RESEARCH ARTICLE

Conditional QTL mapping of protein content in wheat with respect to grain yield and its components

LIN WANG[1,2*], FA CUI[1,3], JINPING WANG[2], LI JUN[1,4], ANMING DING[1,5], CHUNHUA ZHAO[1,3], XINGFENG LI[1], DESHUN FENG[1], JURONG GAO[1] and HONGGANG WANG[1]

[1]*State Key Laboratory of Crop Biology, Shandong Key Laboratory of Crop Biology, Taian Subcenter of National Wheat Improvement Center, College of Agronomy, Shandong Agricultural University, Taian 271018, People's Republic of China*
[2]*Jining Municipal Academy of Agricultural Sciences, Ji'ning 272031, Shandong, People's Republic of China*
[3]*Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Shijiazhuang, 050021, Hebei, People's Republic of China*
[4]*Tianxing Biotechnology, Handian Industrial Zone, 256200 Zouping, Shandong, People's Republic of China*
[5]*Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Qingdao 266101, People's Republic of China*

Abstract

Grain protein content in wheat (*Triticum aestivum* L.) is generally considered a highly heritable character that is negatively correlated with grain yield and yield-related traits. Quantitative trait loci (QTL) for protein content was mapped using data on protein content and protein content conditioned on the putatively interrelated traits to evaluate possible genetic interrelationships between protein content and yield, as well as yield-related traits. Phenotypic data were evaluated in a recombinant inbred line population with 302 lines derived from a cross between the Chinese cultivar Weimai 8 and Luohan 2. Inclusive composite interval mapping using IciMapping 3.0 was employed for mapping unconditional and conditional QTL with additives. A strong genetic relationship was found between protein content and grain yield, and yield-related traits. Unconditional QTL mapping analysis detected seven additive QTL for protein content, with additive effects ranging in absolute size from 0.1898% to 0.3407% protein content, jointly accounting for 43.45% of the trait variance. Conditional QTL mapping analysis indicated two QTL independent from yield, which can be used in marker-assisted selection for increasing yield without affecting grain protein content. Three additional QTL with minor effects were identified in the conditional mapping. Of the three QTLs, two were identified when protein content was conditioned on yield, which had pleiotropic effects on those two traits. Conditional QTL mapping can be used to dissect the genetic interrelationship between two traits at the individual QTL level for closely correlated traits. Further, conditional QTL mapping can reveal additional QTL with minor effects that are undetectable in unconditional mapping.

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Introduction

Protein content is the most important trait in wheat production and the most important factor determining the end-use quality of wheat for pasta making. In addition, it is essential to human nutrition. However, simultaneous increases of seed protein content and seed yield is difficult to achieve in practical breeding programmes, considering this trait is the product of complex interdependencies between plant developmental traits and yield components. The relationships between the protein accumulation and yield components in wheat have been well documented (Bathia and Rabson 1987; Sourdille *et al*. 1999; Zanetti *et al*. 1999; Perretant *et al*. 2000; Börner *et al*. 2002; Groos *et al*. 2003; Blanco *et al*. 2002, 2006). Each developmental process is to a greater or lesser extent under genetic control and is affected by varying degrees by environmental factors (Chee *et al*. 2001; Börner *et al*. 2002; Groos *et al*. 2003; Prasad *et al*. 2003; Quarrie *et al*. 2006). Protein content in winter wheat has been generally regarded as a highly heritable character. Many mapping experiments for protein content in different winter wheat species have been conducted (Dholakia *et al*. 2001; Blanco *et al*. 2002; Prasad *et al*. 2003; Olmos *et al*. 2003; Huang *et al*. 2003, 2004). Quantitative trait locus (QTL) with additive effects,

*For correspondence. E-mail: wanglinwang@126.com.

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as well as epistatic and environmental interactions have been presented by Kulwal *et al*. (2005), Kumar *et al*. (2007), and Mann *et al*. (2009). However, the relationships of mapped QTL with yield components were not examined, although QTL for thousand grain weight, grain number per spike, and grain weight per spike have been extensively studied (Campbell *et al*. 1999; Marza *et al*. 2006; Araki *et al*. 1999). Nonetheless, their genetic relationship with protein content at QTL level are still poorly understood.

