbarc054-Xgwm55</i>	E3	2.59	0.06	8.88
	T3	<i>QGlo-1B</i>	<i>Xwmc406-Xbarc156</i>	E2	4.56	0.05	12.71
		<i>QGlo-1B-2</i>	<i>Xbarc312-Xcfe023.1</i>	E1/E5	2.65/8.71	-0.03/-0.07	6.94/25.13
		<i>QGlo-1D</i>	<i>Xgdm60-Xwmc429</i>	E4	2.58	-0.07	7.62
		<i>QGlo-3B-1</i>	<i>Xgpnw148-Xgpnw4075</i>	E2/E4	3.15/4.04	-0.04/-0.04	8.91/10.08
		<i>QGlo-4B</i>	<i>Xcfd39.2-Xcfd22.2</i>	E2	2.74	-0.03	6.72
		<i>QGlo-4B-1</i>	<i>Xcfd22.2-Xwmc657</i>	E3	4.02	-0.04	11.05
		<i>QGlo-4B-2</i>	<i>Xwmc657-Xwmc48</i>	E4	3.16	-0.03	9.41
		<i>QGlo-3B</i>	<i>Xgwm685-Xwmc615</i>	E6	3.07	-0.04	8.52
T4		<i>QGlo-2A</i>	<i>Xgpnw7218-Xbarc264</i>	E1/E2/E4/E5	2.53/3.17/3.38/3.18	0.05/0.05/0.05/0.05	7.07/7.77/8.19/7.99
		<i>QGlo-3A</i>	<i>Xwmc21-Xwmc664</i>	E6	4.34	-0.06	11.21
		<i>QGlo-4A</i>	<i>Xwmc219-Xwmc776</i>	E2	3.22	0.06	9.01
		<i>QGlo-5A</i>	<i>Xwem32.2-Xwmc59</i>	E4	2.67	0.06	8.12
		<i>QGlo-7D-1</i>	<i>Xgwm676-Xgwm437</i>	E2/E3	3.10/2.83	0.05/0.05	8.73/9.01
		<i>QGlo-7D-2</i>	<i>Xwmc42-Xswes23</i>	E1	2.69	-0.07	7.46
T5		<i>QGlo-6B</i>	<i>GluB-Xswes170.1</i>	E2/E3/E5/E6	5.08/4.32/4.57/4.14	-1.02/0.72/-0.82/-0.69	25.12/18.54/21.43/17.74

(continued)

Table 2.26 (continued)

