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Single nucleotide polymorphisms linked to quantitative trait loci for grain quality traits in wheat

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ABSTRACT

Wheat (*Triticum aestivum* L.) grain quality traits that are controlled by quantitative traits loci (QTL) define suitable growing areas and potential end-use products of a wheat cultivar. To dissect QTL for these traits including protein content (GPC); test weight (TW); single kernel characterization system (SKCS)-estimated kernel weight (SKW); kernel diameter (KD); kernel hardness measured by near-infrared reflectance spectroscopy (NIRS) hardness index (NHI); and SKCS-hardness index (SHI), a high-density genetic map with single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) markers was developed using recombinant inbred lines (RILs) derived from Ning7840 × Clark. The RILs were evaluated for these quality traits in seven Oklahoma environments from 2001 to 2003. A total of 41 QTL with additive effects on different traits were mapped on most wheat chromosomes, excluding 1A, 2A, 3D, 4D, 6D, and 7B. Seven chromosome regions showed either tightly linked QTL or QTL with pleiotropic effects on two to four traits. Ten pairs of QTL showed additive × additive effects (AA), four QTL were involved in additive × environment (AE) effects, and one was involved in AAE effects. Two to eleven QTL for each of the six traits and 139 tightly linked markers to these QTL were identified. The findings shed light on the inheritance of wheat grain quality traits and provide DNA markers for manipulating these important traits to improve quality of new wheat cultivars.

1. Introduction

Grain protein content (GPC), test weight (volumetric grain weight, TW), kernel weight (KW), kernel size (KS), and kernel hardness (KH) are important grain quality traits in bread wheat (*Triticum aestivum* L.). Quantitative trait locus (QTL) analysis for GPC has been extensively studied and a large number of QTL were reported to cover all 21 wheat chromosomes

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[1,2,3,4,5,6,7,8,9,10,11]. Previous studies detected many QTL for TW located on almost all 21 wheat chromosomes except for 6D [3,5,8,12,13,14,15,16]. KW is an important component of not only grain yield but also flour yield. Many studies on QTL for KW have been performed and QTL were detected on all chromosomes except 3D and 6D [8,11,14,15,16,17,18,19,20,21,22,23]. The uniformity of KS or its distribution allows for a more efficient milling and quality control. Different QTL were detected in diverse germplasm lines when different methods were used to assess KS. QTL for KS, which is conditioned by genes independently of those for kernel length and width, were mapped to 16 wheat chromosomes excluding 3A, 3D, 4D, 6D, and 7D [14,20,23,24,25,26,27,28]. Pleiotropic QTL were also identified for KS and kernel weight on chromosomes 2A, 5D, 6A [14] and 2B, 2D, 4B, 5B [20]. KH is an important quality trait of bread wheat (*Triticum aestivum* L.) and determines wheat classification and end-use properties. Previous studies indicated that QTL with large effects on KH were co-located with the *Ha* locus on chromosome 5DS [2,4,13,24,29]. In addition, a number of QTL that affect wheat KH have been identified in different mapping populations and covered all 21 wheat chromosomes except for 3D and 6A [6,9,30,31,32,33,34].

Although QTL on the 21 chromosomes have been identified for grain quality traits in wheat germplasm, epistatic effects among them have not been well documented despite the importance in understanding the genetic basis of complex traits. Also, environments often influence expression of grain quality traits and genotype × environment interaction significantly contributes to phenotypic variations of such traits. Sun et al. [19] used a Ning7840 × Clark recombinant inbred line (RIL) population to construct a SSR and AFLP-based map and identified 25 QTL for quality factors, but they did not consider epistatic effects and QTL × environment interactions. Besides, single nucleotide polymorphisms (SNPs) are the most common polymorphism among individuals of any species with virtually unlimited numbers and constitute the basis of most genetic variation between individuals [35]. The availability of diverse SNP genotyping platforms facilitates genetic dissection, marker discovery and genomic selection of traits in crop plants [36]. In the present study, we used a high-density, SNP and SSR genetic map developed for the Ning7840 × Clark RIL population to identify new additive QTL for wheat grain quality traits and SNP markers closely linked to the QTL, and evaluated interaction effects between QTL and between QTL and environments.

2. Materials and methods

2.1. Plant materials and phenotypic data collection

A population of 127 $F_{10–12}$ RILs was developed from the cross Ning7840 × Clark by single-seed descent. Ning7840 (Avrora/Anhui 11//Sumai 3) is a Chinese hard red wheat breeding line. It has relatively low yield potential, but a high level of resistance to various rust pathogens and *Fusarium graminearum* [37]. Clark is a soft winter wheat cultivar released from Purdue University, IN, with good yield potential [38].

Phenotypic data were collected from field experiments at three Oklahoma locations, Stillwater (ST), Lahoma (LA) and Altus (AL) in three crop years ending in 2001, 2002, and 2003, respectively. The RILs along with the parents were measured for six grain quality traits including GPC, TW, single kernel characterization system (SKCS)-estimated kernel weight (SKW), kernel diameter (KD), SKCS-grain hardness index (SHI), and near-infrared reflectance spectroscopy (NIR)-estimated grain hardness index (NHI). Experiments were conducted in seven combinations of years and locations: Stillwater 2001 to 2003 (ST01 to ST03), Lahoma 2002 and 2003 (LA02 and LA03), and AL02 and AL03 (Altus 2002 and 2003). The RILs were arranged in a replicates-in-sets design with three replicates and a plot size of 1.4 m^2 planted at a density of 58 kg ha^{-1}. The phenotypic data for GPC, TW, SKW, KD, SHI, and NHI were collected as previously described [19].

2.2. DNA extraction and marker analysis

Genomic DNA isolation from both the parents and RILs and PCR for SSR were conducted following previously described protocols [39]. PCR fragments were separated with an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) and scored using GeneMarker version 1.6 (Soft Genetics LLC, State College, PA, USA).

SNP genotyping was performed using Infinium iSelect SNP genotyping assays containing 9000 wheat SNPs developed by Illumina Inc. (San Diego, CA, USA). The assay was designed under protocols of the International Wheat SNP Consortium [40]. SNP call was performed using GenomeStudio v2011.1 software (Illumina Inc.). The genotyping assay was conducted at the USDA Small Grains Genotyping Laboratory at Fargo, ND.

2.3. QTL identification

A linkage map for QTL mapping of grain quality traits was reported previously [41]. This map consisted of 998 markers (594 SNPs and 404 SSRs) in 47 linkage groups that corresponded to all 21 wheat chromosomes and covered 4225.7cM of total genetic distance. This final map was used to map the QTL for grain quality traits. QTL mapping was performed using inclusive composite interval mapping of additive (ICIM-ADD) and epistatic QTL (ICIM-EPI) functionalities in the software QTL IciMapping version 3.2 [42]. Additive QTL were detected using 1.0cM steps. The significance probability was set at 0.001 for stepwise regression. Significant LOD thresholds were determined for each dataset by 1000 permutations. Type I error to determine the LOD thresholds from permutation tests was set at $P < 0.05$. Epistatic QTL were detected using a scanning step of 5.0cM, a probability of 0.0001 in stepwise regression, and a LOD threshold of 5.0 to claim significance.