This study aims to evaluate the genetic influence of variation in yield components on protein content. In the present study, QTL for protein content was mapped in a recombinant inbred line (RILs) population derived from a cross between two varieties. The genetic relationship between protein content QTL and those of yield and yield-related components are investigated. A method for multivariable conditional analysis that was proposed for analysing the contributions of component traits to a complex trait and for investigating the genetic relationship between two traits at the QTL level has been adopted in this study (Wen and Zhu 2005). Thus, protein content conditioning on thousand grain weight allows a protein content analysis to be conducted independently of variation in thousand grain weight if protein content is genetically correlated with a secondary trait, such as thousand grain weight.

The conditional values are estimated for the no variation situation in the secondary trait, a method very similar to the estimation of adjusted values in a covariance analysis. Subsequently, the protein content conditioned on the various other traits can be analysed by QTL mapping in the same way as the original protein content. Genetic interdependence between protein and yield components can be identified at individual QTL level by comparing unconditional and conditional QTL for protein content. The results may provide valuable information on marker-assisted selection (MAS) to improve protein content without negative effects on yield.

Materials and methods

Plant materials and genetic map

A population of 302 RILs ($F_{8:9}$) derived from a cross of Weimai 8 × Luohan 2 was used in this experiment. Weimai 8, as the female parent, is a large-spike type of the ideotype model and was released by Weifang Municipal Academy of Agricultural Sciences, Shandong, China, in 2003; Luohan 2, the male parent, is a multi-spike type, released by Luoyang Municipal Academy of Agricultural Sciences, Henan, China, in 2001. The parents together with 302 RILs, were planted in three environments in Shandong province, China: Tai'an in 2008–2009 (E1), Tai'an in 2009–2010 (E2), and Jining in 2009–2010 (E3). A two-row plot with rows 200-cm long and 30 cm apart was used, and 50 seeds were planted in each row. From each plot, 10 representational leading tillers in the middle of the row were selected before harvest as samples to evaluating grain number per spike (GNPS), a one-row plot with rows 100-cm long as samples to evaluate spike number per row (SNPR), grain yield per plot (YLD), grain weight per spike (GWPS) and thousand grain weight (TGW). Grain protein content (GPC), were measured by near-infrared reflectance spectroscopy (NIRS) on a Perten DA-7200 instrument (Perten Instruments, Huddinge, Sweden) and expressed on a 14% moisture basis. The measurements were calibrated using calibration samples. Indeed, NIRS determinations were highly correlated with traditional methods. Again, previous studies have demonstrated the importance of NIRS on wheat quality traits, especially grain protein content or flour protein content (Sourdille *et al.* 1996; Perretant *et al*. 2000; Blanco *et al*. 2002; Kuchel *et al*. 2006; Suprayogi *et al*. 2009; Sun *et al*. 2010).

Analysis of molecular and biochemical markers

Molecular markers of G-SSR, EST-SSR, ISSR, STS, and SRAP were used to genotype the two parents and their derived lines. Of these, relevant information regarding G-SSR markers, including BARC, CFA, CFD, CFT, GWM, GDM, GPW, WMC and PSP codes, as well as PCR-based STS markers of the MAG code, were taken from the GrainGenes website (http://wheat.pw.usda.gov). Relevant information about EST-SSR markers prefixed CFE, KSUM and CNL are publicly available (http://wheat.pw.usda.gov/ITMI/EST-SSR/). EST-SSR markers of SWES and WW codes were developed and kindly provided by Professor Sishen Li, College of Agronomy, Shandong Agricultural University, China. EST-SSR markers with the prefixes CWEM, EDM and CWM were published in reference articles by Peng and Lapitan (2005), Mullan *et al*. (2005) and Gao *et al*. (2004), respectively. ISSR markers were developed by the University of British Columbia Biotechnology Laboratory (UBCBL) (Nagaoka and Ogihara 1997). Relevant information about chromosome 1RS-specific markers of rye were detailed by Zhao *et al*. (2009), and functional markers were detailed by Liu *et al*. (2008) and Liang *et al*. (2010). The differences of high molecular weight glutenin subunits (HMW-GS) at Glu-a1, Glu-b1 and Glu-d1 between the parents were detected and used as biochemical markers.

Each PCR reaction for G-SSR, EST-SSR and PCR-based STS markers was conducted in a total volume of 25 μL in a TaKaRa PCR thermal cycler or a Bio-Rad 9600 thermal cycler. PCR reaction mixture was compounded according to the formula described by Röder *et al*. (1998). Amplifications were performed using a touchdown PCR protocol detailed by Hao *et al*. (2008). PCR reaction mixture, as well as PCR protocol for SRAP and ISSR markers followed the formula and the procedure detailed by Li *et al*. (2007). The PCR products were separated in 6% nondenaturing polyacrylamide gels. Gels were then silver stained and photographed. The types of HMW-GS were detected by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Singh and Shepherd 1988). Markers of BARC, CFA, CFD, GWM, GDM and WMC codes were also screened on the

Table 1. Phenotypic value of parental lines, and the RILs population in the mean across three environments.