Protein-fraction content	Stage	Locus	Marker interval	Environment ^a	LOD score	Additive ^b	PVE (%) ^c
Gliadin content	T1	<i>QGli-1B</i>	<i>Xwmc128-Xbarc312</i>	E6	2.52	-0.02	7.82
		<i>QGli-1B-2</i>	<i>Xgwm582-Xgpn7388</i>	E3	2.96	-0.02	10.24
		<i>QGli-1B-4</i>	<i>Xswes98-Xswes649</i>	E2/E4	3.14/3.84	-0.02/-0.02	8.76/10.07
		<i>QGli-6A</i>	<i>Xgpn322-Xgpn7683</i>	E3/E4	2.56/2.80	-0.02/-0.02	7.75/8.03
		<i>QGli-6B</i>	<i>Glub-Xswes170.1</i>	E1/E6	2.34/2.72	-0.08/-0.09	8.34/8.84
		<i>QGli-7A</i>	<i>Xbarc070-Xbarc250</i>	E3	2.52	0.02	7.26
		<i>QGli-1B-1</i>	<i>Xbarc120.3-Xbarc008</i>	E1/E6	2.75/3.27	-0.03/-0.03	7.67/9.17
	T2	<i>QGli-1B-3</i>	<i>Xgpn7388-Xgpn2258</i>	E3	4.52	-0.05	16.27
	T3	<i>QGli-7B</i>	<i>Xgwm333-Xwmc10</i>	E1	2.65	0.04	12.37
	T4	<i>QGli-2A</i>	<i>Xwmc177-Xgpn2321</i>	E1/E2/E3/E4/E5/E6	2.74/3.08/3.44/4.79/4.85/4.98	0.06/0.06/0.07/0.08/0.09/0.09	8.70/9.22/11.02/14.18/15.18/16.21
		<i>QGli-2B</i>	<i>Xwmc489.1-Xcwm5</i>	E5	2.73	-0.07	6.84
		<i>QGli-4A</i>	<i>Xwmc497-Xwmc219</i>	E6	2.59	0.05	6.42
		<i>QGli-4A-1</i>	<i>Xwmc776-Xbarc362</i>	E3	3.41	0.06	8.47
		<i>QGli-5A</i>	<i>Xgwm186-Xcfe223</i>	E1/E4	3.54/2.72	-0.08/-0.05	13.61/7.16
		<i>QGli-6A-1</i>	<i>Xgpn3238-Xswes170.2</i>	E6	2.68	0.05	11.44
		<i>QGli-7A-1</i>	<i>Xwmc530-Xcfa2123</i>	E2/E3/E5/E6	2.52/3.70/3.14/2.92	0.08/0.10/0.08/0.08	8.28/12.01/10.10/8.55
	T5	<i>QGli-2D</i>	<i>Xgpn3041-Xwmc658.1</i>	E4	2.85	0.29	7.10
		<i>QGli-3B</i>	<i>Xgwm533-Xbarc251</i>	E5	2.76	-0.15	9.82
		<i>QGli-6B</i>	<i>Glub-Xswes170.1</i>	E1/E6	3.61/4.34	-0.21/-0.24	11.89/12.39
		<i>QGli-7B</i>	<i>Xgwm333-Xwmc10</i>	E1/E4	3.04/3.52	0.21/0.18	11.00/9.93
							(continued)

Table 2.26 (continued)

Protein-fraction content	Stage	Locus	Marker interval	Environment ^a	LOD score	Additive ^b	PVE (%) ^c
Glutenin content	T1	<i>QGlu-1A</i>	<i>GluA1-Xwmc550</i>	E1	3.88	0.36	20.08
		<i>QGlu-3A</i>	<i>Xwmc21-Xwmc664</i>	E1/E3/E6	4.18/3.06/3.38	-0.05/-0.05/-0.06	10.71/9.01/9.32
		<i>QGlu-6B</i>	<i>Xbarc247-Xbarc1129</i>	E1	2.59	0.09	9.69
		<i>QGlu-6D-1</i>	<i>Xswes861.1-Xgwm681</i>	E2	6.98	-0.57	35.02
	T2	<i>QGlu-3A</i>	<i>Xwmc21-Xwmc664</i>	E3/E6	2.56/2.7	-0.07/-0.06	7.16/6.87
		<i>QGlu-3B</i>	<i>Xgwm566-Xcfe009</i>	E2/E3	2.90/2.54	-0.07/-0.06	7.90/6.63
		<i>QGlu-3D</i>	<i>Xbarc1119-Xcfd4</i>	E1/E4	2.57/2.79	-0.06/-0.07	6.62/7.56
		<i>QGlu-4D</i>	<i>Xcfe188-Xbarc1009</i>	E6	2.62	0.06	6.97
		<i>QGlu-6D</i>	<i>Xgwm133.2-Xswes861.1</i>	E3	2.55	-0.19	14.71
	T3	<i>QGlu-6A</i>	<i>Xbarc023-Xbarc1077</i>	E2	2.52	0.07	8.29
		<i>QGlu-6D-1</i>	<i>Xswes861.1-Xgwm681</i>	E5	4.90	1.22	20.02
	T4	<i>QGlu-5D</i>	<i>Xbarc1097-Xcfd8</i>	E5	2.69	-0.09	11.66
		<i>QGlu-6B</i>	<i>Xbarc1129-Xcfa2257</i>	E4	2.62	-0.11	7.54
	T5	<i>QGlu-7B</i>	<i>Xgwm333-Xwmc10</i>	E2/E3/E4/E5	4.24/3.76/4.24/2.72	0.15/0.15/0.14/0.11	14.34/14.11/16.06/9.83
		<i>QGlu-1D</i>	<i>Xwmc93-GluD1</i>	E2/E4/E5	3.65/3.45/3.35	-0.17/-0.15/-0.14	15.55/11.34/11.24
		<i>QGlu-3A-1</i>	<i>Xwmc489.2-Xwmc489.3</i>	E1/E2/E3/E6	3.23/3.64/4.43/3.4	-0.11/-0.14/-0.15/-0.17	8.26/8.89/10.53/9.41
		<i>QGlu-5A</i>	<i>Xcfe223-Xwmc273.3</i>	E3/E6	4.02/2.52	-0.14/-0.11	9.49/6.04
		<i>QGlu-7A</i>	<i>Xbarc157.2-Xgwm60</i>	E6	2.71	0.11	6.97
		<i>QGlu-7A-1</i>	<i>Xwmc530-Xcfa2123</i>	E1	3.12	0.14	9.17
		<i>QGlu-7B</i>	<i>Xgwm333-Xwmc10</i>	E2/E5	3.05/3.25	0.13/0.13	8.43/8.53

