Australian Journal of Crop Science AJCS
AJCS 7(6):820-829 (2013) ISSN:1835-2707

Quantitative trait loci controlling amino acid contents in wheat (*Triticum aestivum* L.)

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Abstract

Quantitative trait locus/loci (QTL) for amino acid content (AAC), 17 individual amino acid and total amino acid (TAA) contents of wheat were studied using a doubled haploid (DH) population containing 168 progeny lines derived from a cross between 'Huapei 3' and 'Yumai 57', Chinese bread wheat cultivars. The inclusive composite interval mapping (ICIM) was applied on wheat meals from 2008 and 2009 in Shandong province, China using QTL IciMapping 2.2. The results indicated that total 32 QTL were detected in wheat meals from 2008, contributing to 4.86-30.95% of total phenotypic variation. Total 53 QTL, accounting for 4.39-23.87% of total phenotypic variance, were detected in wheat meals from 2009. Most QTL were co-localized, forming 13 QTL clusters in two cropping seasons, whereas 4 QTL clusters were coincident in two years. Especially, the loci near marker Xbarc86 on chromosome 3A detected in both years influenced 13 amino acids, and also controlled protein and wet gluten contents, which could be used for marker for protein and amino acids contents.

Keywords: Amino acid content; DH population; QTL; Wheat (*Triticum aestivum* L.).
Abbreviation: AAC-Amino acid content; Ala-Alanine; Arg-Arginine; Asp-Aspartic acid; Cys-Cysteine; DH-Doubled haploid; EAA-Essential amino acid; EST -Expressed sequence tag; Glu-Glutamic acid; Gly-Glycine; GMP-Glutenin macro-polymer; His-Histidine; ICIM-Inclusive composite interval mapping; Ile-Isoleucine; ISSR - Inter-simple sequence repeat; Leu-Leucine; LOD-Logarithm of odds; Lys-Lysine; Met-Methionine; PC-Protein content; Phe-Phenylalanine; Pro-Proline; QTL-Quantitative trait locus/loci; RFLP-Restriction fragment length polymorphisms; Ser-Serine; SSR-Simple sequence repeats; SV-Sedimentation value; TAA-Total amino acid; TEAA-Total essential amino acid; Thr-Threonine; Tyr-Tyrosine; Val-Valine ; WGC-Wet gluten content

Introduction

Wheat, the most important cereal crop in the world (Peňa et al., 2006), is the principal source of energy, protein, and dietary fiber for a major portion of the world's population (Abdel-Aal and Hucl, 2002). Wheat protein quality is mainly influenced by protein content and the balance of amino acid composition in the wheat (Liu et al., 2002; Li and Zhang, 2000). Amino acid composition in wheat protein is unbalanced, especially for the total essential amino acid (TEAA) content, which is only 42% of that in egg and milk proteins (Zhai, 1988). In particular, lysine, threonine, and isoleucine are the main limiting amino acids that wheat protein alone can not meet human nutritional needs (Myer et al., 1996). Balancing amino acid contents in a diet is important. Recently, with the development of molecular markers such as restriction fragment length polymorphisms (RFLPs) and simple sequence repeats (SSRs), elucidating the genetic basis of quantitatively inherited traits can be achieved for the most of the agricultural and quality traits (Huang et al., 2003). QTL mapping for lots of wheat traits such as protein content (Olmos et al., 2003; Blanco et al., 2006; Kulwal et al., 2005), yield (Reif et al., 2011; Kumar et al., 2006a; Marza et al., 2006), kernel weight (Kumar et al., 2006b), and hardness (Crepieux et al., 2005) had been studied. Amino acid content (AAC) in wheat is quantitative traits, controlled by many genes. Despite its genetic basis, it had been studied by only few researchers with a classical genetic method (Dong et al., 1993; Zheng, 1989; Zhou et al., 1990) that little information is available on the genetic region or single gene controlling AAC in wheat. Several studies on QTL mapping for amino acid contents in rice (Zheng et al., 2008; Wang et al., 2008; Tang, 2007) and soybean (Panthee et al., 2006a, b) are available. Wang et al. (2008) identified 18 chromosomal regions for 19 components of AAC in rice by using 190 recombinant inbred lines (RILs), and the most QTL were co-localized, forming ten QTL clusters in 2002 and six in 2004. Panthee et al. (2006) used 101 F_6-derived RILs to identify the QTL controlling AAC in soybean, and at least one QTL for each amino acid was detected, which explained phenotypic variation ranged from 9.4 to 45.3%. However, no studies were found related to QTL mapping for AAC of wheat. Therefore, the study was to dissect the genetic basis of 17 amino acid and total amino acid contents in wheat using DH population under two different environments.

Results

Phenotypic traits

Total 18 traits including 17 individual amino acids, TAA of the DH population, and the parents under two environments were described in Table 1. The most of AAC was significantly ($p<0.05$) different between the two parents.

Table 1. Amino acid content of wheat for DH population and parents in 2008 and 2009 (mg/g).