	Parent		RILs population ($n = 302$)			
Trait	Luohan 2	Weimai 8	Mean	SD	Min.	Max.
GPC	14.76	12.06	13.52	0.81	11.17	16.42
GNPS	42.25	54.44	53.58	6.36	38.30	68.34
HGW	41.42	49.08	42.20	4.92	30.58	55.63
GWPS	1.27	1.89	1.64	0.27	1.07	2.79
SNPR	133.25	111.50	117.45	20.67	66.50	157.50
YLD	120.54	145.62	160.49	27.54	94.81	235.07

GNPS, grain number per spike; HGW, thousand-grain weight (g); GPC, grain protein content (%); GWPS, grain weight per spike; SNPR, spike number one-meter-long row; YLD, grain yield of one-meter-long-row plot (g).

nullisomic–tetrasomic stocks of Chinese Spring (CSNT) to assign them to chromosomes, where possible.

Construction of the genetic linkage map

Linkage groups were constructed by Mapmaker 3.0 (Lander *et al*. 1987). First, the 'Anchor' command was used to locate marker loci on chromosomes based on the CSNT identification and the public genetic maps in GrainGenes 2.0 (http://wheat.pw.usda.gov/GG2/index.shtml). Then, the assignment of the remaining loci to chromosomes was made using the 'Assign' command at a LOD score of 3.0. Based on the linkage group defined above, JoinMap, v3.0 (Biometris, Wageningen, The Netherlands; http://www.joinmap.nl), was used to construct linkage map, and centiMorgan units were calculated using the Kosambi mapping function (Kosambi 1944).

Data analysis and QTL mapping

To estimate variance and covariance components, all traits were first analysed with the Minque method proposed by Zhu (1992). Phenotypic correlation coefficients between GPC and yield-related traits were calculated from the trait means for the three environments. Basic statistical analysis was implemented by the software SPSS v13.0 (SPSS, Chicago, USA). Conditional genetic analysis was conducted based on the phenotypic values of GPC conditioned on each of YLD related traits, 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|GNPS = protein content conditioned on grain number per spike). Phenotypic variances were calculated from trait means over three locations. Inclusive composite interval mapping by IciMapping 3.0 (http://www.isbreeding.net) was used based on stepwise regression of simultaneous consideration of all marker information (Li *et al*. 2007). The walking speed for all QTL was 1.0 cM. A LOD score of 2.5 was set as a threshold for declaring the presence of a QTL.

Results

Phenotypic variation of traits and correlations to protein content

Table 1 shows the mean, maximal, and minimal trait values calculated from averages over three locations for GPC and yield-related traits. Strong transgressive segregations were observed for all traits; which showed that alleles with positive effects were distributed among the parents. The evaluation of the phenotypic correlation between GPC and different traits (table 2) showed highly significant negative correlations with a high correlation coefficient to YLD and a

Table 2. Phenotypic correlations between protein content and the other traits, and phenotypic variances of protein content and protein content conditioned on the other traits.

		Variance		
Trait	Phenotypic correlation	Direct	Conditioned on	±%
GPC	–	0.963		
GNPS	−0.150**		1.056	+9.028
TGW	0.164**		1.054	+8.747
GWPS	−0.103		0.935	−3.519
SNPR	−0.077		1.001	+3.295
YLD	−0.188**		0.887	−8.525

For abbreviation see table 1. $^{**}P \leq 0.01$.

Table 3. Unconditional QTL for grain protein content and conditional QTL when protein content conditioned on yield-related traits.