QTL × environment interactions were detected using the Multi-Environment Trials (MET) functionality. Additive × environment (AE) effects and additive × additive × environment (AAE) effects were identified using ICIM-ADD and ICIM-EPI functionalities in the software QTL IciMapping [42]. AE and AAE interactions were detected using 1.0cM steps in scanning, a probability of 0.001 for stepwise regression, and a LOD threshold of 2.5 for claiming significant QTL in each dataset. Significant AE interactions were claimed at $P < 0.05$ (LOD = 3.8) and significant AAE interactions were claimed at $P < 0.001$ (LOD = 10.2).

3. Results

3.1. Additive QTL for wheat grain quality traits

Forty-one putative QTL with additive effects on different traits were distributed on all of the wheat chromosomes except 1A, 2A, 3D, 4D, 6D, and 7A (Table 1, Fig. 1). Five QTL on chromosome arms 2DL, 5AS, 6AL, and 7AL showed simultaneous additive effects on two kernel size traits (SKW-KD), one QTL on chromosome 5DS showed an additive effect on both measurements of kernel hardness (SHI-NHI), and two QTL on chromosome arms 4BS and 5AL showed a simultaneous additive effect on four traits (GPC-TW-SKW-KD). Among the 41 QTL, 17 (41.5%) were located in the A genome, 16 (39.0%) in the B genome, and 8 (19.5%) in the D genome. The numbers of additive QTL on homoeologous groups one to seven were 6, 4, 4, 6, 13, 4, and 4, respectively. A total of 139 markers (106 SNPs and 33 SSRs) showed tight linkage to the QTL, and most of them might be useful for marker-assisted selection.

GPC, SKW, KD, SHI, and NHI were measured in five environments (ST01–ST03, AL02, and LA03), whereas TW was measured in all seven environments. Four QTL for GPC were identified on chromosomes 3A, 4BS, 5AL, and 5BL with the high GPC alleles all from Ning7840 (Table 1). *QGpc.hwwgr-4BS* was detected consistently across three environments and had the most significant effect on wheat GPC. This QTL was located between SNP markers *IWA1846* and *IWA4662* on chromosome 4BS and explained 20.1% to 22.0% of the phenotypic variance. Another three QTL, *QGpc.hwwgr-3A*, *QGpc.hwwgr-5AL*, and *QGpc.hwwgr-5BL* were detected in single environments, explaining 11.5% to 21.5% of the phenotypic variance.

Nine additive QTL for TW were detected on chromosomes 1DL, 3BL, 4AS, 4BS, 5AL, 5AS, 5BS, and 7DS with increased TW alleles at *QTw.hwwgr-5AS* and *QTw.hwwgr-5AL.2* from Clark, but from Ning7840 at other QTL. Among these QTL, *QTw.hwwgr-4BS* with the most significant effect on TW across three environments was flanked by markers *IWA4662* and *IWA482* on chromosome 4BS, and accounted for 29.5% (AL02), 12.4% (ST03), and 13.3% (ST02) of the phenotypic variance. *QTw.hwwgr-5AL.1* in the interval *IWA649–IWA6988* and *QTw.hwwgr-4AS* in interval *Xbarc206-Xwmc96* were significant in two environments. *QTw.hwwgr-5AL.1* explained 29.4% (ST03) and 15.5% (AL02) of the phenotypic variance, respectively; *QTw.hwwgr-4AS* explained 11.4% (ST02) and 9.1% (LA03) of the phenotypic variance, respectively. Other QTL detected in single environments explained 8.3% to 26.4% of the phenotypic variance.

Ten QTL for SKW, were mapped to chromosomes 1BL, 1BS, 2DL, 4BS, 5AL, 5AS, 6AL, 6B, 7AL, and 7DL. Among them, *QSkw.hwwgr-7AL* between the markers *IWA7325* and *IWA6535* and *QSkw.hwwgr-6AL* in the interval *Xbarc1055–IWA6962* were detected in four and three environments, respectively. Increased SKW alleles of these QTL were contributed by Clark and accounted for 6.2% to 27.6% and 4.9% to 9.6% of the phenotypic variance, respectively. *QSkw.hwwgr-4BS* between *IWA4662* and *IWA1846*, and *QSkw.hwwgr-1BS* interval *Xwmc818.1–IWA3620* were detected in ST02 and AL02 with increased SKW coming from Ning7840. *QSkw.hwwgr-4BS* and *QSkw.hwwgr-1BS* explained 27.6% and 11.7% of the phenotypic variance, respectively, in ST02, and 13.0% and 10.2% in AL02. *QSkw.hwwgr-2DL* between markers *IWA5252* and *Xgwm539* was significant in two environments (ST01 and AL02) with an increased SKW allele from Clark that accounted for 12.0% (ST01) and 5.9% (AL02) of the phenotypic variance. Other QTL, *QSkw.hwwgr-1BL*, *QSkw.hwwgr-5AL*, *QSkw.hwwgr-5AS*, *QSkw.hwwgr-6B*, and *QSkw.hwwgr-7DL*, were significant in single environments and explained 4.8% to 13.8% of the phenotypic variance.

Eleven QTL for KD were identified on chromosomes 1BL, 2BS, 2DL, 3BS, 4AL, 4BS, 5AL, 5AS, 6AS, 6AL, and 7AL. Among them, *QKd.hwwgr-5AL* was detected consistently across four environments and had the second largest effect on KD. This QTL was located between markers *IWA649* and *Xcfa2149.1* with the larger KD allele coming from Ning7840 and explaining 9.7% to 22.9% of the phenotypic variance. *QKd.hwwgr-5AS* in marker interval *Xwmc96.1-IWA4710* was detected in three environments, explaining 7.6% (ST01), 8.9% (ST03) and 9.4% (AL02) of the phenotypic variance with Clark contributing the positive allele. *QKd.hwwgr-4AL*, *QKd.hwwgr-4BS*, *QKd.hwwgr-6AS*, and *QKd.hwwgr-7AL* were identified in two environments; among them *QKd.hwwgr-6AS* between markers *IWA5239* and *IWA731* and *QKd.hwwgr-7AL* between the markers *IWA6670* and *IWA6535* contained increased KD alleles from Clark with *QKd.hwwgr-6AS* explaining 13.9% (AL02) and 24.0% (ST03) of the phenotypic variance and *QKd.hwwgr-7AL* accounting for 11.7% (ST01) and 7.4% (ST02) of the phenotypic variance; *QKd.hwwgr-4BS* in the marker interval *IWA482–IWA1846* and *QKd.hwwgr-4AL* in marker interval of *Xwmc376.3–Xwmc313* had increased KD alleles from Ning7840 with *QKd.hwwgr-4BS* explaining 9.0% (ST02) and 13.4% (AL02) of the phenotypic variance and *QKd.hwwgr-4AL* explaining 6.6% (AL02) and 12.5% (ST03) of the phenotypic variance. *QKd.hwwgr-1BL*, *QKd.hwwgr-2BS*, *QKd.hwwgr-2DL*, *QKd.hwwgr-3BS*, and *QKd.hwwgr-6AL* were detected in single environments and explained 5.1 to 8.9% of the phenotypic variance.