Trait	2008					2009				
	Parents		DH Population			Parents		DH Population		
	'Huapei 3'	'Yumai57'	Mean	Min	Max	'Huapei 3'	'Yumai 57'	Mean	Min	Max
Lys	2.66a	2.67a	2.80	1.86	3.66	2.59a	2.38a	2.53	1.58	3.12
Thr	3.04a	3.00a	3.20	2.15	3.90	3.06a	2.82a	2.92	1.77	3.78
Ile	6.72a	6.28b	6.68	3.69	7.99	6.68a	6.14b	6.20	3.04	9.46
Phe	4.47a	4.12b	4.93	3.36	6.27	4.80a	4.27b	4.61	2.52	8.69
Val	8.02a	7.55b	7.86	4.66	9.27	7.61a	6.79b	6.86	2.42	9.95
Met	7.42a	6.70b	6.59	2.16	8.11	6.67a	5.87b	6.45	2.21	11.81
Leu	10.65a	10.80a	10.28	6.28	12.26	10.38a	9.01b	9.76	6.53	12.99
Glu	29.28b	30.28a	32.23	22.72	40.03	28.72a	26.27b	28.40	17.85	36.39
Pro	10.88a	9.68b	10.58	6.98	15.10	10.48b	11.17a	8.40	4.23	12.55
Cys	7.15a	6.74b	6.85	3.20	8.90	5.44a	5.24a	5.99	2.87	10.62
Asp	5.85a	5.98a	6.28	4.24	7.40	5.53a	5.23b	5.73	3.31	6.99
Ser	4.42a	4.44a	4.67	3.05	6.13	4.47a	4.08b	4.56	2.63	5.97
Gly	4.16a	4.36a	4.34	2.81	5.08	4.28a	4.17a	4.19	2.43	5.15
Ala	4.46a	4.28b	4.43	2.82	5.53	4.68a	4.36b	4.60	2.62	5.83
Tyr	3.66a	3.53a	3.60	2.34	4.43	2.70a	2.47a	3.41	1.71	5.66
His	2.54a	2.43a	2.73	1.80	4.96	2.46a	2.22b	2.21	1.36	3.15
Arg	5.25a	4.81b	5.48	3.38	6.64	4.71a	4.91a	4.61	2.68	6.23
TAA	120.63a	117.66a	123.52	83.39	146.29	115.25a	107.40b	111.40	70.34	147.37

The different alphabets in right side of the same line show significant difference ($p<0.05$).

'Huapei 3' had higher concentration for the majority of amino acids. Differences were also found for the 18 components of AAC among DH lines under the two environments. The DH population showed transgressive segregations in both directions for all traits in this study (Fig. 1).

QTL analysis

Additive QTL for AAC of wheat in 2008

32 individual additive QTL (LOD>2.0) were detected for the 18 components of AAC in the DH population in 2008, ranging from one to four QTL for each trait (Table 2). The total phenotypic variation explained by the individual QTL for the 18 components of AAC varied from 4.86% for Asp content to 30.95% for Ser content. Most of the 32 QTL mainly distributed on chromosomes 3A, 6D and 7D. Most of the QTL detected tended to be co-localized within the genome, and thus 16 chromosome regions were identified in the study in 2008 wheat, including seven QTL clusters and nine single QTL. The allelic effects of each QTL in the same QTL cluster were all in the same direction. The QTL cluster, flanked by Xbarc356-Xwmc489.2 on chromosome 3A, was the largest in number, which consisted of nine individual QTL (associated with Lys, Thr, Val, Pro, Asp, Gly, Ala, Arg, and TAA), followed by the QTL cluster near Xswes861.1-Xgwm681 on chromosome 6D, consisting of four amino acids (Ile, Phe, Leu, Asp). However, according to the average phenotypic variation explained by individual QTL (R^2) , the QTL cluster flanked by Xgwm428-Xcfd175 on chromosome 7D was the largest, accounting for 17.99% of total variation, followed by the QTL clusters near Xswes861.1-Xgwm681 on chromosome 6D and near Xswes107- Xbarc86 on chromosome 3A (R^2=12.15%, 11.66% , respectively). Ten main effect QTL (R^2>10%) associated with Gly, Ile, Leu, Ser, His, and Arg contents were identified in the population in 2008 wheat. Especially, the QTL for Ser flanked by Xgwm428-Xcfd175 on 7D chromosome could explained 30.95% of total phenotypic variation, and the other QTL for His linked to Xwmc415-Xswes170.1 on chromosome 6B, whose R^2 was 30.18%. For Lys and Thr contents, only one QTL distributed on chromosome 3A linked with Xbarc356-Xwmc489.2 were detected, explaining 5.96% and 7.31% of total variation, respectively, and the positive alleles originated from 'Yumai 57'. For TAA, only one QTL flanked by Xbarc356-Xwmc489.2 on chromosome 3A were identified, explaining 6.58% of total variation.

Additive QTL for AAC in wheat grain in 2009

53 individual additive QTL (LOD>2.0) were detected for the 18 components of AAC in the DH population in 2009 wheat, ranging from one to six QTL for each trait (Table 3). The total phenotypic variation explained by the individual QTL for the 18 components of AAC varied from 4.39% (Asp) to 23.87% (Ser). Most of the QTL mainly distributed on chromosomes 1A, 3A, 4A, 4B, 5B, 6A, 6B, 6D and 7D. These QTL detected also tended to be co-localized within the genome, and thus ten QTL clusters were identified in the population. The QTL cluster, flanked by Xwmc74-Xgwm58 on chromosome 6B, was the largest in number, which consisted of six individual QTL, followed by the QTL cluster, near Xbarc36-Xbarc140 on chromosome 5B, consisting four individual QTL. Nine main effect QTL (R^2>10%) associated with Asp, Ser, Glu, Gly, Phe, His, and Lys contents were identified in the DH population in 2009 wheat. Especially, the QTL for Asp flanked by Xwmc74-Xgwm58 on chromosome 6B could explain 23.87% of total phenotypic variation, and the other QTL for Lys linked to Xwmc415-Xswes170.1 on chromosome 6B, whose R^2 was 21.08%, and the positive allele originated from Yumai57. For Lys content, five QTL distributed on chromosome 1A, 6B, 6A, and 2A, were detected, jointly explaining 51.00% of the total variation, and especially, *QLys1A-2* had the highest phenotypic contribution (R^2=9.02%). Two QTL were identified for TAA, located on chromosome 4B and 7D, explaining 13.91% of total variation.