^aEnvironments are as follows: E1: Baoding in 2011; E2: Xingtai in 2011; E3: Cangzhou in 2011; E4: Baoding in 2012; E5: Xingtai in 2012; and E6: Cangzhou in 2012
^bPositive additive effects are associated with increased effects from Huapei-3 alleles, and negative additive effects are associated with increased effects from Yumai-57 alleles
^cPVE: phenotypic variance explained.

Table 2.27 Conditional QTLs for protein-fraction contents detected between two sampling stages

Protein-fraction content	Stage	Locus	Marker interval	Environment ^a	LOD score	Additive ^b	PVE (%) ^c
Albumin content	T2 T1	<i>QAlu-6A-2</i>	<i>Xcfe179.2-Xcfe179.1</i>	E3	3.75	0.33	9.78
		<i>QAlu-6A-1</i>	<i>Xcfe179.1-Xgpnw3238</i>	E2/E6	4.19/3.68	0.34/0.27	12.46/16.21
		<i>QAlu-7B-1</i>	<i>Xgpnw2224-Xgpnw3256</i>	E1/E2/E3/E4/E5	2.67/2.57/3.10/3.20/3.17	-0.41/-0.38/-0.37/-0.34/0.23	8.59/8.18/9.42/10.83/8.59
	T3 T2	<i>QAlu-1B</i>	<i>Xwmw406-Xbarr156</i>	E1/E2/E4	3.65/4.05/3.02	0.25/0.31/0.20	10.28/11.33/8.14
		<i>QAlu-6A-1</i>	<i>Xcfe179.1-Xgpnw3238</i>	E5	2.51	0.28	15.64
		<i>QAlu-7B</i>	<i>Xgwm333-Xwmw10</i>	E2	2.77	0.20	10.96
	T4 T3	<i>QAlu-7B-2</i>	<i>Xgpnw3226-Xgpnw2224</i>	E1/E2/E4	3.76/4.21/4.35	-0.24/-0.20/-0.25	10.44/11.91/14.82
		<i>QAlu-1B-1</i>	<i>Xcwmw9-Xbarr120.3</i>	E2/E3/E4/E5/E6	2.41/3.07/2.56/3.39/2.99	0.17/0.23/0.20/0.08/0.07	6.44/8.51/7.36/9.09/8.35
		<i>QAlu-1B-3</i>	<i>Xgpnw7388-Xgpnw2258</i>	E1/E2/E3/E4/E5/E6	3.82/4.69/5.33/5.51/5.34/5.42	0.24/0.29/0.27/0.29/0.36/-0.32	12.41/14.56/15.64/16.85/11.67/8.42
	T5 T4	<i>QAlu-7D-1</i>	<i>Xgwm676-Xgwm437</i>	E1	3.34	-0.24	9.86
		<i>QAlu-1B-3</i>	<i>Xgpnw7388-Xgpnw2258</i>	E1/E2/E3/E4/E5/E6	3.79/3.89/2.93/2.75/2.72/2.55	0.08/0.07/0.07/0.08/0.20/0.23	11.23/10.71/9.31/8.57/7.88/8.59
		<i>QAlu-2B</i>	<i>Xbarr200-Xwmw770</i>	E2/E3/E4/E6	3.13/3.41/2.91/3.03	-0.36/-0.28/-0.33/0.28	8.54/9.00/8.11/15.64
		<i>QAlu-3A</i>	<i>Xgwm155-Xcfe2170</i>	E1	2.58	-0.24	6.97