QTL on chromosomes 5DS and 1BL were detected with both increased NHI alleles coming from 'Ning 7840'. *QNhi.hwwgr-5DS* between markers *Xgwm190* and *Xcfd18* spanning about 7.2cM was significant across all five environments and explained 52.9% to 61.4% of the variance for NHI in different environments. *QNhi.hwwgr-1BL* was located between loci *IWA4151* and *Xgwm153* on the chromosome 1BL and explained 5.7% (ST01) and 5.6% (AL02) of the phenotypic variance.

Five additive QTL for SHI were identified on chromosomes 1DL, 2BL, 3AS, 5BL, and 5DS. *QShi.hwwgr-5DS* was the most significant QTL identified across all five environments. This QTL was located between *Xgwm190* and *Xcfd18*; and the increased SHI allele was from Ning7840 and explained 60.2% to 70.4% of the phenotypic variance in various environments. Three QTL on chromosomes 1DL, 2BL, and 3AS were detected in two environments with the increased SHI alleles at *QShi.hwwgr-2BL* and *QShi.hwwgr-3AS* coming from Ning7840, and that at *QShi.hwwgr-1DL* was from Clark. *QShi.hwwgr-2BL* between markers *IWA8195* and *IWA7850* explained 3.6% (ST01) and 4.7% (ST02) of the phenotypic variance, *QShi.hwwgr-3AS* in the interval *IWA6387–Xbarc12* accounted for 4.2% (AL02) and 5.6% (ST03) of the phenotypic variance, and

Table 1 – Chromosome locations, marker intervals, interval distances, associated markers, LOD values and phenotypic variance explained by (PVE %) and additive effects (ADD) for QTL and additive × environment effects (AE) detected for wheat grain quality traits in three Oklahoma environments, Stillwater (ST), Lahoma (LA), and Altus (AL), from 2001 to 2003.

QTL	Env	Peak position (cM)	Marker interval	Interval in cM	LOD[a]	PVE %	ADD[b]	Linked marker	AE[c]	Common QTL reported previously[d]
Grain protein content (GPC)										
QGpc.hwwgr-3A	ST01	65	*IWA3069–IWA2023*		3.9	11.5	0.3	*IWA3069, IWA2023, IWA7011, IWA3070, IWA1487, IWA7387, IWA2153*	–	Sun et al. [19]
QGpc.hwwgr-4BS	ST02	41	*IWA482–IWA1846*	1.0	7.9	22.0	0.3	*IWA482,IWA1846, IWA6850*	–	Sun et al. [19]
	AL02	38	*IWA4662–IWA482*	11.0	5.4	20.1	0.2	*IWA4662, IWA482*		
	ST03	41	*IWA482–IWA1846*	1.0	7.3	20.5	0.3	*IWA482, IWA1846, IWA6850*		
QGpc.hwwgr-5AL	ST01	40	*IWA649–IWA7509*	9.6	6.7	21.5	0.4	*IWA649, IWA7509, IWA648, IWA3335*	–	–
QGpc.hwwgr-5BL	ST02	119	*IWA197–IWA6713*	4.9	5.5	14.7	0.3	*IWA197, IWA6713, IWA936, IWA4635, IWA4634, IWA7708*	–	Li et al. [6]
Test weight (TW)										
QTw.hwwgr-1DL	ST02	40	*Xwmc429–IWA3446*	8.1	3.7	10.5	0.8	*Xwmc429, IWA3446*	–	Sun et al. [19]
QTw.hwwgr-3BL	ST01	193	*IWA6254–IWA2400*	3.4	4.9	10.3	0.5	*IWA6254, IWA2400*	–	Reif et al. [8]
QTw.hwwgr-4AS	ST02	17	*Xbarc206–IWA3902*	1.3	4.2	11.4	0.8	*Xbarc206, IWA3902, IWA3584, IWA4480*	–	Sun et al. [19]
	LA03	31	*IWA826–Xwmc96*	0.4	3.5	9.1	0.5	*Xwmc96, IWA826, IWA1178, IWA3027*		
QTw.hwwgr-4BS	AL02	37	*IWA4662–IWA482*	11.0	8.4	29.5	1.0	*IWA4662, IWA482*	–	Sun et al. [19]
	ST03	37	*IWA4662–IWA482*	11.0	3.7	12.4	0.6	*IWA4662, IWA482*		
	ST02	37	*IWA4662–IWA482*	11.0	4.0	13.3	0.9	*IWA4662, IWA482*		
QTw.hwwgr-5AL.1	ST03	40	*IWA649–IWA7509*	9.6	9.4	29.4	0.9	*IWA649, IWA7509, IWA648, IWA3335*	–	Sun et al. [19]
	AL02	41	*IWA7509–IWA6988*	0.8	6.3	15.5	0.7	*IWA7509, IWA6988, IWA2642, IWA6082, IWA2645, IWA2641*		
QTw.hwwgr-5AL.2	AL03	108	*IWA122–Xgwm156*	1.3	4.4	18.0	−0.6	*Xgwm156, IWA122, IWA121*	–	Reif et al. [8]
QTw.hwwgr-5AS	LA03	138	*IWA5395–IWA3263*	1.3	9.1	26.4	−0.9	*IWA5395, IWA3263, IWA4454, IWA7777*	–	Sun et al. [19]
QTw.hwwgr-5BS	ST01	6.5	*Xcfd2–Xwmc73*	1.7	6.2	13.3	0.6	*Xcfd2, Xwmc73, Xgwm372*	–	Sun et al. [19]
QTw.hwwgr-7DS	ST01	1	*Xwmc335.2–Xwmc376.1*	5.1	3.8	8.3	0.5	*Xwmc335.2, Xwmc376.1*	–	–
SKCS-kernel weight (SKW)										
QSkw.hwwgr-1BL	ST03	65	*IWA6479–Xgwm403*	2.6	5.7	13.8	1.0	*Xgwm403, IWA6479, IWA4939, IWA2889, IWA2040*	–	Sun et al. [19]
QSkw.hwwgr-1BS	ST02	35	*Xwmc818.1–IWA7398*	1.7	5.0	11.7	0.9	*Xwmc818.1, IWA7398, IWA3123*	–	Sun et al. [19]
	AL02	36	*IWA7398–IWA3620*	2.9	7.6	10.2	0.9	*IWA7398, IWA3620, IWA3123, IWA4975, IWA8619*		
QSkw.hwwgr-2DL	ST01	14	*IWA5252–Xgwm539*	10.4	7.8	12.0	−1.1	*Xgwm539, IWA5252*		Ramya et al. [20]
	AL02	13	*IWA5252–Xgwm539*	10.4	4.6	5.9	−0.7	*Xgwm539, IWA5252*		
QSkw.hwwgr-4BS	ST02	39	*IWA4662–IWA482*	11.0	9.7	27.6	1.4	*IWA4662, IWA482*	–	Ramya et al. [20]
	AL02	41	*IWA482–IWA1846*	1.0	9.3	13.0	1.1	*IWA482, IWA1846, IWA6850*		Sun et al. [19]
QSkw.hwwgr-5AL	ST01	41	*IWA7509–IWA6988*	0.8	8.0	11.1	1.1	*IWA7509, IWA6988, IWA2642, IWA6082, IWA2645, IWA2641*	–	–
QSkw.hwwgr-5AS	ST01	130	*Xwmc96.1–IWA3775*	1.3	6.1	8.5	−0.9	*Xwmc96.1, IWA3775, IWA3776, IWA7980*	–	Sun et al. [19]
QSkw.hwwgr-6AL	ST03	89	*Xbarc1055–IWA5421*	4.0	4.1	9.6	−0.8	*Xbarc1055, IWA5421, IWA4370, IWA3782, IWA1285*	1.75	Sun et al. [19]
	AL02	101	*Xwmc807–IWA6962*	4.0	6.9	9.5	−0.9	*Xwmc807, IWA6962, IWA2812, IWA3463*		
	ST01	93	*IWA2367–IWA7431*	4.4	3.7	4.9	−0.7	*IWA2367, IWA7431*		
QSkw.hwwgr-6B	LA03	88	*Xbarc216.3–Xgwm191.2*	6.2	3.9	12.9	−0.7	*Xbarc216.3, Xgwm191.2*	–	–
QSkw.hwwgr-7AL	ST01	97	*IWA6670–IWA6535*	2.5	15.2	25.3	−1.7	*IWA6670, IWA6535, IWA5913, IWA4196, IWA7409*	–	Sun et al. [19]
	AL02	95	*IWA7406–IWA6670*	4.0	4.5	6.2	−0.7	*IWA7406, IWA6670, IWA7407*		
	ST03	94	*IWA7406–IWA6670*	4.0	8.2	20.6	−1.2	*IWA7406, IWA6670, IWA7407*		
	ST02	69	*IWA7325–IWA4626*	7.7	5.6	14.2	−1.0	*IWA7325, IWA4626*		
QSkw.hwwgr-7DL	AL02	2	*Xwmc150–Xgwm121*	2.2	3.9	4.8	0.6	*Xwmc150, Xgwm121*	–	–