Comparison of QTL detected in 2008 and 2009 wheat

In total, 13 QTL clusters for the 18 components of AAC were identified in the population, with three specific to 2008 wheat

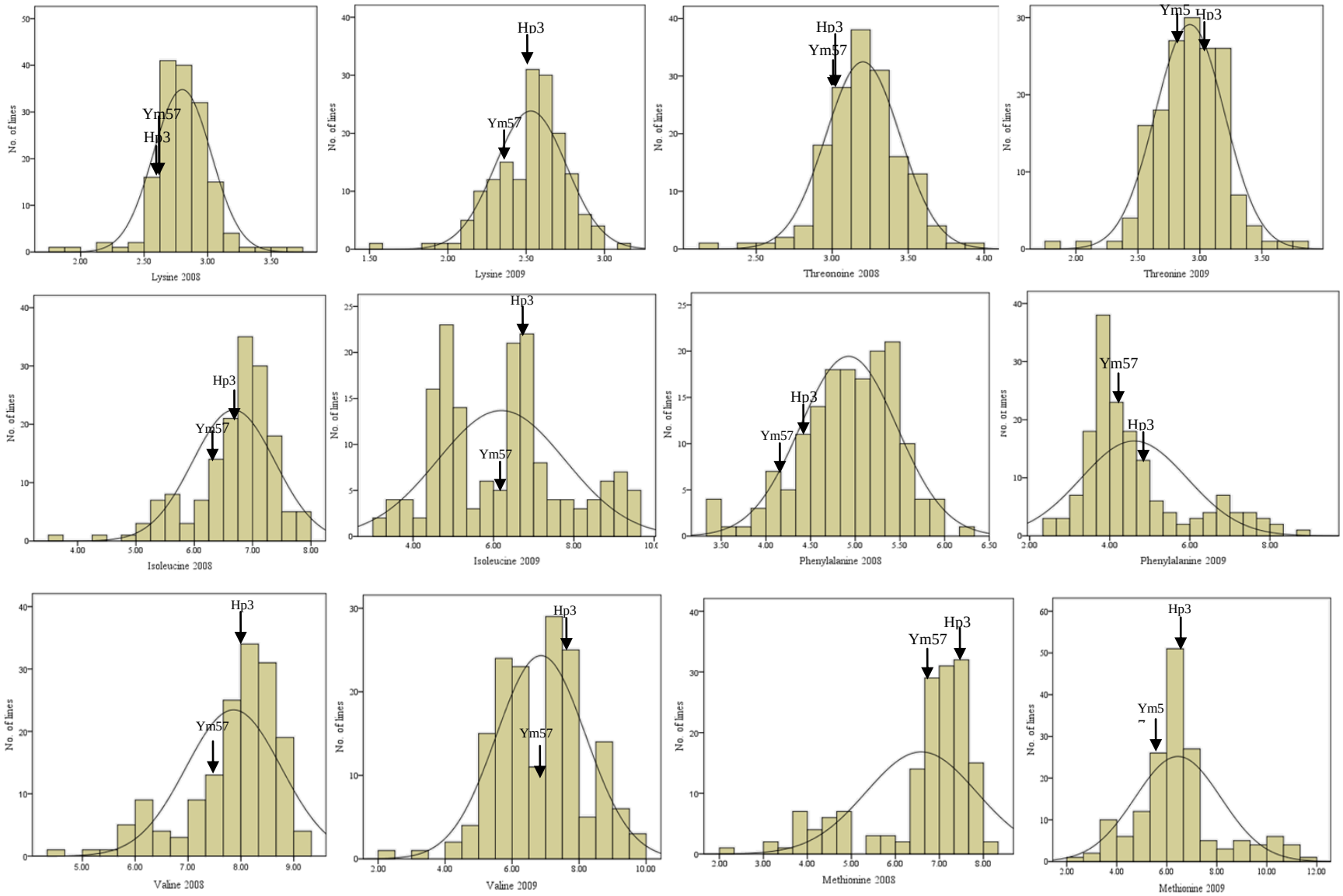

Fig 1. Distribution of wheat amino acid content of DH population in 2008 and 2009.

