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Mapping of Quantitative Traits Loci for Grain Protein Content in Common Wheat

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ABSTRACT

The main objective of the present study was to identify alleles of quantitative trait loci (QTLs) for grain protein content (GPC) in 95 doubled haploid (DH) lines of a mapping population derived from a cross between common wheat lines Chinese Spring and SQ1 grown in Southeast Kazakhstan. The GPC of DH lines was significantly different between rainfed and irrigated sites ($P<0.05$). In total, 10 QTLs for GPC were found under the two treatments for moisture availability. Two QTLs for GPC under rainfed conditions were predicted to be novel in comparison to those reported earlier. The novel QTLs were mapped onto chromosomes 2BS and 5DL in the population grown under rainfed conditions. Closely-linked DNA markers were identified for the majority of mapped QTLs. The results could be implemented in a local breeding program for the wheat grain quality improvement by using marker-assisted selection. This study is further contribution towards better understanding of the genetic control of GPC in common wheat.

Keywords: DNA markers, genetic map, grain quality traits, *Triticum aestivum* L.
Abbreviations: AFLP, amplified fragment length polymorphism; **DH**, doubled haploid; **GPC**, grain protein content; **LOD**, logarithm of odds; **MAS**, marker-assisted selection; **QTL**, quantitative trait locus; **RFLP**, restriction fragment length polymorphism; **SSR**, simple sequence repeat

INTRODUCTION

Grain protein content (GPC) in common wheat *Triticum aestivum* L. is one of the important grain quality traits determining its nutritional and end-use value. In wheat, grain proteins categorized based on their solubility in different solvents on albumins, globulins, gliadins, and glutenins. The variation in protein content as well as their composition can significantly modify grain quality for bread-making (Weegels *et al.* 1996; Branlard *et al.* 2001). Although grain protein composition s primarily controlled by genotype, it is also significantly affected by environmental factors and their interactions (Dholakia *et al.* 2001; Zhu and Khan 2001; Triboi *et al.* 2003).

Grain protein quantity is a typical quantitative trait controlled by a complex genetic system. The genetic components of GPC have been extensively studied in durum and bread wheat (Joppa *et al.* 1997; Perretant *et al.* 2000; Zanetti *et al.* 2001; Blanco *et al.* 2002; Groos *et al.* 2003; Olmos *et al.* 2003; Prasad *et al.* 2003; Blanco *et al.* 2006; Huang *et al.* 2006). Application of molecular markers, including RFLPs (restriction fragment length polymorphisms), AFLPs (amplified fragment length polymorphisms), and SSRs (simple sequence repeats) has helped to facilitate the construction of detailed chromosomal maps and allowed to map large number of quantitative trait loci (QTLs) on wheat chromosomes. In several research publications (Joppa *et al.* 1997; Perretant *et al.* 2000; Dholakia *et al.* 2001; Zanetti *et al.* 2001; Blanco *et al.* 2002; Borner *et al.* 2002; Groos *et al.* 2003; Prasad *et al*. 2003; Blanco *et al.* 2006) it was shown that factors influencing grain protein concentration in wheat are located in almost all wheat chromosomes. Joppa *et al.* (1997) had detected a major QTL explaining 66% of the phenotypic variation for GPC located on chromosome 6B of durum wheat. Blanco *et al.* (2002) detected seven QTLs for GPC, located on the chromosome arms 4BS, 5AL, 6AS (two loci), 6BS, 7AS and 7BS, which were significant in at least one environment at $P<0.001$ or in at least two environments at $P<0.01$. Groos *et al*. (2003) have identified 'stable' QTLs (i.e. detected in at least four of six field locations) for GPC on chromosomes 2A, 3A, 4D and 7D, each explaining about 10% of the phenotypic variation of GPC. Among these four 'stable' QTLs for GPC detected by these authors, none were co-located with any of the known storage protein loci, located on chromosome groups 1 and 6, or with previously described QTLs for GPC (Joppa *et al.* 1997; Prasad *et al.* 1999; Perretant *et al.* 2000; Zanetti *et al.* 2001). Distelfeld *et al.* (2006) exploiting extensive wheat-rice colinearity, developed a high-density molecular map of the wheat chromosome 6BS region and mapped the QTL as a simple Mendelian locus designated Gpc-B1. To tag this QTL the authors had developed a high-throughput codominant marker Xuhw89. A 4-bp deletion present in the *Triticum turgidum* ssp. *dicoccoides* accession (DIC) allele was absent in a collection of 117 cultivated tetraploid and hexaploid wheat germplasm, suggesting that this marker will be useful to incorporate the high GPC allele from the DIC accession studied into commercial wheat varieties (Distelfeld *et al*. 2006). Despite the progress in dissecting QTLs for protein content, the fact that a large number of a cultivars with inevitably different alleles determining GPC grow under various environmental conditions means that validation of detected QTLs and identification of new genes regulating GPC in a particular environment is still far from complete.