Kernel diameter (KD)										
QKd.hwwgr-1BL	ST02	14	*Xbarc80–IWA7892*	8.8	3.7	5.4	0.04	*Xbarc80*, *IWA7892*	–	
QKd.hwwgr-2BS	ST02	259	*IWA2988–IWA2973*	8.1	3.6	5.1	0.04	*IWA2988*, *IWA2973*	–	
QKd.hwwgr-2DL	ST01	13	*IWA5252–Xgwm539*	10.4	4.0	6.1	−0.04	*Xgwm539*, *IWA5252*	–	Breseghello et al. [26] Ramya et al. [20]
QKd.hwwgr-3BS	ST02	33	*Xgwm389–IWA195*	1.8	6.1	8.9	−0.05	*Xgwm389*, *IWA195*	–	
QKd.hwwgr-4AL	AL02	199	*Xwmc376.3–Xwmc313*	4.6	4.6	6.6	0.04	*Xwmc376.3*, *Xwmc313*, *Xwmc313.5*, *Xwmc307*, *Xwmc313.1*	–	Sun et al. [19]
	ST03	202	*Xwmc376.3–Xwmc313*	4.6	5.7	12.5	0.04	*Xwmc376.3*, *Xwmc313*, *Xwmc313.5*, *Xwmc307*, *Xwmc313.1*		
QKd.hwwgr-4BS	ST02	41	*IWA482–IWA1846*	1.0	6.2	9.0	0.05	*IWA482*, *IWA1846*, *IWA6850*	–	Ramya et al. [20]
	AL02	41	*IWA482–IWA1846*	1.0	8.4	13.4	0.05	*IWA482*, *IWA1846*, *IWA6850*		
QKd.hwwgr-5AL	ST01	41	*IWA7509–IWA6988*	0.8	12.9	22.9	0.07	*IWA7509*, *IWA6988*, *IWA2642*, *IWA6082*, *IWA2645*, *IWA2641*	–	Sun et al. [19]
	ST02	40	*IWA649–IWA7509*	9.6	7.7	11.8	0.05	*IWA649*, *IWA7509*, *IWA648*, *IWA3335*		
	AL02	42	*IWA6988–Xcfa2149.1*	1.3	6.1	9.7	0.05	*Xcfa2149.1*, *IWA6988*, *IWA6082*, *IWA2645*, *IWA2641*		
	ST03	40	*IWA649–IWA7509*	9.6	5.3	11.1	0.04	*IWA649*, *IWA7509*, *IWA648*, *IWA3335*		
QKd.hwwgr-5AS	ST01	130	*Xwmc96.1–IWA3775*	1.3	5.0	7.6	−0.04	*Xwmc96.1*, *IWA3775*, *IWA3776*, *IWA7980*	–	Sun et al. [19]
	AL02	148	*IWA6405–IWA5728*	1.7	6.1	9.4	−0.05	*IWA6405*, *IWA5728*, *IWA4465*, *IWA4068*		
	ST03	133	*IWA3775–IWA4710*	2.1	4.3	8.9	−0.04	*IWA3775*, *IWA4710*, *IWA3776*, *IWA7980*		
QKd.hwwgr-6AL	ST01	93	*IWA2367–IWA7431*	4.4	4.7	7.2	−0.04	*IWA2367*, *IWA7431*	–	
QKd.hwwgr-6AS	AL02	56	*IWA5239–IWA731*	3.4	8.5	13.9	−0.06	*IWA5239*, *IWA731*, *IWA5238*, *IWA1875*, *IWA1874*, *IWA1873*, *IWA1276*, *IWA1049*, *IWA1048*, *IWA902*, *IWA6311*, *IWA1589*, *IWA1903*	2.07	Sun et al. [19]
	ST03	56	*IWA5239–IWA731*	3.4	10.2	24.0	−0.06	*IWA5239*, *IWA731*, *IWA5238*, *IWA1875*, *IWA1874*, *IWA1873*, *IWA1276*, *IWA1049*, *IWA1048*, *IWA902*, *IWA6311*, *IWA1589*, *IWA1903*		
QKd.hwwgr-7AL	ST01	97	*IWA6670–IWA6535*	2.5	7.1	11.7	−0.05	*IWA6670*, *IWA6535*, *IWA5913*, *IWA4196*, *IWA7409*	–	
	ST02	96	*IWA6670–IWA6535*	2.5	5.2	7.4	−0.04	*IWA6670*, *IWA6535*, *IWA5913*, *IWA4196*, *IWA7409*		
NIR-hardness index (NHI)										
QNhi.hwwgr-1BL	ST01	84	*IWA4154–Xgwm153*	1.3	4.4	5.7	5.2	*Xgwm153*, *IWA4154*, *IWA415*	–	Li et al. [6]
	AL02	84	*IWA4154–Xgwm153*	1.3	4.3	5.6	3.9	*Xgwm153*, *IWA4154*, *IWA415*		
QNhi.hwwgr-5DS	ST01	25	*Xgwm190–Xcfd18*	7.2	26.9	52.9	15.7	*Xgwm190*, *Xcfd18*	2.83	Sun et al. [19]
	ST02	25	*Xgwm190–Xcfd18*	7.2	20.8	58.1	9.8	*Xgwm190*, *Xcfd18*		Kunert et al. [4]
	AL02	25	*Xgwm190–Xcfd18*	7.2	28.4	57.3	12.2	*Xgwm190*, *Xcfd18*		Li et al. [6]
	ST03	25	*Xgwm190–Xcfd18*	7.2	24.8	61.4	12.8	*Xgwm190*, *Xcfd18*		
	LA03	25	*Xgwm190–Xcfd18*	7.2	23.6	60.0	11.1	*Xgwm190*, *Xcfd18*		
SKCS-hardness index (SHI)										
QShi.hwwgr-1DL	ST01	0	*Xgdm126–Xbarc66*	9.2	7.3	6.5	−5.7	*Xgdm126*, *Xbarc66*	–	Sun et al. [19]
	AL02	0	*Xgdm126–Xbarc66*	9.2	7.4	7.2	−5.4	*Xgdm126*, *Xbarc66*		
QShi.hwwgr-2BL	ST01	111	*IWA8195–IWA7850*	4.1	4.3	3.6	4.3	*IWA8195*, *IWA7850*, *IWA933*	–	–
	ST02	113	*IWA8195–IWA7850*	4.1	4.1	4.7	4.8	*IWA8195*, *IWA7850*, *IWA933*		
QShi.hwwgr-3AS	AL02	5	*IWA6387–Xbarc12*	4.5	4.6	4.2	4.1	*Xbarc12*, *IWA6387*	–	–
	ST03	5	*IWA6387–Xbarc12*	4.5	4.1	5.6	4.4	*Xbarc12*, *IWA6387*		
QShi.hwwgr-5BL	ST01	46	*IWA5950–IWA2565*	1.2	4.8	3.9	4.4	*IWA5950*, *IWA2565*, *IWA1774*	–	Sun et al. [19]
QShi.hwwgr-5DS	ST01	26	*Xgwm190–Xcfd18*	7.2	37.6	60.2	17.3	*Xgwm190*, *Xcfd18*	1.53	Sun et al. [19]
	ST02	25	*Xgwm190–Xcfd18*	7.2	34.1	63.8	17.3	*Xgwm190*, *Xcfd18*		Kunert et al. [4]
	AL02	25	*Xgwm190–Xcfd18*	7.2	36.7	60.5	15.6	*Xgwm190*, *Xcfd18*		Li et al. [6]
	ST03	24	*Xgwm190–Xcfd18*	7.2	27.3	62.6	14.6	*Xgwm190*, *Xcfd18*		
	LA03	25	*Xgwm190–Xcfd18*	7.2	25.3	70.4	14.1	*Xgwm190*, *Xcfd18*		