(continued)

Table 2.27 (continued)

Protein-fraction content	Stage	Locus	Marker interval	Environment ^a	LOD score	Additive ^b	PVE (%) ^c
Globulin content	T2 T1	<i>QGlo-1B-3</i>	<i>Xgwm582-Xgpnw7388</i>	E1/E2/E4/E5	2.73/3.52/2.76/3.11	-0.07/-0.15/-0.07/-0.14	8.85/13.66/8.96/10.62
		<i>QGlo-1B-4</i>	<i>Xgpnw2258-Xcfe026.2</i>	E1	3.09	0.17	10.33
		<i>QGlo-2D</i>	<i>Xharc129.2-Xwmc658.2</i>	E6	3.01	-0.05	8.62
		<i>QGlo-6A</i>	<i>Xgpnw3238-Xswest170.2</i>	E2/E3/E5/E6	2.68/2.81/2.67/2.70	0.04/0.05/0.04/0.04	8.18/8.55/8.17/8.47
		<i>QGlo-6B</i>	<i>GluB-Xswest170.1</i>	E2/E5	2.63/2.61	-0.03/-0.03	6.94/6.53
		<i>QGlo-6D</i>	<i>Xharc054-Xgwm55</i>	E3	2.67	0.06	9.39
	T3 T2	<i>QGlo-1B</i>	<i>Xwmc406-Xharc156</i>	E2	4.27	0.04	11.82
		<i>QGlo-1B-2</i>	<i>Xharc312-Xcfe023.1</i>	E1/E5	2.56/8.42	-0.03/-0.06	6.69/24.21
		<i>QGlo-1D</i>	<i>Xwmc222-Xgdm60</i>	E4	2.57	-0.07	7.35
		<i>QGlo-3A-1</i>	<i>Xharc157.1-Xharc1177</i>	E6	2.83	-0.03	7.45
		<i>QGlo-3B</i>	<i>Xgwm685-Xwmc615</i>	E6	3.09	-0.04	8.55
		<i>QGlo-3B-1</i>	<i>Xgpnw1148-Xgpnw4075</i>	E2/E4	3.06/4.04	-0.04/-0.04	8.68/10.08
	T4 T3	<i>QGlo-4B</i>	<i>Xcfd39.2-Xcfd22.2</i>	E2	2.74	-0.03	6.72
		<i>QGlo-4B-1</i>	<i>Xcfd22.2-Xwmc657</i>	E3	4.08	-0.04	11.22
		<i>QGlo-6B</i>	<i>GluB-Xswest170.1</i>	E2/E5	2.50/2.63	-0.49/-0.51	9.17/9.32
		<i>QGlo-2A</i>	<i>Xgpnw7218-Xharc264</i>	E1/E2/E4/E5	2.52/3.15/3.36/3.17	0.05/0.05/0.05/0.05	7.04/7.74/8.15/7.95
		<i>QGlo-3A</i>	<i>Xwmc21-Xwmc664</i>	E6	3.42	-0.06	8.80
		<i>QGlo-3A-2</i>	<i>Xgpnw1108-Xgpnw1107</i>	E3	2.79	0.05	7.86
		<i>QGlo-4A</i>	<i>Xwmc219-Xwmc776</i>	E2	3.74	0.07	10.36
		<i>QGlo-6B</i>	<i>GluB-Xswest170.1</i>	E2/E5	2.63/2.65	-0.04/-0.04	6.74/6.84
		<i>QGlo-6D-1</i>	<i>Xgwm681-Xtbc808</i>	E3	2.51	0.11	9.57
		<i>QGlo-7D</i>	<i>Xgwm295-Xgwm676</i>	E2/E3	2.91/2.93	0.05/0.06	9.27/8.76
		<i>QGlo-7D-1</i>	<i>Xgwm676-Xgwm437</i>	E2/E3	2.59/3.18	0.04/0.05	6.72/8.95
	T5 T4	<i>QGlo-6B</i>	<i>GluB-Xswest170.1</i>	E2/E3/E5/E6	4.93/4.19/4.44/4.02	-0.78/-0.55/-0.62/-0.52	15.01/11.08/12.81/10.61