Unconditional QTL	Conditional QTL	Env.[a]	Chr.[b]	Position	Marker interval	LOD	PVE (%)[c]	Additive effect[d]
	QGpc|tgw.WL-1D[e]	E2	1D	89	*Xgpw7082.2–Xgdm33.2*	3.1331	5.1250	0.2362
QGpc.WL-2A		E3	2A	85	*Xbarc010–Xcfa2263.2*	3.7574	6.8151	−0.2568
	QGpc|gwps.WL-2A	E3	2A	84	*Xbarc010–Xcfa2263.2*	3.3707	6.4381	−0.2499
	QGpc|gwps.WL-2B	E2	2B	182	*Xcfe140–WW7.1*	2.6313	4.7345	−0.2109
	QGpc|tgw.WL-2B	E2	2B	181	*Xcfe140–WW7.1*	4.3109	8.3382	−0.2972
	QGpc|yld.WL-2B	E2	2B	180	*Xcfe140–WW7.1*	2.7428	6.6070	−0.2428
QGpc.WL-3B		P	3B	100	*Xissr850–Xcau6.2*	2.5768	4.1477	0.1898
	QGpc|tgw.WL-3B	P	3B	100	*Xissr850–Xcau6.2*	3.0727	4.7911	0.1999
	QGpc|snpr.WL-3B	P	3B	100	*Xissr850–Xcau6.2*	2.8279	4.5429	0.1978
QGpc.WL-4A		E1	4A	28	*Xwmc516–BE517017*	3.6091	6.1173	−0.2849
	QGpc|gwps.WL-4A	E1	4A	29	*BE517017–BF293342*	3.5106	7.4797	−0.3145
	QGpc|tgw.WL-4A	E1	4A	29	*BE517017–BF293342*	3.0046	6.5591	−0.2848
	QGpc|gnps.WL-4A	E1	4A	29	*BE517017–BF293342*	3.6870	7.9591	−0.3122
	QGpc|yld.WL-4A	P	4A	80	*Xbarc140.2–Xbarc106*	2.8675	4.8070	0.1831
QGpc.WL-5B		E3	5B	90	*Xbarc110–Xgwm544.1*	3.2196	5.9728	0.3466
	QGpc|tgw.WL-5B	E3	5B	90	*Xbarc110–Xgwm544.1*	3.4176	6.3092	0.3488
	QGpc|yld.WL-5B	E3	5B	90	*Xbarc110–Xgwm544.1*	2.9404	5.2268	0.3271
	QGpc|snpr.WL-5B	E3	5B	90	*Xbarc110–Xgwm544.1*	3.9298	6.9254	0.3777
QGpc.WL-5D		E2	5D	72	*Xcfd78-T2*	2.6071	4.2239	0.3407
	QGpc|gnps.WL-5D	E2	5D	72	*Xcfd78-T2*	2.7002	4.3611	0.3636
	QGpc|snpr.WL-5D	E2	5D	72	*Xcfd78-T2*	2.5437	4.1144	0.3442
QGpc.WL-6B		P	6B	14	*Xcfe254.4–Xswes180.2*	2.9081	9.7311	0.2816
	QGpc|gwps.WL-6B	P	6B	15	*Xcfe254.4–Xswes180.2*	3.0508	10.9021	0.2986
	QGpc|gnps.WL-6B	P	6B	14	*Xcfe254.4–Xswes180.2*	2.9037	10.1436	0.2839
	QGpc|snpr.WL-6B	P	6B	15	*Xcfe254.4–Xswes180.2*	2.7886	9.2516	0.2764
QGpc.WL-7A		P	7A	64	*Xwmc475.1–Xsec-P3.1*	2.6811	6.4528	−0.2718
	QGpc|gwps.WL-7A	P	7A	64	*Xwmc475.1–Xsec-P3.1*	2.7423	6.9296	−0.2786
	QGpc|tgw.WL-7A	P	7A	64	*Xwmc475.1–Xsec-P3.1*	3.1219	7.4074	−0.2861
	QGpc|yld.WL-7A	P	7A	64	*Xwmc475.1–Xsec-P3.1*	2.6089	6.3123	−0.2631
	QGpc|gnps.WL-7A	P	7A	64	*Xwmc475.1–Xsec-P3.1*	2.6512	6.4937	−0.2697
	QGpc|snpr.WL-7A	P	7A	64	*Xwmc475.1–Xsec-P3.1*	2.6774	6.3526	−0.2683
	QGpc|tgw.WL-7A	E2	7A	62	*Xmag600.1–Xwmc475.1*	2.8061	6.2471	−0.3294

[a]Env, environment; [b]Chr, chromosome; [c]PVE, phenotypic variation explained; [d]Positive values indicate that Weimai 8 alleles increase the protein content, negative values indicate that Weimai 8 alleles reduce protein content. [e]*Gpc| gnps*, *Gpc| tgw*, *Gpc|gwps*, *Gpc|snpr*, *Gpc|yld* indicates protein content conditioned on GNPS, TGW, GWPS, SNPR, YLD, respectively (for abbreviations see table 1).