QShi.hwwgr-1DL between *Xgdm126* and *Xbarc66* explained 6.5% and 7.2% of the phenotypic variation in ST01 and AL02, respectively. The smallest QTL, *QShi.hwwgr-5BL*, positioned in marker interval *IWA5950–IWA2565* was detected in a single environment (ST01), with increased SHI allele from Ning7840 explaining 3.9% of the phenotypic variance.

3.2. Epistatic QTL for grain quality traits

Ten digenic epistatic QTL for grain quality traits were identified on wheat chromosomes 4BL/5DL, 3BL/3DS, 1DL/7DL, and 7BL/3BL for GPC, 7BL/3BS for TW, 2BL/5BS, 5DL/1DS, and 2AS/1DL for SKW, 1BL/3AS for NHI, and 4BL/5DL for SHI (Table 2). Among the four pairs of epistatic loci associated with GPC, three pairs (*QGpc.hwwgr-3BL/3DS*, *QGpc.hwwgr-1DL/7DL*, and *QGpc.hwwgr-7BL/3BL*) showed increased GPC and explained 6.9% to 13.8% of the phenotypic variance, whereas one pair (*QGpc.hwwgr-4BL/5DL*) showed reduced GPC and accounted for 17.7% of the phenotypic variance. *QTw.hwwgr-7BL/3BS* contributed 6.9% of the phenotypic variance for TW. Among the three pairs of epistatic QTL for SKW, *QSkw.hwwgr-2BL/5BS* and *QSkw.hwwgr-2AS/1DL* showed reduced SKW that accounted for 16.0% and 4.2% of the phenotypic variance, respectively, whereas *QSkw.hwwgr-5DL/1DS* showed increased SKW that explained 4.5% of the phenotypic variance. *QNhi.hwwgr-1BL/3AS* and *QShi.hwwgr-4BL/5DL* explained 1.8% and 0.9% of the phenotypic variance for NHI and SHI, respectively.

3.3. Interactions between QTL and environments

Four QTL with AE interactions and one pair of QTL with an AAE interaction were identified for SKW, KD, NHI, SHI, and GPC, respectively (Tables 1 and 2). AE interactions for four QTL (*QSkw.hwwgr-6AL*, *QKd.hwwgr-6AS*, *QNhi.hwwgr-5DS*, and *QShi.hwwgr-5DS*) accounted for 1.53% to 2.83% of the phenotypic variance (Table 1). The QTL pair, *QGpc.hwwgr-1DL/7DL*, was involved in an AAE interaction, and explained 0.42% of the phenotypic variation for GPC (Table 2).