(continued)

Table 2.27 (continued)

Protein-fraction content	Stage	Locus	Marker interval	Environment ^a	LOD score	Additive ^b	PVE (%) ^c
Gliadin content	T2 T1	<i>QGli-1B-1</i>	<i>Xbarc120.3-Xbarc008</i>	E1/E6	2.89/2.75	-0.03/-0.03	8.11/7.67
		<i>QGli-1B-3</i>	<i>Xgwm7388-Xgwm2258</i>	E3	4.30	-0.05	15.57
	T4 T3	<i>QGli-2A</i>	<i>Xwmc177-Xgwm2321</i>	E1/E2/E3/E4/E5/E6	2.58/2.89/3.44/3.51/4.50/4.94	0.06/0.06/0.07/0.07/0.08/0.08	8.56/8.86/11.02/12.59/13.62/14.46
		<i>QGli-2B</i>	<i>Xwmc489.1-Xwmc5</i>	E5	2.73	-0.07	6.84
		<i>QGli-4A</i>	<i>Xwmc776-Xbarc362</i>	E6	3.41	0.06	8.47
		<i>QGli-5A</i>	<i>Xgwm186-Xcfe223</i>	E1/E4	3.32/3.14	-0.07/-0.06	12.47/9.70
		<i>QGli-6A</i>	<i>Xgwm3238-Xwmc170.2</i>	E5/E6	2.51/2.68	0.06/0.05	11.37/11.44
	T5 T4	<i>QGli-7A</i>	<i>Xwmc530-Xcfa2123</i>	E2/E3/E5/E6	2.61/3.90/3.25/2.92	0.09/0.10/0.08/0.08	8.57/12.68/10.45/8.55
		<i>QGli-2D</i>	<i>Xgwm3041-Xwmc658.1</i>	E4	2.84	0.29	7.05
		<i>QGli-3A</i>	<i>Xgwm155-Xcfa2170</i>	E3	2.64	-0.29	7.54
		<i>QGli-3B</i>	<i>Xgwm533-Xbarc251</i>	E5	2.73	-0.15	9.72
		<i>QGli-6B</i>	<i>GluB-Xwmc170.1</i>	E1/E6	3.61/4.47	-0.21/-0.24	11.89/12.70
		<i>QGli-7B</i>	<i>Xgwm333-Xwmc10</i>	E1/E4	3.18/3.52	0.21/0.18	11.80/9.93
Glutenin content	T2 T1	<i>QGlu-3B</i>	<i>Xgwm566-Xcfe009</i>	E2/E3	2.56/3.12	-0.06/-0.07	6.66/9.14
		<i>QGlu-3D</i>	<i>Xbarc1119-Xcfa4</i>	E1/E4	2.63/3.23	-0.06/-0.07	6.73/8.06
		<i>QGlu-4D</i>	<i>Xcfe188-Xbarc1009</i>	E6	2.62	0.06	6.97
		<i>QGlu-5D</i>	<i>Xbarc320-Xwmc215</i>	E5	2.80	0.07	6.76
		<i>QGlu-6D</i>	<i>Xgwm133.2-Xwmc861.1</i>	E3	2.51	-0.19	14.52
	T3 T2	<i>QGlu-6D-1</i>	<i>Xwmc861.1-Xgwm681</i>	E5	4.89	1.21	20.09
		<i>QGlu-2D</i>	<i>Xgwm539-Xcfa168</i>	E4	2.54	-0.09	7.35
	T5 T4	<i>QGlu-7B</i>	<i>Xgwm333-Xwmc10</i>	E2/E3/E4/E5	3.75/3.24/4.38/2.72	0.14/0.14/0.14/0.11	12.73/12.21/16.08/9.83
		<i>QGlu-1D</i>	<i>Xwmc93-GluD1</i>	E2/E4/E5	3.65/3.45/3.35	-0.17/-0.15/-0.14	15.55/11.34/11.24
		<i>QGlu-3A-1</i>	<i>Xwmc489.2-Xwmc489.3</i>	E1/E2/E3/E6	3.29/3.64/4.50/3.46	-0.11/-0.14/-0.15/-0.17	8.39/8.89/10.71/9.57
		<i>QGlu-5A</i>	<i>Xcfe223-Xwmc273.3</i>	E3/E6	3.93/2.43	-0.14/-0.10	9.22/5.84
	T4 T3	<i>QGlu-7A</i>	<i>Xbarc157.2-Xgwm60</i>	E6	2.71	0.11	6.97
		<i>QGlu-7A-1</i>	<i>Xwmc530-Xcfa2123</i>	E1	2.91	0.13	7.86
		<i>QGlu-7B</i>	<i>Xgwm333-Xwmc10</i>	E5	3.25	0.13	8.53