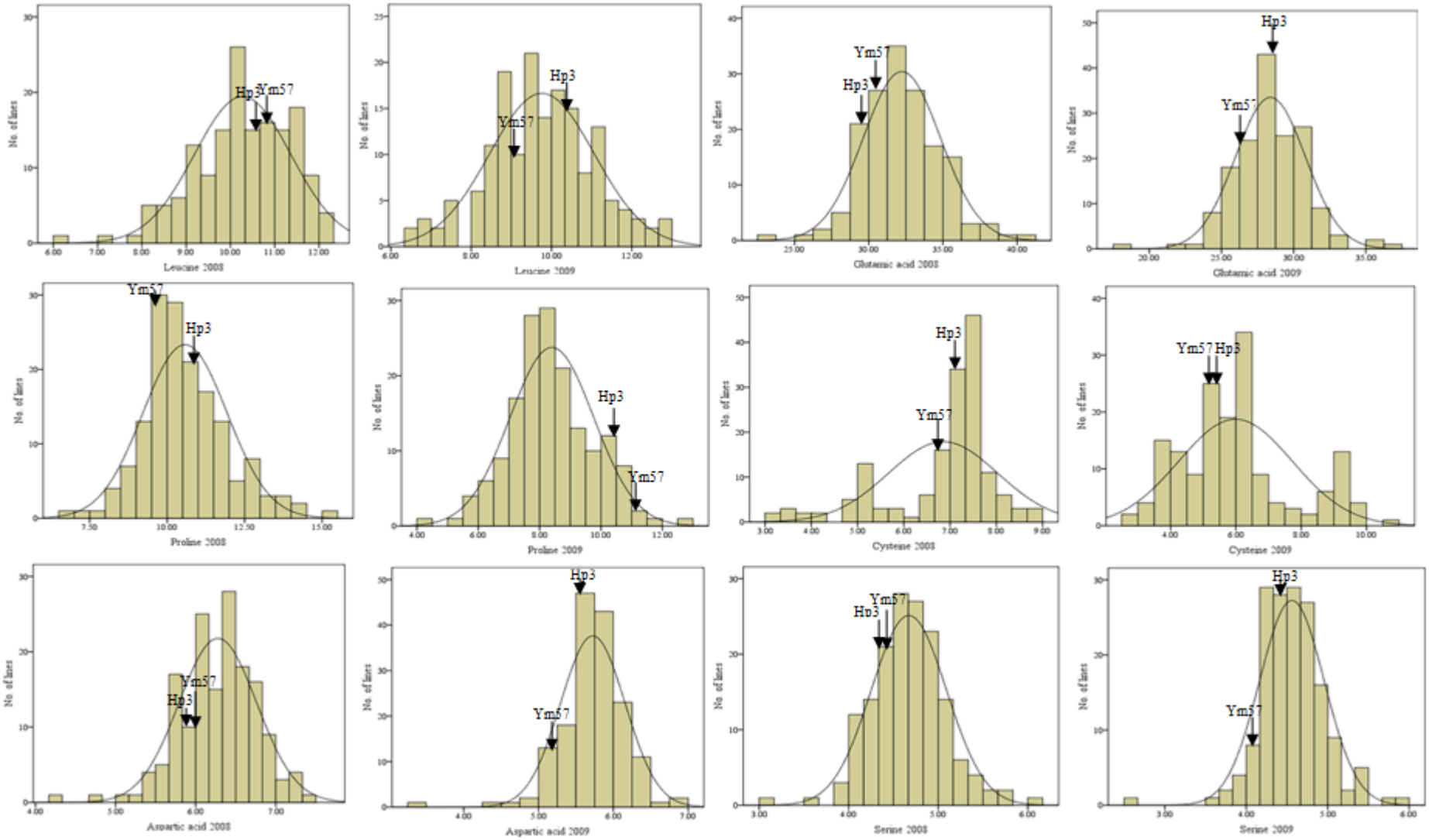

Fig 1. Continued.