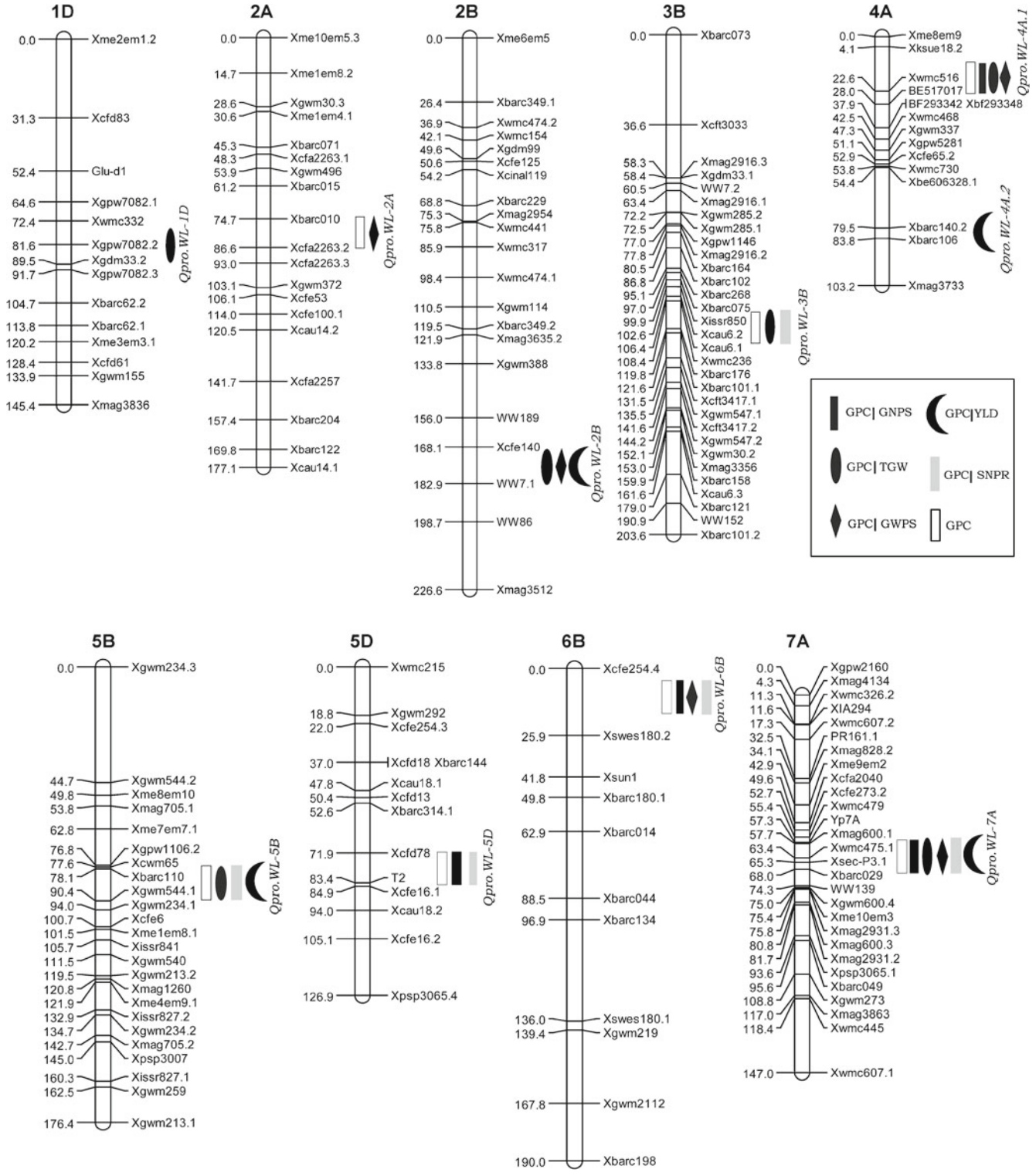

Figure 1. Genetic linkage map and location of putative QTL for grain protein content on the genetic map of 302 RILs derived from the cross Weimai 8 × Luohan 2. The positions of marker loci on chromosomes are listed on the left of the corresponding chromosomes. Map distances are shown in centiMorgans and were calculated using the Kosambi (1944) mapping function. A putative QTL with LOD > 2.5 is placed on its corresponding flanking markers. QTL symbols are described below chromosome 4A.

smaller correlation coefficient to GNPS. In addition, highly significant positive correlations to TGW were observed, whereas no significant correlations were found with GWPS and SNPR.