^aEnvironments are as follows: E1: Baoding in 2011; E2: Xingtai in 2011; E3: Cangzhou in 2011; E4: Baoding in 2012; E5: Xingtai in 2012; and E6: Cangzhou in 2012
^bPositive additive effects are associated with increased effects from Hupei-3 alleles, and negative additive effects are associated with increased effects from Yumai-57 alleles
^cPVE: phenotypic variance explained.

2.7.2.2.2 Unconditional and Conditional QTLs for Globulin Content

Twenty-two different unconditional additive QTLs for globulin content were identified at five filling stages between 2 years at three locations (Table 2.26). These QTLs were mapped to chromosomes 1B, 1D, 2A, 2B, 2D, 3A, 3B, 4A, 4B, 5A, 6A, 6B, 6D, 7B, and 7D and accounted for 6.72–25.13 % of the phenotypic variation. Among them, *QGlo1B-3* was consistently detected at two filling stages. Seven major QTL (*QGlo1B*, *QGlo1B-2*, *QGlo1B-3*, *QGlo3A*, *QGlo3B-1*, *QGlo4B-1*, and *QGlo6B*) were detected. The major QTL *QGlo1B-2* accounted for the largest amount of phenotypic variation (25.13 %) at the five filling stages.

We detected 21 conditional additive QTLs for globulin content during four filling stages (Table 2.27) and mapped them to chromosomes 1B, 1D, 2A, 2D, 3A, 3B, 4A, 4B, 6A, 6B, 6D, and 7D. These QTLs accounted for 6.53–24.21 % of the phenotypic variation. Among them, *QGlo6B* was consistently detected at four filling stages. Eight major QTLs (*QGlo1B*, *QGlo1B-2*, *QGlo1B-3*, *QGlo1B-4*, *QGlo4A*, *QGlo3B-1*, *QGlo4B-1*, and *QGlo6B*) were detected, with *QGlo1B-2* responsible for the largest amount of phenotypic variation (25.13 %) at the five filling stages.

2.7.2.2.3 Unconditional and Conditional QTLs for Gliadin Content

A total of 18 unconditional additive QTLs for this trait were identified at five filling stages between 2 years at three locations (Table 2.26). These QTLs were mapped to chromosomes 1B, 2A, 2B, 2D, 3B, 4A, 5A, 6A, 6B, 7A, and 7B and accounted for 6.41–16.27 % of the phenotypic variation. Two of these QTLs, *QGli6B* and *QGli7B*, were consistently detected at two filling stages. Nine major QTLs (*QGli1B-2*, *QGli1B-3*, *QGli1B-4*, *QGli2A*, *QGli5A*, *QGli6A-1*, *QGli6B*, *QGli7A-1*, and *QGli7B*) were detected, of which *QGli2A* accounted for the largest amount of phenotypic variation (16.21 %) at the five filling stages.