The objectives of this study were (1) to identify and map QTLs for grain protein content in a bread wheat DH population grown under different water regimes in Southeast Kazakhstan, and (2) to identify DNA markers tightly linked to GPC QTLs and potentially suitable for marker-assisted selection for better grain quality of wheat.

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Original Research Paper

MATERIALS AND METHODS

Plant material

The genetic map was constructed earlier using a population of 95 doubled haploid lines from the cross between two common wheat (*T. aestivum* L.) varieties Chinese Spring and SQ1 (CSDH lines; Quarrie *et al.* 2005). The map consists of 567 RFLP, AFLP, SSR, morphological and biochemical markers covering all 21 chromosomes, with a total map length of 3522 cM (Quarrie *et al.* 2005). In 1999 and 2005 CSDH lines, 'Chinese Spring' and 'SQ1' were tested in randomized single-row plots with 10 cm between plants and 30 cm between rows in two replicated field experiments carried out under rainfed and irrigated conditions at two experimental farms of the Kazakhstan Research Institute of Agriculture, Almaty region, Kazakhstan.

Quality analyses

GPC was determined using near-infrared reflectance (NIR) spectroscopy on a Pacific Scientific 4250 using a 9-g ground-wheat grain sample from each plot (Gomez-Bessera *et al.* 2009). The method had been calibrated on a sample set of wheat genotypes assayed in parallel with the Kjeldahl method. GPC was calculated by multiplying nitrogen contents in seeds using a conversion factor of N × 5.7 expressed on a 14% moisture basis.

Statistical analysis and QTL mapping

Standard statistical analyses were performed using GraphPad InStat 3.06 (San Diego, CA) and QSTATS software (QTL CARTOGRAPHER suite; Wang *et al*. 2006). The presence of QTLs was determined using composite interval mapping method with the computer package QTL CARTOGRAPHER v 2.5 (Wang *et al.* 2006). The minimum LOD threshold was 2.0.

RESULTS

The GPC of DH lines was tested in 1999 and 2005 in non-irrigated and irrigated sites. In 1999, the GPC for DH lines ranged from 15.6 to 24.7% in the rainfed site and from 14.6 to 25.1% in the irrigated site (**Fig. 1**). Mean GPC differed between rainfed (20.7%) and irrigated (21.3%) sites (n=77, $P<0.05$). In 2005, the difference in mean GPC between non-irrigated and irrigated sites was extremely significant (n=71, $P<0.0001$), with GPC in the non-irrigated site varying from 14.2 to 25.3%, and in the irrigated site from 18.1 to 26.4% (**Fig. 1**). In rainfed sites the highest GPCs were recorded in