Relationship of QTL for GPC to the other evaluated traits

Table 3 and figure 1 show the unconditional QTL for GPC and conditional QTL when protein content was conditioned on yield and yield-related traits. Table 4 compares the variance of the conditional QTL for grain protein content conditioned on yield and different yield related traits. Unconditional mapping identified seven QTL for GPC, with additive effects ranging in absolute size from 0.1898% to 0.3407% protein content, together accounting for 43.4456% of the trait variance. Three of the seven QTL did not show significant effects when GPC was conditioned on GNPS, indicating a strong genetic association between these two traits. Four QTL were detected in conditional mapping, with additive effects ranging in absolute size from 0.2697% to 0.3636%, together accounting for 28.9575% of the variance of the conditional trait. Three of the seven QTL failed to show significant effects when GPC was conditioned on TGW, and two additional QTL were detected in conditional mapping, with additive effects ranging in absolute size from 0.2362% to 0.2972%, together comprising 38.9234% of the conditional trait variance, values close to the 43.4456% that was found for the unconditioned GPC.

QTL mapping for GPC conditioned on GWPS showed one QTL each in linkage groups 2A, 4A, 6B, and 7A, respectively, which were independent from these two traits. Meanwhile, the QTL on linkage groups 3B, 5B, and 5D showed no significant effects when conditioned on GWPS. And one additional QTL was detected with additive effects of 0.2109% in absolute size, yielding five QTLs in all, which, together, comprised 36.8610% of the conditional trait variance. Two QTL showed no significant effects when GPC was conditioned on SNPR, whereas the remaining five QTL comprised 31.1869% of the conditional trait variance. However, only two QTL have shown significant effects when GPC was conditioned on YLD. Moreover, two additional QTL were detected with additive effects. Together, these four QTL accounted for only 22.9531% of the conditional trait variance, a significantly smaller fraction.

Discussion

Genetic relationship between GPC and YLD and its components

Zhu (1995) first introduced a new methodology for conditional genetic analysis which was later used to study developmental quantitative genetics in mice (Atchley and Zhu 1997), rice (Shi *et al*. 2001), and cotton (Zhu 1995; Ye *et al*. 2003). A method for multivariable conditional analysis for analysing the contributions of component traits to a complex trait was also proposed (Wen and Zhu 2005). Further, by combining the conditional genetic analysis with unconditional QTL mapping, it was extended to map conditional QTL for a molecular dissection of the development of traits like plant height and tiller number during plant growth in several studies on rice (Yan *et al*. 1998a,b; Cao *et al*. 2001; Wu *et al*. 2002) and to evaluate the genetic contributions of yield components to yield (Guo *et al*. 2005). This methodology was used in the present study to analyse the interrelationships between protein content and yiled and yield-related traits in wheat.

In the present study, the close relationship between GPC and YLD was reflected in the strong negative correlation between the two traits and in the strong reduction of variance (tables 2 and 4) when GPC was conditioned on YLD. Accordingly, five of the seven initially mapped QTL for GPC failed to show significant effects in the conditional data.

Table 4. Phenotypic variation explained when grain protein content conditioned on yield and different yield-related traits.

			Conditional QTL (%)				
QTL	Marker interval	Unconditional QTL (%)	GPC|GNPS[a]	GPC|TGW	GPC|GWPS	GPC|SNPR	GPC|YLD
QGpc|tgw.WL-1D	*Xgpw7082.2–Xgdm33.2*			5.1250			
QGpc.WL-2A	*Xbarc010–Xcfa2263.2*	6.8151			6.4381		
QGpc|yld.WL-2B	*Xcfe140-WW7.1*			8.3382	4.7345		6.6070
QGpc.WL-3B	*Xissr850–Xcau6.2*	4.1477		4.7911		4.5429	
QGpc.WL-4A	*Xwmc516–BE517017*	6.1173	7.9591	6.5591	7.4797		
QGpc|yld.WL-4A	*Xbarc140.2–Barc106*						4.8070
QGpc.WL-5B	*Xbarc110–Xgwm544.1*	5.9728		6.3092		6.9254	5.2268
QGpc.WL-5D	*Xcfd78-T2*	4.2239	4.3611			4.1144	
QGpc.WL-6B	*Xcfe254.4–Xswes180.2*	9.7311	10.1436		10.9021	9.2516	
QGpc.WL-7A	*Xwmc475.1–Xsec-P3.1*	6.4528	6.4937	7.4074	6.9296	6.3526	6.3123
Total		43.4456	28.9575	38.9234	36.8610	31.1869	22.9531

[a]*Gpc\|gnps*, *Gpc\|tgw*, *Gpc\|gwps*, *Gpc\|snpr*, *Gpc\|yld* indicates protein content conditioned on GNPS, TGW, GWPS, SNPR, YLD, respectively (for abbreviations see table 1).