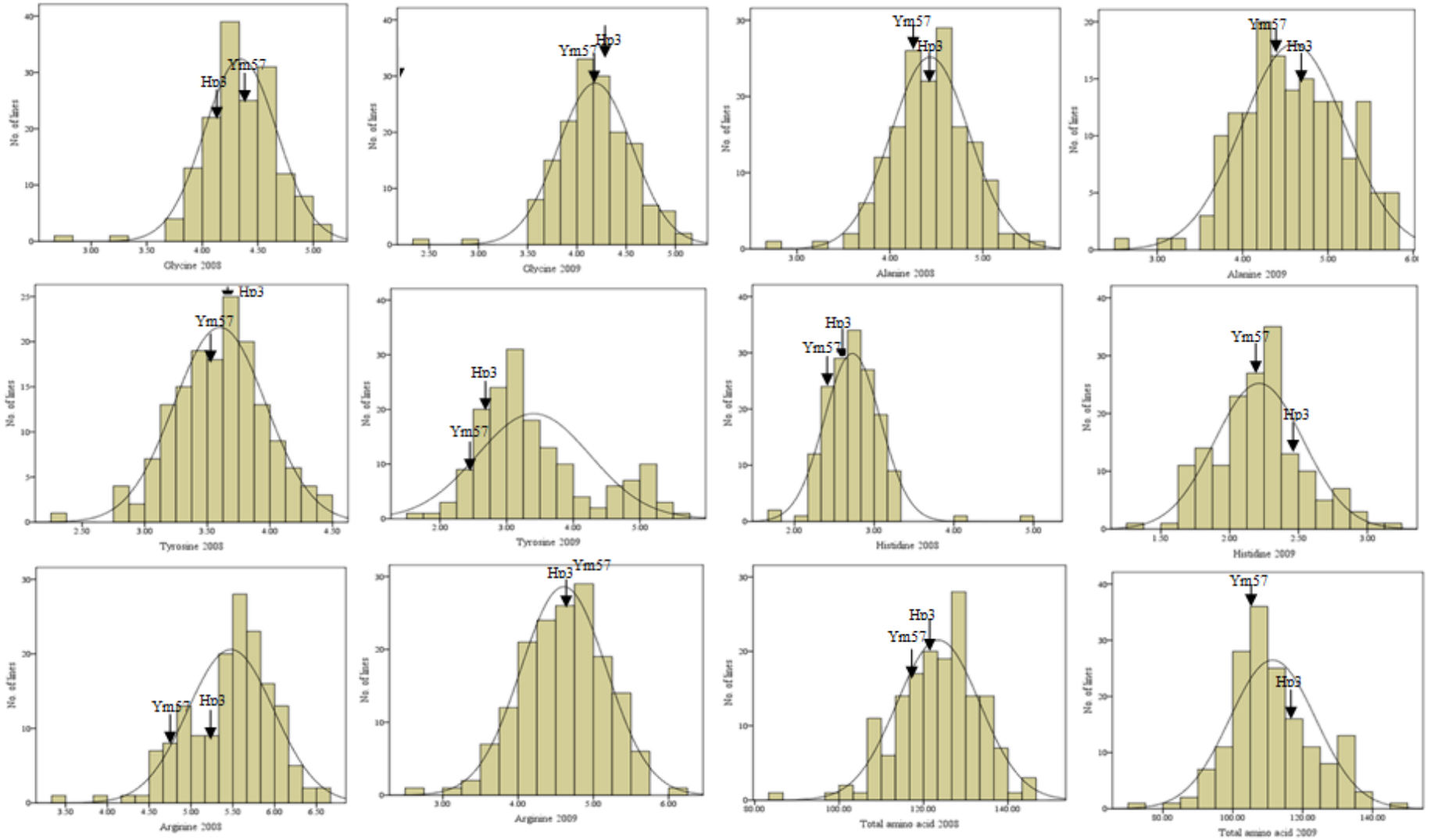

Fig 1. Continued.

and six specific to 2009 wheat. And, four QTL clusters flanked by Xbarc356-Xwmc489.2, Xbarc86-Xwmc21, Xwmc74-Xgwm58, and Xgdm67-Xwmc634, which distributed on chromosome 3A, 6B, and 7D, respectively, were detected consistently in two years. The QTL clusters in the interval Xbarc356-Xwmc489.2 and Xbarc86-Xwmc21 on chromosome 3A were 7 cM apart from each other, and this region associated with 13 amino acid contents, and the positive alleles came from 'Yumai 57'.

Discussion

Significance of mapping QTL for AAC in wheat

Improving nutritional quality of wheat is one of the major objectives for wheat breeding. Most researchers focused on content of protein and starch for nutritional quality of wheat (Cavanagh et al., 2010; Hristov et al., 2010; Sun et al., 2010; Zhang et al., 2011; Zlatska, 2005). Amino acids are the materials for protein synthesis, decomposed products and are the main form of using protein in human and animal bodies. Wheat protein quality is manifested in the type and ratio of amino acids. Amino acid composition of the most cereal crop protein is unbalanced for people's needs. For example, lysine is the most lacking in wheat protein, which seriously affects absorption and utilization of wheat protein. Therefore, it has an important significance for genetic control and improving of amino acid composition in wheat protein.

Since determination of AAC using an amino acid analyzer, is operationally complex, time consuming and expensive, few researches were focused on QTL mapping for AAC by using genetic population. To our knowledge, there were several researches on QTL mapping for AAC in rice (Zheng et al., 2008; Wang et al., 2008; Tang, 2007) and soybean (Panthee, 2006a,b), however, similar studies in wheat has not been reported up to the present. The loci and markers associated with AAC detected in the study can be easily used in screening and identification of wheat with high AAC, and can be used in marker-assisted selection of hybrids, which has significance for breeding new wheat varieties with high nutritional value.

Pleiotropic effects of QTL for AAC in wheat

Most of the genes governing correlated characters were on the same or near genomic regions (Xiao et al., 1996; Tan et al., 1999), which was deemed as "pleiotropic effects" or "linkage of gene". QTL detected in the study tended to be co-localized within the genome, and thus 13 QTL clusters were identified, and four QTL clusters were consistently in the DH population in two years. Especially, two QTL clusters linked to the interval Xbarc356-Xwmc489.2 and the marker Xbarc86, respectively, and with the same effects direction were closely linked each other on chromosome 3A, which were detected commonly in two years. It is likely that the two QTL clusters were one gene or two genes linked closely. Furthermore, this genomic region controlled multiple amino acid contents, which should be taken seriously by breeders. Moreover, a genomic region was close to Xgwm681 on chromosome 6B associated with six amino acid contents, which was also identified commonly in two years. It was shown that some of the QTL for AAC detected in this study was "pleiotropic effects", and each of them controlled multiple amino acid contents with the same effect direction. Hence, manipulation of these genomic regions may lead to changing the concentration of several amino acids simultaneously in wheat grain, which may improve breeding efficiency.

Comparison of the present study with previous researches

To our knowledge, this study is the first to identify QTL for 17 amino acids and total amino acid content in wheat. Therefore, comparison of the present study with previous researches was undertaken by using the previous results on QTL for protein content (PC), wet gluten content (WGC), sedimentation value (SV). AAC tested in the present study was obtained by protein hydrolyzed, and amount of the free amino acid was little in wheat (Zhang et al., 1998). Therefore, it was likely that the genomic region controlling amino acid contents was mapped to vicinity to that associated with protein content and other correlated traits. Some loci detected here were co-mapped to the loci for PC detected in the previous studies. The QTL clusters linked to Xbarc86-Xwmc21 on chromosome 3A and Xcfd42-Xcfd13 on chromosome 6D detected in this study was also identified for controlling PC reported by Zhao et al. (2010). The loci near Xgwm428 controlling Glu and Ser were detected in this study, which also associated with PC researched by Groos et al. (2004). In addition, the QTL linked to markers Xgwm388 (chromosome 2B) and Xgwm428 (chromosome 7D) were co-mapped with the QTL for PC detected by Charmet et al. (2005). Furthermore, the loci near Xgwm 219 for Asp detected here, which also identified for GMP (glutenin macro-polymer) in wheat reported by Li et al. (2006). In addition, Zhang (2008) has identified six QTL for WGC by using the same DH population. Comparing with this result, five of the six QTL was detected for AAC in the study. It is noted that, the loci near Xbarc86 associated with 13 amino acid contents in the study, which also controlled WGC. Zhao (2009) has found QTL for SV by using the same DH population, too, and four QTL were detected. After comparing with the result of AAC, three QTL was co-mapped, which linked to Xcfe023.2, Xbarc358.2 and Xwmc93, respectively.