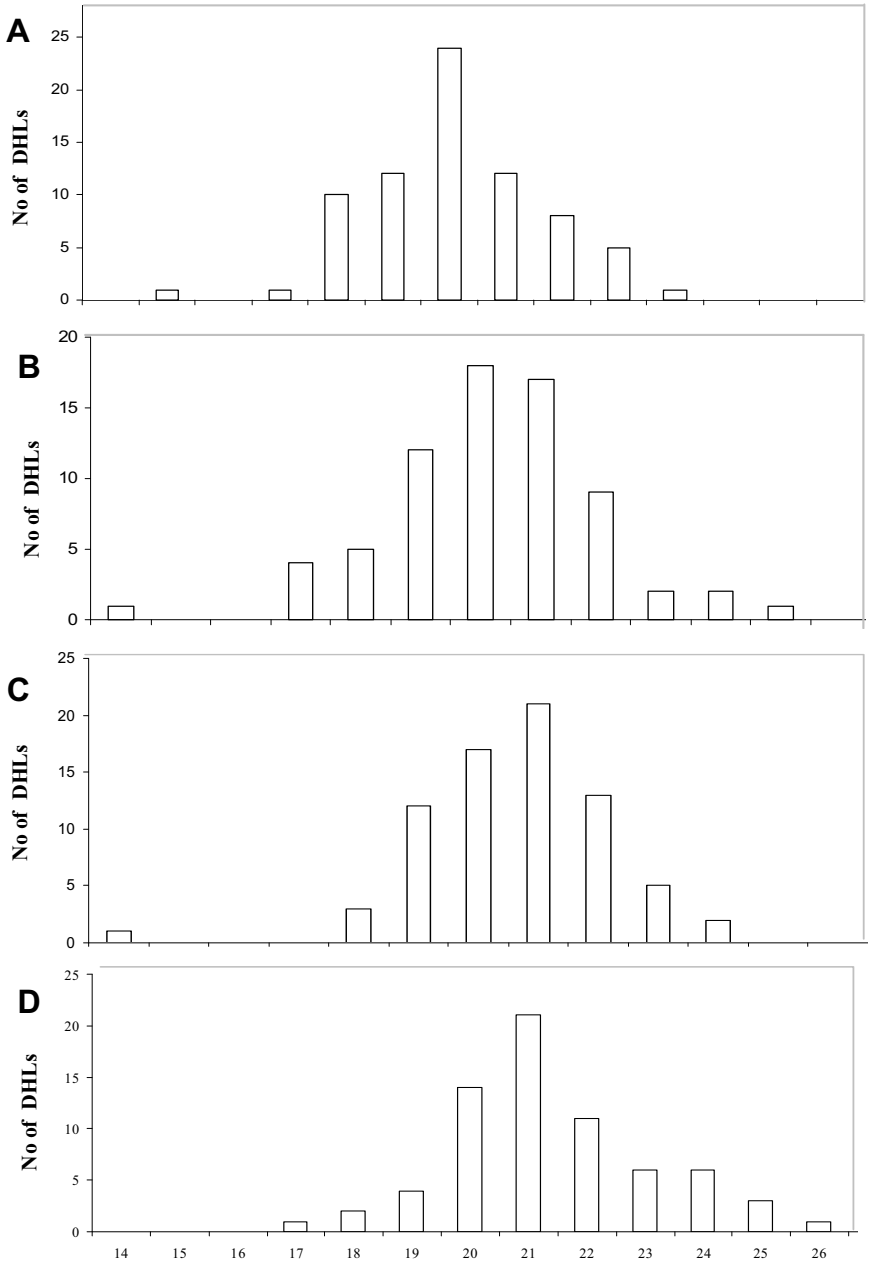

Fig. 1 The grain protein content in DH lines of Chinese Spring x SQ1 in irrigated (A, C) and non-irrigated (B, D) conditions in 1999 (A, B) and 2005 (C, D).

Table 1 Quantitative trait loci for grain protein content detected in the doubled haploid *Chinese Spring x SQ-1* wheat population and their characteristics using composite interval mapping.