4. Discussion

4.1. QTL for grain quality traits

Improved GPC is one of the primary objectives of wheat quality breeding. QTL for GPC were previously identified on several chromosomes [9,10,11], suggesting that multiple loci controlled wheat GPC. Even in studies where parental lines had small differences in GPC, QTL were still detected. In our study QTL for GPC were identified on chromosomes 4BS, 5AL, 5BL, and 3A, and the parent Ning7840 contributed the alleles for increased GPC. Among them, *QGpc.hwwgr-4BS* and *QGpc.hwwgr-3A* were the same as those reported by Sun et al. [19]. *QGpc.hwwgr-4BS* was not reported in other populations and may be a novel QTL for GPC. Four SNP markers were identified to closely link the QTL. We could not determine whether *QGpc.hwwgr-5AL* and *QGpc.hwwgr-3A* are the same QTL as reported previously [4,8,28], because different flanking markers were involved. However the QTL on 5BL was the same QTL as previously reported by Li et al. [6] because the QTL detected in both studies were located near SSR markers *Xwmc28* and *Xbarc59*. Zhao et al. [7] identified two pairs of epistatic effects that increased GPC and explained 24.0% of the phenotypic variance. In the present study, we detected four QTL pairs with a significant epistatic effect. Three of the QTL increased GPC, whereas one had the largest negative effect on GPC and explained 17.7% of the phenotypic variance. One pair of epistatic QTL was involved in weak AAE interaction. These results suggested that although there were both additive and epistatic effects for GPC, additive effects played a major role in conditioning wheat GPC.

TW is considered an important trait in determining the market price of wheat. QTL for TW were previously identified on various chromosomes in different populations. For an example, Sun et al. [14] mapped seven QTL on chromosomes 2A, 3B, 4A, 5D, 6A, 6B, and 7B using recombinant inbred lines; Reif et al. [8] used association mapping to identify 12 QTL on chromosomes 1A, 3A, 5A (two), 7A, 1B, 3B, 6B, 1D, 3D, 4D, and 7D. Among them, only one QTL on chromosome 3B was identical in two studies, suggesting that TW was controlled by many genes. Using the Ning7840 × Clark RIL population, Sun et al. [19] mapped eight QTL on chromosomes 1DL, 2DL, 4AS, 4B, 5AS, 5AL, 5BS, and 6AS and six of them, except for the QTL on 2DL and 6AS, were found in the present study. In addition, three new QTL were detected on chromosomes 3BL, 5AL, and 7DS. Among of them, *QTw.hwwgr-5AL.2* might be the QTL identified by Reif et al. [8] because the two closely linked markers to each QTL were also closely linked. Only one epistatic QTL was found in this study and it explained 6.9% of the phenotypic variation, and AE or AAE interactions were not significant, showing that an additive effect contributed to the major genetic variance of TW.

Ten QTL for SKW were identified on chromosomes 1BL, 1BS, 2DL, 4BS, 5AL, 5AS, 6AL, 6B, 7AL, and 7DL. Ning7840 contributed 4.8% to 27.6% of phenotypic variation at five QTL and Clark alleles contributed 4.9% to 25.3% of phenotypic variation at other five. This result indicated that both parents contributed alleles for increased SKW. The most significant QTL was *QSkw.hwwgr-4BS*. This QTL explained 27.6% (ST02) and 13.0% (AL02) of the variation for SKW and is likely the same QTL as reported by Ramya et al. [20]. Another prominent QTL, *QSkw.hwwgr-7AL*, that explained 6.2% to 20.6% of the phenotypic variance for SKW in this study,

Notes to Table 1:
[a] LOD value at the center of the additive QTL.
[b] Additive effect; a positive value implies the Ning7840 allele increased phenotypic value, whereas a negative value indicates the Clark allele increased phenotypic value.
[c] Phenotypic variance explained by the additive QTL × environment interaction. '–' indicates no additive × environment effect.
[d] Previously reported in the same chromosome region. '–' indicates that the QTL was not reported previously.

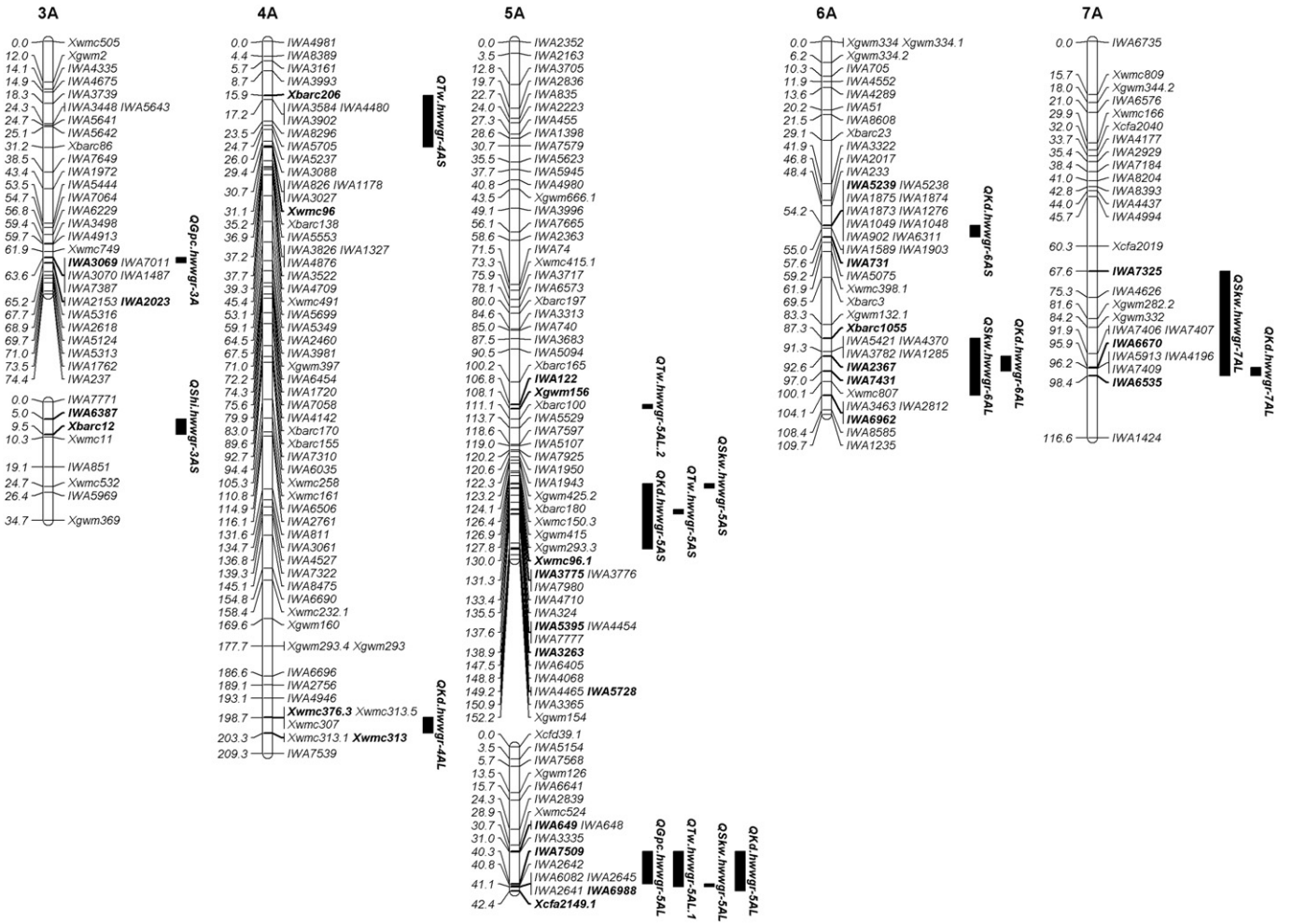

Fig. 1 – Additive QTL for grain-quality traits in the Ning7840 × Clark recombinant inbred population. QTL confidence intervals are indicated by vertical bars and bold scripts.