Materials and methods

Plant materials

A population of 168 DH lines generated from a cross between two Chinese bread wheat cultivars 'Huapei 3'/ 'Yumai 57' was used for the construction of a genetic linkage map. The DH population and parents were provided by Henan Academy of Agricultural Sciences, Zhengzhou, China. 'Huapei 3' and 'Yumai 57', winter wheat, were registered by Henan province in 2006 (Hai and Kang, 2007) and by China in 2003 (Guo et al., 2004).

Field experiment

The field experiment was conducted under different environmental conditions on the experimental farm at Shandong Agricultural University, Tai'an (36.18^{o} N, 117.13^{o} E), Shandong Province, China in 2008 and 2009. A completely randomized block design was used with three replications in two years, and all lines and parental lines were grown in 2-m-long four-row plots (25 cm apart). Crop managements were carried out following the local practices. The lines were harvested individually at maturity, and cleaned prior to milling.

Table 2. Additive QTL for Amino acid content in the DH population in 2008 wheat.

Trait	QTL	Chr.	Site(cM)	Flanking-marker	A	LOD	R^2 (%)
Lys	QLys3A	3A	96	Xbarc356-Xwmc489.2	-0.05	2.12	5.96
Thr	QThr3A	3A	91	Xbarc356-Xwmc489.2	-0.07	2.86	7.31
Ile	QIle6D	6D	112	Xswes861.1-Xgwm681	-0.99	3.93	18.12
Phe	QPhe6D-1	6D	38	Xcfd42-Xcfd13	0.13	2.16	5.48
	QPhe6D-2	6D	113	Xswes861.1-Xgwm681	-0.51	2.19	8.68
Val	QVal2A	2A	74	Xgwm448-Xwmc455	-0.22	2.55	6.23
	QVal3A	3A	97	Xbarc356-Xwmc489.2	-0.22	2.54	6.12
Mat	QMet3D	3D	10	Xgdm72-Xbarc1119	0.29	2.10	5.59
Leu	QLeu3A	3A	86	Xbarc86-Xwmc21	-0.30	2.74	8.06
	QLeu6D	6D	112	Xswes861.1-Xgwm681	-1.31	2.61	14.18
Glu	QGlu3A	3A	72	Xswes107-Xbarc86	-0.82	3.04	10.20
	QGlu7D	7D	164	Xgwm428-Xcfd175	-0.57	2.21	5.04
Pro	QPro3A	3A	91	Xbarc356-Xwmc489.2	-0.37	3.18	7.66
	QPro6A	6A	107	Xcfe179.1-Xswes170.2	0.37	2.35	8.03
Cys	QCys3D	3D	10	Xgdm72-Xbarc1119	0.32	2.82	7.44
Asp	QAsp3A	3A	91	Xbarc356-Xwmc489.2	-0.12	2.58	6.05
	QAsp6B	6B	0	Xcfa2187-Xgwm219	0.11	2.13	4.86
	QAsp6D	6D	113	Xswes861.1-Xgwm681	-0.43	2.04	7.61
Ser	QSer3A	3A	76	Xswes107-Xbarc86	-0.15	4.60	13.12
	QSer7D	7D	164	Xgwm428-Xcfd175	-0.23	12.03	30.95
Gly	QGly3A	3A	92	Xbarc356-Xwmc489.2	-0.10	3.95	10.55
	QGly6B	6B	56	Xwmc74-Xgwm58	0.65	3.06	18.20
	QGly7D	7D	152	Xgdm67-Xwmc634	-0.08	2.47	6.63
Ala	QAla3A	3A	92	Xbarc356-Xwmc489.2	-0.12	3.36	8.89
Tyr	QTyr3A	3A	85	Xbarc86-Xwmc21	-0.09	2.28	6.55
His	QHis1B	1B	50	Xgwm582-Xcfe026.2	-0.10	2.35	7.94
	QHis6B	6B	54	Xwmc415-Xswes170.1	-0.87	5.08	30.18
Arg	QArg2B-1	2B	42	Xwmc661-Xbarc200	-0.20	5.51	16.03
	QArg2B-2	2B	76	Xgwm388-Xbarc101	0.14	3.32	8.14
	QArg3A	3A	95	Xbarc356-Xwmc489.2	-0.18	4.76	12.24
	QArg7D	7D	151	Xgdm67-Xwmc634	-0.11	2.17	4.94
Total	QTotal3A	3A	91	Xbarc356-Xwmc489.2	-2.50	2.48	6.58

Milling and determination of the amino acid content

The whole wheat meals were prepared by milling the wheat with a 3100 Mill (Perten, Sweden). Wheat (200g) was added into the feeder, then the mill was started and continued to run for 1 min after all the wheat pasted the roller. Amino acid composition was obtained using an amino acid analyzer (Biochram 30; Amersham, Britain) using Chinese standard method GB7649-87 1987. In a test tube, 10 mL of 6 *N* HCl were added to a 50-mg sample. The test tube was evacuated and flushed with nitrogen, sealed, and placed in an oven at 110°C for 24 hr, then cool to room temperature. The hydrolyzate was filtered to remove the visible sediments and evaporated under vacuum at 60°C. The hydrolyzate was dissolved in 1 mL of buffer **(**pH 2.2). A known volume (20μL**)** was injected into the amino acid analyzer to estimate the amino acid profile for each sample. Each sample was replicated.

Data analysis

The result of each material was presented on dry matter basis. The normal distribution test and paired-samples t test for 17 individual amino acid and total amino acid content of DH lines and parents were analyzed using the SPSS 16.0.