These QTL are likely to represent genes involved in protein synthesis. However, two other QTL with very small reductions were found in the conditional additive effects. These additional QTL should represent genes influencing protein content independently from YLD formation. Such genes are of special interest in high-protein content wheat breeding because they would allow increased protein content with a concomitant increase in high yield.

Similarly, the close relationship between GPC and GNPS was reflected in the strong negative correlation (tables 2 and 4) between the two traits and in the strong reduction of variance when GPC was conditioned on GNPS. Of the seven initially identified QTL for GPC, three failed to show significant effects. The expression of these genes was presumably affected by the genes affecting grain number formation. Nevertheless, four other QTL with very small changes in conditional additive effects were identified. These QTL should influence protein content independently from GNPS formation. Such QTL are potentially useful in high-protein content wheat breeding because they would allow an increase in protein content with a concomitant increase in GNPS, which have a positive correlation with yield.

In the RIL population, GPC showed significantly positive correlation to TGW (table 2). This relationship was reflected by the larger location change and number of QTL identified when GPC was conditioned on TGW (table 4). Three QTL failed to show significant effects, whereas two additional QTL were identified in conditional mapping. These QTL have effects on both GPC and TGW. In addition, four QTL showed only a small change in variance in conditional mapping, indicating that the expression of these genes on protein formation was independent of the expression of the genes affecting TGW. Similarly, such genes are important because they improve protein content independent of TGW, which is positively correlated with both yield and most variable yield component trait (Chastain *et al*. 1995; Donmez *et al*. 2001; Groos *et al*. 2003).

The RIL population analyses showed broad segregation in GWPS (table 1), due to parental lines that were strongly divergent in two traits. Despite this broad variation, the evidence for a genetic interrelationship between GPC and GWPS was weak (table 2). In addition, the reduction in variance (table 4) when GPC was conditioned on GWPS was negligible, indicating that most of the protein content variation occurred independently of GWPS variation. Among the seven QTL for GPC, only three failed to show significant effects when GPC was conditioned on GWPS. Of these three, one showed the smallest effects, whereas the other two had relatively larger effects. Further, one additional QTL was detected by conditional mapping. These four QTL may be involved in an interaction between GPC and GWPS. Considering that the correlation between GPC and GWPS was not significant, the three QTL, especially the two QTL with larger effects involved in the interaction between the two traits identified in this study, can be valuable in high GPC wheat breeding. They would allow a simultaneous increase in GPC and GWPS, with the latter trait being the most important component of YLD. Several studies have found GWPS to be positively correlated with YLD (Ashfaq *et al.* 2003; Knežević *et al.* 2008).

Apart from GWPS, SNPR showed the least significant correlations (table 2) to GPC in the segregating RIL population. Although the reduction in variance was small (table 4) when GPC was conditioned on SNPR, there were two QTL failed to show significant effects in conditional mapping. These two QTL may be involved in an interaction between GPC and GWPS. Meanwhile, the other five QTL showed nearly large effects in unconditional mapping. The correlation between GPC and SNPR was not significant, thus, QTL, the effects of which remained unchanged in this study, can be useful in high-yield and high-quality wheat breeding, because they would permit GPC and SNPR to increase simultaneously.

Comparison of the present study with previous studies

GPC and yield and its components in wheat has been subjected to monosomic or QTL analysis in many other reports. In most cases, they were reported individually, but not simultaneously in one report (Prasad *et al*. 1999, 2003; Perretant *et al*. 2000; Campbell *et al*. 2001; Olmos *et al*. 2003; Pushpendra *et al*. 2007). Although several other reports have simultaneously documented QTL for GPC, YLD, and its components either by traditional QTL or association mapping analysis, no conditional QTL mapping method was implemented to dissect their genetic relationships (Prasad *et al*. 1999; Groos *et al*. 2003; Blanco *et al*. 2006). The present study first evaluated the genetic relationship between GPC and YLD and its components at the individual QTL level using a combined unconditional and conditional QTL mapping method, thus, enhancing the understanding of the genetic control system involved in GPC and YLD synthesis.