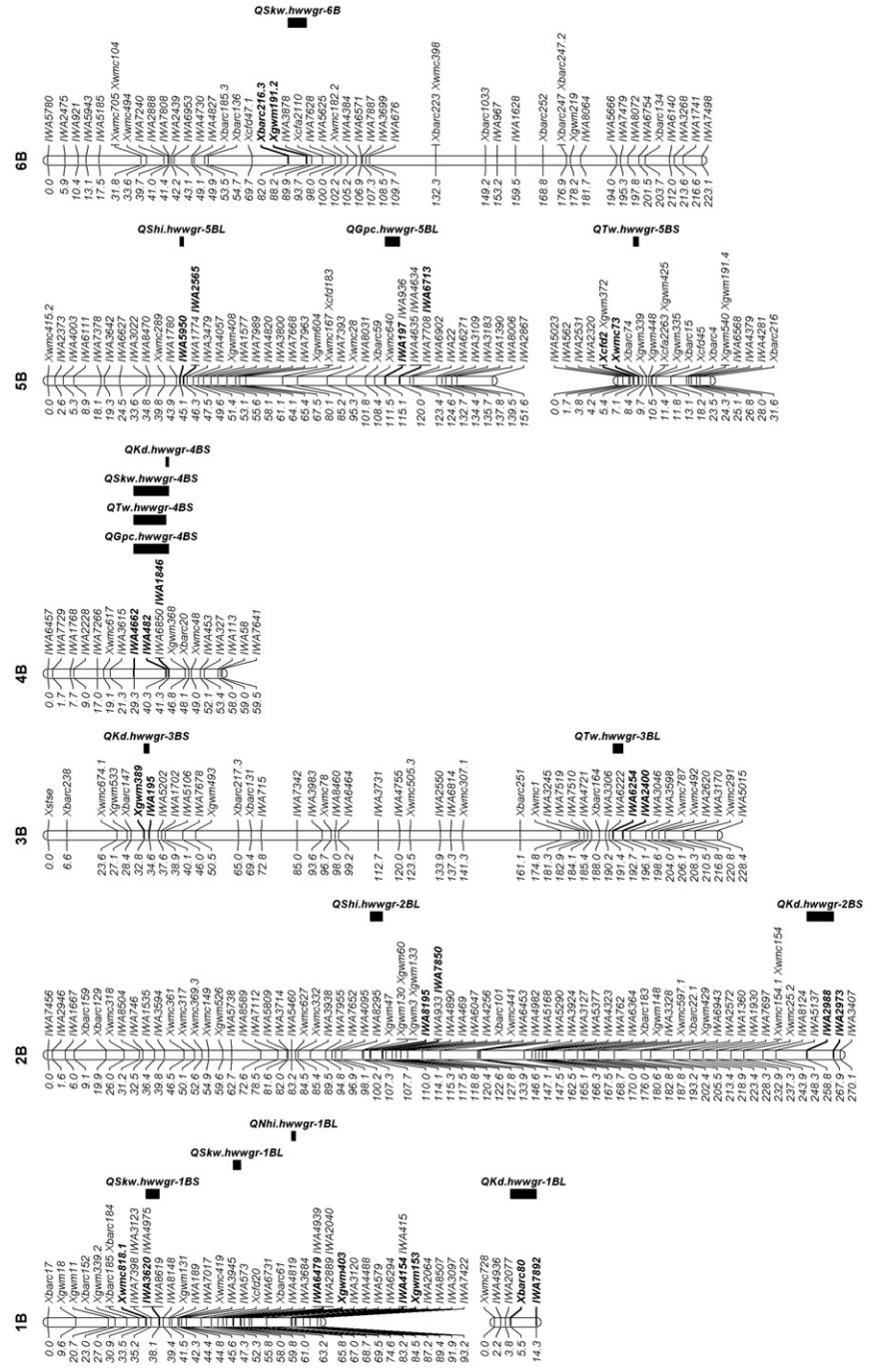

Fig. 1 (*continued*).

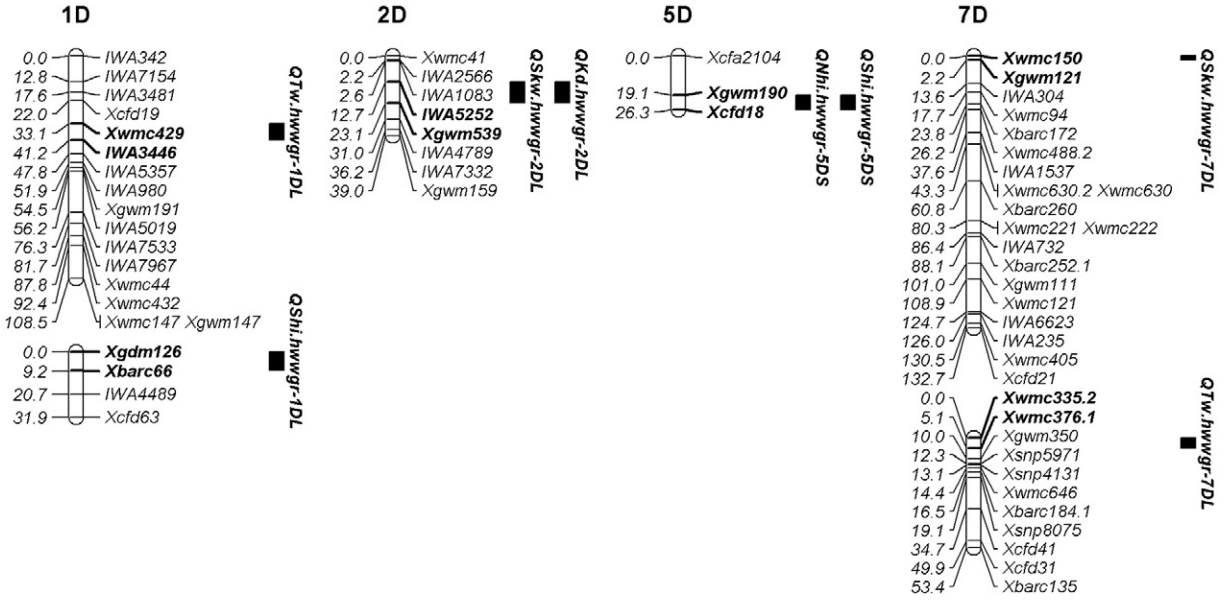

Fig. 1 (*continued*).

had not been reported in other populations and therefore may be novel. Ramya et al. [20] reported a TKW-associated marker *Xgwm539* on 2D, suggesting commonality with *QSkw.hwwgr-2DL* identified in this study. Three pairs of epistatic QTL were identified and explained 4.2% to 16.0% of the phenotypic variance for SKW. One additive QTL, *QSkw.hwwgr-6AL*, was involved in AE interaction that contributed 1.75% to the phenotypic variance. Thus, additive QTL mainly controls SKW, however, digenic epistasis and environments may also affect expression of some QTL.