QTL analysis

A genetic linkage map of the DH population with 323 markers, including 284 simple sequence repeat (SSR) loci, 37 expressed sequence tag (EST) loci, one inter-simple sequence repeat (ISSR) locus, and one high-molecular-weight glutenin subunit locus, was used in this study. This linkage map covered a total length of 2,485.7 cM, with an average distance of 7.67 cM between adjacent markers. The inclusive composite interval mapping (ICIM) was applied by means of the QTL IciMapping 2.2 (Li et al 2007) to identify additive QTL for amino acid contents. A logarithm of odds (LOD) of 2.0 was set to declare QTL as significant. QTL effects were estimated as the proportion of phenotypic variance (R^2) explained by the QTL.

Conclusion

Four QTL clusters and a number of major QTL detected in two wheat cultivars produced consequently in two years should be applied in wheat breeding. Especially, the loci tightly linked to Xbarc86 on chromosome 3A, governing 13 amino acid contents, was commonly detected in the two environments. Furthermore, the loci also associated with PC and WGC with the same effects direction, and the positive alleles came from ‘Yumai 57’.

Table 3. Additive QTL for Amino acid content in the DH population in 2009 wheat.

Trait	QTL	Chr.	Site(cM)	Flanking-marker	A	LOD	R^2 (%)
Lys	QLys1A-1	1A	49	Xwmc550-Xbarc269	0.07	4.41	9.79
	QLys1A-2	1A	65	Xgwm498-Xcwem6.2	-0.10	9.02	21.08
	QLys2A	2A	101	Xwmc455-Xgwm515	0.06	2.64	6.24
	QLys6A	6A	43	Xgwm82-Xwmc553	-0.05	2.53	5.43
	QLys6B	6B	55	Xwmc74-Xgwm58	0.29	3.00	8.47
Thr	QThr1A	1A	65	Xgwm498-Xcwem6.2	-0.07	2.66	5.83
	QThr3A	3A	91	Xbarc356-Xwmc489.2	-0.07	3.14	7.03
	QThr5B	5B	2	Xbarc36-Xbarc140	0.08	3.22	8.10
	QThr6B	6B	55	Xwmc74-Xgwm58	0.28	3.07	7.07
Ile	QIle3A	3A	159	Xgwm155-Xcfa2170	-0.37	2.04	5.56
	QIle4B	4B	8	Xwmc413-Xcfd39.2	0.42	2.79	7.18
Phe	QPhe4B	4B	7	Xwmc47-Xwmc413	0.38	4.06	8.46
	QPhe4D	4D	155	Xcfe254-BE293342	-0.30	2.68	5.39
	QPhe5A-1	5A	6	Xswes45-Xbarc180	-0.46	4.48	12.54
	QPhe5A-2	5A	35	Xcwem40-Xbarc358.2	0.29	2.39	4.82
	QPhe6A	6A	73	Xwmc553-Xgwm732	-0.34	2.42	6.92
	QPhe6D	6D	124	Xubc808-Xswes679.1	-0.50	2.73	7.06
Val	QVal3A	3A	159	Xgwm155-Xcfa2170	-0.34	2.44	6.62
	QVal4B	4B	0	Xwmc125-Xwmc47	0.33	2.56	6.42
Mat	QMet2B	2B	85	Xcwem55-Xbarc129.1	0.43	2.42	6.46
Leu	QLeu7D	7D	162	Xgdm67-Xwmc634	-0.39	3.50	9.48
Glu	QGlu1D	1D	32	Xwmc429-Xcfd19	0.54	2.37	4.84
	QGlu3A	3A	91	Xbarc356-Xwmc489.2	-0.80	5.25	10.80
	QGlu4A	4A	32	Xwmc313-Xwmc497	0.52	2.33	4.63
	QGlu6B-1	6B	42	Xbarc1129-Xcfa2257	0.90	2.22	6.75
	QGlu6B-2	6B	55	Xwmc74-Xgwm58	2.25	2.85	5.80
Pro	QPro2D-1	2D	45	Xcfd53-Xwmc18	-0.40	3.19	8.53
	QPro2D-2	2D	114	Xcfd161-Xgwm311.2	0.41	3.84	9.10
	QPro6D	6D	114	Xgwm681-Xubc808	-0.89	2.34	8.10
Cys	QCys2D	2D	67	Xwmc170.2-Xgwm539	-0.42	2.41	6.08
Asp	QAsp3A	3A	92	Xbarc356-Xwmc489.2	-0.13	4.34	9.15
	QAsp4A-1	4A	10	Xbarc343-Xwmc313	-0.12	3.02	7.44
	QAsp4A-2	4A	39	Xbarc362-Xbarc078	0.14	5.09	10.36
	QAsp6B	6B	56	Xwmc74-Xgwm58	0.96	7.35	23.87
	QAsp7B	7B	12	Xwmc273.1-Xcfd22.1	0.09	2.22	4.39
Ser	QSer3A	3A	84	Xbarc86-Xwmc21	-0.14	5.10	13.08
	QSer6D	6D	114	Xgwm681-Xubc808	-0.31	2.74	9.40
Gly	QGly3A	3A	90	Xbarc86-Xwmc21	-0.12	4.90	10.77
	QGly5B	5B	5	Xbarc36-Xbarc140	0.10	2.71	7.71
	QGly6B	6B	56	Xwmc74-Xgwm58	0.74	3.02	14.55
	QGly7D	7D	162	Xgdm67-Xwmc634	-0.09	2.90	6.27
Ala	QAla3A	3A	160	Xgwm155-Xcfa2170	-0.15	2.61	6.79
Tyr	QTyr3A	3A	162	Xcfa2170-Xbarc51	-0.23	3.04	7.74
	QTyr6A	6A	64	Xwmc553-Xgwm732	-0.25	2.87	9.25
His	QHis1A	1A	65	Xgwm498-Xcwem6.2	-0.08	3.07	6.23
	QHis1B	1B	82	Xbarc061-Xwmc766	-0.11	5.55	11.94
	QHis5B	5B	3	Xbarc36-Xbarc140	0.09	3.47	8.64
	QHis6A	6A	72	Xwmc553-Xgwm732	-0.09	3.08	8.63
	QHis7B	7B	69	Xgwm333-Xwmc10	0.09	2.88	8.30
Arg	QArg5B	5B	0	Xbarc36-Xbarc140	0.13	2.12	5.15
	QArg6B	6B	55	Xwmc74-Xgwm58	0.54	2.00	6.21
Total	QTotal4B	4B	9	Xwmc413-Xcfd39.2	2.86	2.42	5.62
	QTotal7D	7D	162	Xgdm67-Xwmc634	-3.48	3.56	8.29