Most QTL reported in this study were consistent with previous reports. *QGpc.WL-1D*, an extra conditional QTL for GPC without the influences of TGW, was mapped to a position similar to the QTL for GPC reported by Kulwal *et al.* (2005). Pushpendra *et al*. (2007) also located a QTL for GPC on 1D. *QGpc.WL-2A* corresponds to QTL for GPC reported by Joppa and Cantrell (1990) and Groos *et al*. (2003). At the same interval, Blanco *et al*. (2006) detected a QTL for GPC with the common flanking marker, *Xcfa2263*, which accounts for about 16.8% of phenotypic variance. This QTL appeared to be the most promising because of a lack of association with low GWPS. More recently, Suprayogi *et al*. (2009) reported a protein content QTL on 2AS. Single marker analysis indicated that this QTL was not associated with kernel weight variation, and hence, would be potentially useful in breeding programmes. *QGpc.WL-3B* was coincident with the QTL reported by Peleg *et al*. (2009). In addition, Sun *et al*. (2008) located a QTL for GPC also at this interval with additive effect values of 0.26%, accounting for 13.34% of the phenotypic variance of the trait. *QGpc|yld.WL-4A*, a conditional QTL for GPC without the influences of YLD was

highly congruent to the QTL for GPC detected by Peleg *et al*. (2009). *QGpc.WL-5B* corresponded to the QTL for GPC on 4A reported by Peleg *et al*. (2009) using a RIL population from a cross between durum wheat and wild emmer wheat. *QGpc.WL-5D* was in agreement with the QTL for GPC identified by Law *et al*. (1978). Turner *et al*. (2004) showed that a RIL population revealed one significant QTL influencing GPC on the long arm of 5D with an additive effect of 0.096%, constituting 19.4% of the population variation. In addition, Mansur *et al*. (1990) located a QTL for flour protein content at the same interval. *QGpc.WL-6B* was mapped to a position similar to that of the QTL for GPC detected by Blanco *et al*. (1996), Joppa *et al*. (1997), Olmos *et al*. (2003), Prasad *et al*. (2003), and Turner *et al*. (2004). Peleg *et al*. (2009) detected a main QTL for GPC in the same region with effects accounting for 12.0% to 13.7% of the phenotypic variance. In addition, Sun *et al*. (2008) located a QTL for GPC on 6B with a common flanking marker (*Xswes180*), however, this QTL was located on the near end of the long arm instead. Suprayogi *et al*. (2009) also identified a location for GPC on 6BL. *QGpc.WL-7A* corresponds to the QTL for GPC reported by Sourdille *et al*. (1999), Börner *et al*. (2002), Prasad *et al*. (2003), Groos *et al*. (2003), Kumar *et al*. (2007), and Peleg *et al*. (2009). Turner *et al*. (2004) reported a QTL for GPC, contributing 8.8% to 10.3% of the variation between years. Suprayogi *et al*. (2009) detected a QTL expressed consistently in four of six environments, with effects ranging from 0.18% to 0.46%.

QTL expression can be influenced by inner and external factors, such as environment, genotype, development stage, and related traits. Such QTL are unstable and specific and may be suitable in specific environments and development stages, or may be suitable in only one population for MAS. In the present study, we found one 'stable' QTL on 7A, accounting for about 0.27% of the additive effect of GPC. This QTL exhibited similar effects when protein content was conditioned on four yield-related traits, indicating that this QTL is not affected by yield. Such QTL are thus important for MAS in wheat breeding.

In total, three additional QTL for GPC undetected in unconditional mapping can be detected in conditional mapping. In QTL mapping, the likelihood of detecting a QTL depends on the ratio between the variance caused by the QTL effects and the total variance of the trait, as well as on the mapping population size (Lander and Botstein 1989). In conditional QTL analysis, the effects of conditional traits on QTL are reduced, whereas QTL with effects below a certain threshold become virtually undetectable. The QTL for protein content initially mapped in the unconditioned data showed effects ranging from 0.19% to 0.35%, indicating a detection threshold at about 0.18%. With allelic effects ranging from 0.19% to 0.25%, the effects of the QTL detected only in the conditional mapping are smaller, and in part, well below the detection threshold in unconditional mapping. Thus, in TGW for instance, the strong reduction in variance observed after conditioning GPC on TGW has obviously allowed the mapping of QTL with smaller effects. In the case of two strongly correlated traits, such as protein content and TGW, conditional mapping can therefore be used to reveal additional QTL that would have otherwise remained below the detection threshold in unconditional mapping.

In conclusion, new QTL controlling the target trait, which were previously undetected by unconditional QTL mapping, were detected by conditional QTL mapping via the combined unconditional and conditional QTL mapping method. Thus, the application of unconditional and conditional mapping method can reflect the net contribution of each causal traits to the resultant trait at QTL level, which may be useful in improving the desirable (resultant) breeding traits.

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