Among 11 QTL identified for KD, six (*QKd.hwwgr-4AL*, *QKd.hwwgr-5AL*, *QKd.hwwgr-5AS*, *QKd.hwwgr-6A*, *QKd.hwwgr-4BS*, and *QKd.hwwgr-7AL*) were significant in more than two environments and four of them were detected by Sun et al. [19] in the same population, indicating these QTL are relatively stable. Moreover, these QTL contributed major genetic effects to phenotypic variance for KD and may be new QTL for KD except for *QKd.hwwgr-4BS* that might be the same QTL as reported by Ramya et al. [20] because of linked common marker *Xwmc617*. Five QTL on chromosomes 1BL, 2BS, 2DL, 3BS, and 6AL were

Table 2 – Marker intervals, LOD values, phenotypic variance attributable to additive × additive effects (AE) of epistatic QTL, and additive × additive × environment effects (AAE) detected for grain quality traits in three Oklahoma locations from 2001 to 2003.

QTL pair	Env.	Marker interval (Chr.1)	Marker interval (Chr.2)	LOD [a]	PVE (%) [b]	AA [c]	AAE [d]
GPC							
QGpc.hwwgr-4BL/5DL	LA03	*Xgwm251–Xgwm301*	*Xgdm116–Xcfd2.1*	5.3	17.7	−0.2	–
QGpc.hwwgr-3BL/3DS	AL02	*IWA2620–IWA3170*	*Xgwm191.1–Xgwm132*	5.5	13.8	0.2	–
QGpc.hwwgr-1DL/7DL	ST02	*Xgdm126–Xbarc66*	*Xwmc634–Xgwm428*	5.6	8.4	0.3	0.42
QGpc.hwwgr-7BL/3BL	ST02	*Xwmc396–IWA116*	*IWA3951–IWA1756*	5.3	6.9	0.2	–
TW							
QTw.hwwgr-7BL/3BS	LA03	*IWA1722–IWA5129*	*IWA6464–IWA3731*	5.9	6.9	−0.6	–
SKW							
QSkw.hwwgr-2BL/5BS	LA03	*IWA3938–IWA7955*	*IWA6946–IWA421*	5.6	16.0	−0.7	–
QSkw.hwwgr-5DL/1DS	AL02	*IWA1681–Xcfd26*	*Xgwm191–IWA5019*	5.0	4.5	0.8	–
QSkw.hwwgr-2AS/1DL	AL02	*Xwmc522–IWA5023*	*Xgdm126–Xbarc66*	5.8	4.2	−0.8	–
NHI							
QNhi.hwwgr-1BL/3AS	ST03	*Xwmc728–IWA4936*	*IWA7771–IWA6387*	5.3	1.8	−3.9	–
SHI							
QShi.hwwgr-4BL/5DL	AL02	*IWA908–Xbarc163*	*Xgwm273–Xgwm654*	5.4	0.9	−4.2	–

[a] LOD score for epistatic effect.
[b] Phenotypic variance explained by epistatic QTL.
[c] Epistatic effect between two loci; a negative number indicates decreased trait value; a positive number indicates increased trait value.
[d] Phenotypic variance explained by the epistatic QTL × environment interaction. '–' indicates no additive × additive × environment effect.

significant only in single environments and explained 5.1% to 8.9% of the phenotypic variance. Among them, *QKd.hwwgr-2DL*, closely linked to *Xgwm539*, was coincident with QTL reported by Breseghello et al. [26] and Ramya et al. [20]. In addition, six QTL for KD were located on the same chromosome regions as the QTL for SKW, agreeing with a previous report that QTL for KD is a major contributor to kernel weight [14]. In the present study, epistasis was not identified for KD and only one QTL, *QKd.hwwgr-6AS*, was involved in AE interaction, explaining only 2.07% of the phenotypic variation, and thus indicating that additive effect mainly contributes to genetic variation of KD.

In this study grain hardness was estimated using both SKCS-hardness index (SHI) and NIR-hardness and only one common QTL with a major effect was located at the *Ha* locus on chromosome 5DS. This QTL was significant across all five environments and explained the highest variation in hardness (52.9% to 61.4% for NHI and 60.2% to 70.4% for SHI). This QTL was reported in many previous studies [4,6], and is the major genetic determinant of grain hardness in wheat. This QTL was also involved in AE interaction, and explained 2.8% of the NHI variance and 1.5% of the SHI variance. A minor QTL on 1BL (*QNhi.hwwgr-1BL*) was detected only by NHI and accounted for about 6.0% of the phenotypic variance in two environments. This QTL likely corresponds to a major QTL reported by Li et al. [6]. For SHI, four additional minor QTL were found on chromosomes 1DL, 2BL, 3AS and 5BL but were significant in only one or two environments and explained 3.9% to 7.2% of the phenotypic variance. The results confirmed that the kernel hardness is mainly controlled by *Ha*locus on 5DS, but some minor QTL may also modify the expression of the gene in different environments.

4.2. QTL with pleiotropic effects

Previous researches showed that QTL/genes in wheat are often located in gene-rich regions and some QTL/genes controlling different traits may be mapped in the same genomic region to form clusters [14,43]. Such QTL may have consistent effect across different traits, and can be used in marker-assisted selection after further validation in relevant populations [14,28,33]. In this study, seven coincident QTL for different traits were detected on chromosomes 2DL, 4BS, 5AL, 5AS, 5DS, 6AL, and 7AL. Among them, two stable QTL clusters influencing GPC, TW, SKW, and KD were mapped on chromosomes 4BS and 5AL, and Ning7840 contributed the favorable alleles, suggesting that the two QTL clusters may in fact be pleiotropic effects. It is also possible that they represent linked genes, especially between QTL for GPC and other three traits in the clusters. Both QTL not only showed major effects on the four traits but were also stable across environments. Several closely linked SNP markers in both clusters (four for the 4BS cluster and nine for the 5AL cluster) suggest that these SNPs might be useful for marker-assisted pyramiding of these QTL to improve grain quality.

Four co-located QTL for SKW and KD were located on chromosomes 2DL, 5AS, 6AL, and 7AL. Clark alleles increased KD at these loci, thus increasing SKW. This is understandable because Clark has larger KD and SKW than Ning7840. Sixteen closely linked SNPs found for these pleiotropic QTL can be used to select new cultivars with larger/heavier seeds.

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