Thirteen conditional additive QTLs were identified as having an effect on gliadin content during four filling stages (Table 2.27). These QTLs were mapped to chromosomes 1B, 2A, 2B, 2D, 3A, 3B, 4A, 5A, 6A, 6B, 7A, and 7B and were responsible for 6.84–15.57 % of the phenotypic variation. We detected seven major QTLs: *QGli1B-3*, *QGli2A*, *QGli5A*, *QGli6A*, *QGli6B*, *QGli7A*, and *QGli7B*. The major QTL *QGli1B-3* accounted for the largest amount of phenotypic variation (15.57 %) at all five filling stages.

2.7.2.2.4 Unconditional and Conditional QTLs for Glutenin Content

Seventeen unconditional additive QTLs for glutenin content were identified at five filling stages between 2 years at three locations (Table 2.26). These QTLs were mapped to chromosomes 1B, 1D, 3A, 3B, 3D, 4D, 5A, 5D, 6A, 6B, 6D, 7A and 7B and accounted for 6.03–35.02 % of the phenotypic variation. Among them, *QGlut*-

3A, *QGlu6D-1*, and *QGlu-7B* were consistently detected at two filling stages. Eight major QTLs (*QGlu1A*, *QGlu1D*, *QGlu3A*, *QGlu3A-1*, *QGlu5D*, *QGlu6D*, *QGlu6D-1*, and *QGlu7B*) were detected, with *QGlu6D-1* having the largest contribution to phenotypic variation (35.02 %) at the five filling stages.

Thirteen conditional additive QTLs were identified as having an effect on glutenin content during four filling stages (Table 2.27). These QTLs were mapped to chromosomes 1D, 2D, 3A, 3B, 3D, 4D, 5A, 5D, 6D, 7A, and 7B and were responsible for 6.04–20.10 % of the phenotypic variation. Among them, *QGlu-7B* was consistently detected at two filling stages. Five major QTLs (*QGlu1D*, *QGlu3A-1*, *QGlu6D*, *QGlu6D-1*, and *QGlu7B*) were detected; the major QTL *QGlu6D-1* accounted for the largest amount of phenotypic variation (20.09 %) at the five filling stages.

2.7.3 Comparison of the Results with the Previous Studies

In previous studies (Charmet et al. 2005; Zhang et al. 2011), QTLs mapping of wheat protein content were carried out by unconditional mapping method, and most of them were focused on a single developmental stage, usually the harvest stage, and thus did not reveal QTL dynamic expression during trait development. However in the present study, we used the unconditional QTL mapping methods and conditional QTL mapping method to identify the dynamic expression of QTLs associated with protein-fraction contents. Most of the QTLs uncovered in this study mapped to new marker regions. The exceptions were three loci located in adjacent marker regions: *QGlu-1D* in marker interval Xwmc93-GluD1 (accounting for 11.24–15.55 % of observed phenotypic variation in three environments), *QGlu3A* in marker interval Xwmc21-Xwmc664 (accounting for 6.87–10.71 % of phenotypic variation at stage 1 in three environments and stage 2 in two environments), and *QGlu3A-1* in marker interval Xwmc489.2-Xwmc489.3 (accounting for 8.26–10.53 % of the phenotypic variation in five environments). QTLs for glutenin content with large effects have been previously detected in comparable regions of these three loci in many environments (Zhang et al. 2011).

Using our DH population, Zhu et al. (2011) have also identified some regions were associated with the grain protein content in wheat. Some loci detected in our study co-mapped to loci for protein-fraction content. Examples include a QTL for albumin content at *Xgdm67-Xwmc634* on 7D, two QTLs for globulin content at *Xgdm60-Xwmc429* on 1D and *Xwmc657-Xwmc48* on 4B, two QTLs for gliadin content at *Xwmc497-Xwmc219* and *Xwmc776-Xbarc362* on 4A, and a QTL for glutenin content at *Xbarc1097-Xcfd8* on 5D. These results suggest that QTLs controlling different protein-fraction content may be combined with protein content through marker-assisted selection in wheat breeding programs.

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