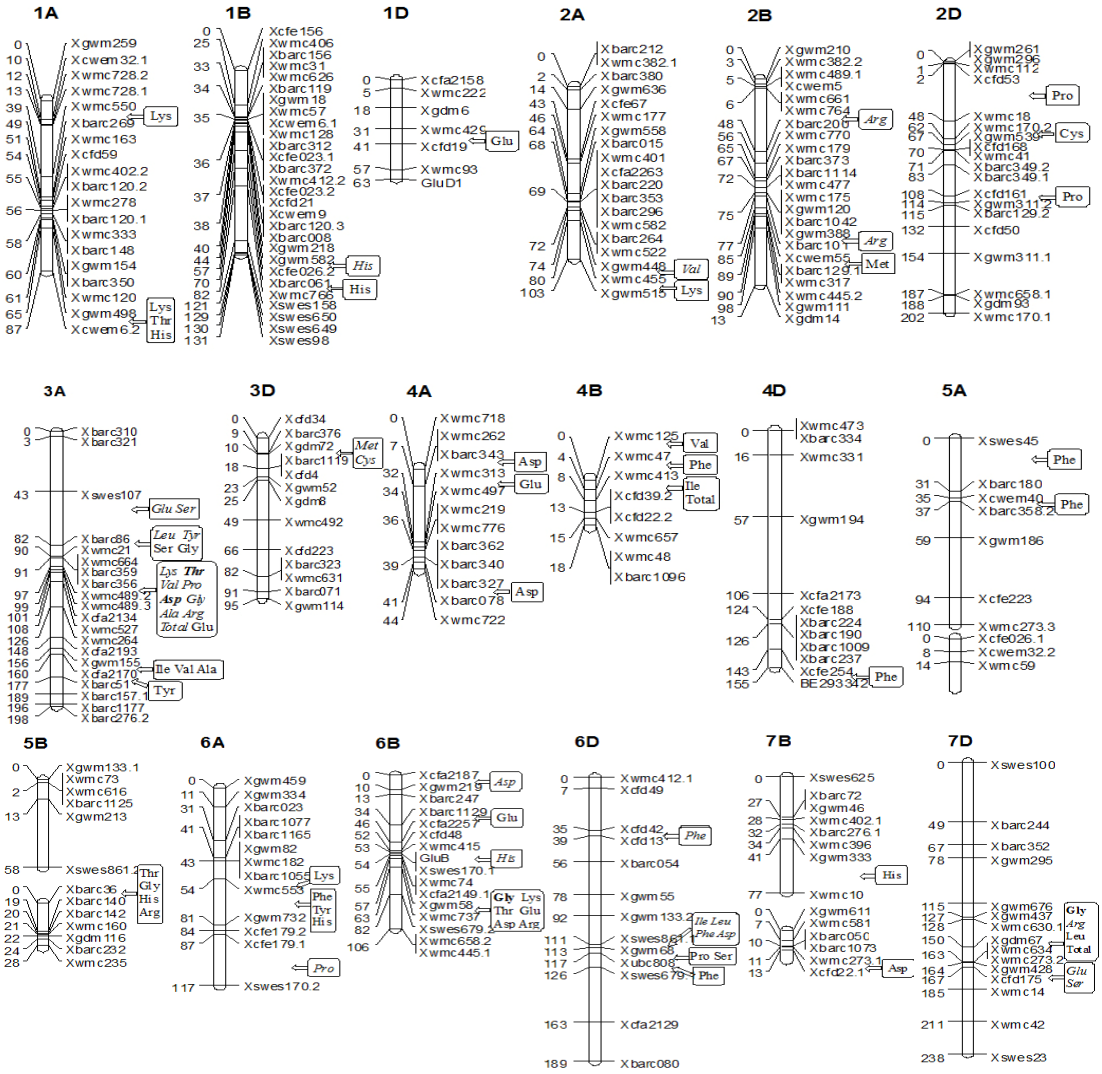

Fig 2. QTL for amino acid content in the DH population in 2008 and 2009 wheat. The italic and normal mean the QTL for amino acid contents detected in 2008 and 2009 wheat, respectively; the bold format means the QTL for amino acid contents detected in two years

Therefore, these loci should be considered to be used in MAS or in positional cloning programs.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (No. 31171554 and 30971764), the National Basic Research Program of China (2009CB118301), the National Major Projects of Cultivated Transgenic New Varieties Foundation of China (2008ZX08002-004 and 2009ZX08002-017B), and the Shandong Provincial Agriculture Liangzhong Project Foundation of China.

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