QTL	Trial	LOD	DNA marker	R^2	Additive effect
QGpc.csdh-1BL	R05	2.40	m92p78.2	0.09	0.55
QGpc.csdh-2BS	R99	4.10	barc124**	0.11	-0.56
QGpc.csdh-5AL	I05	5.62	psr575.2***	0.23	0.89
QGpc.csdh-5BL	R05	3.10	m51p65.4**	0.12	0.66
QGpc.csdh-5DL	R99	2.70	gwm292**	0.08	0.46
QGpc.csdh-6AS	R05	3.10	m87p78.7	0.18	1.03
QGpc.csdh-6BS	I99	3.00	psp3118*	0.10	-0.05
QGpc.csdh-7AL	I99	3.92	wmc273**; m51p65.7****	0.14	0.64
QGpc.csdh-7AL	R99	7.00	wmc273**; m51p65.7****	0.26	0.82
QGpc.csdh-7DL	R99	6.63	wmc273***	0.20	0.72
QGpc.csdh-7DS	I05	4.20	barc 154	0.16	-0.71

QTL: qualitative trait loci, LOD: Logarithm of odds, R: rainfed conditions, I: irrigated conditions
*, **, ***, **** – Significantly linked at the 0.05, 0.01, 0.001, and 0.0001 probability levels, respectively, *QGpc.csdh-7AL* at I99 and R99 plots is considered as the same QTL

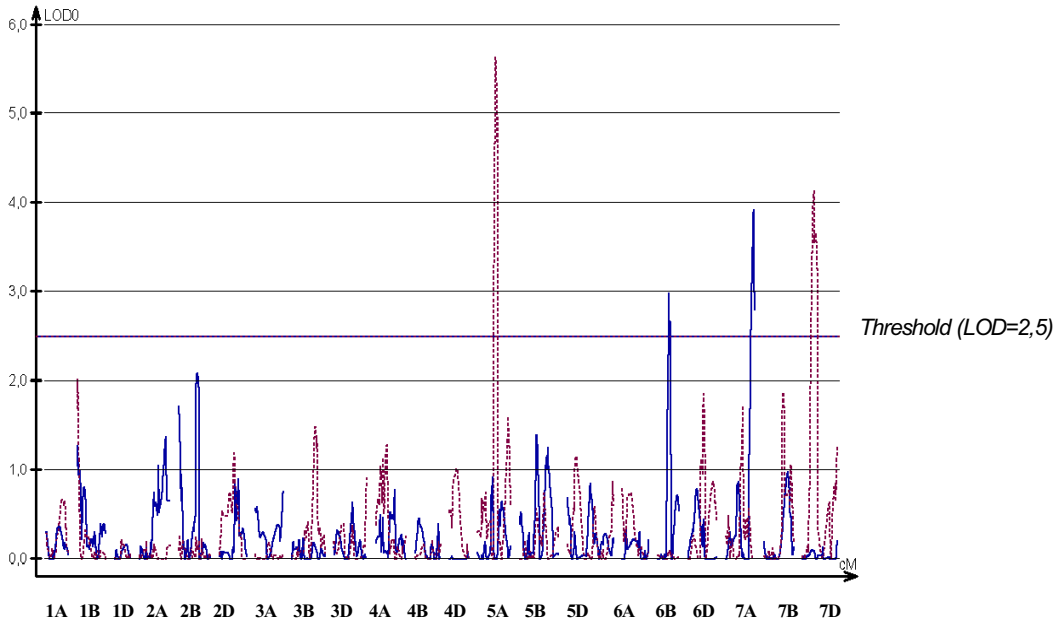

Fig. 2 QTLs for grain protein content localized on the chromosomes of genetic map of hexaploid wheat (*Triticum aestivum* L.) *Chinese Spring x SQ1* in irrigated conditions of 1999 (solid line) and 2005 (dotted line).

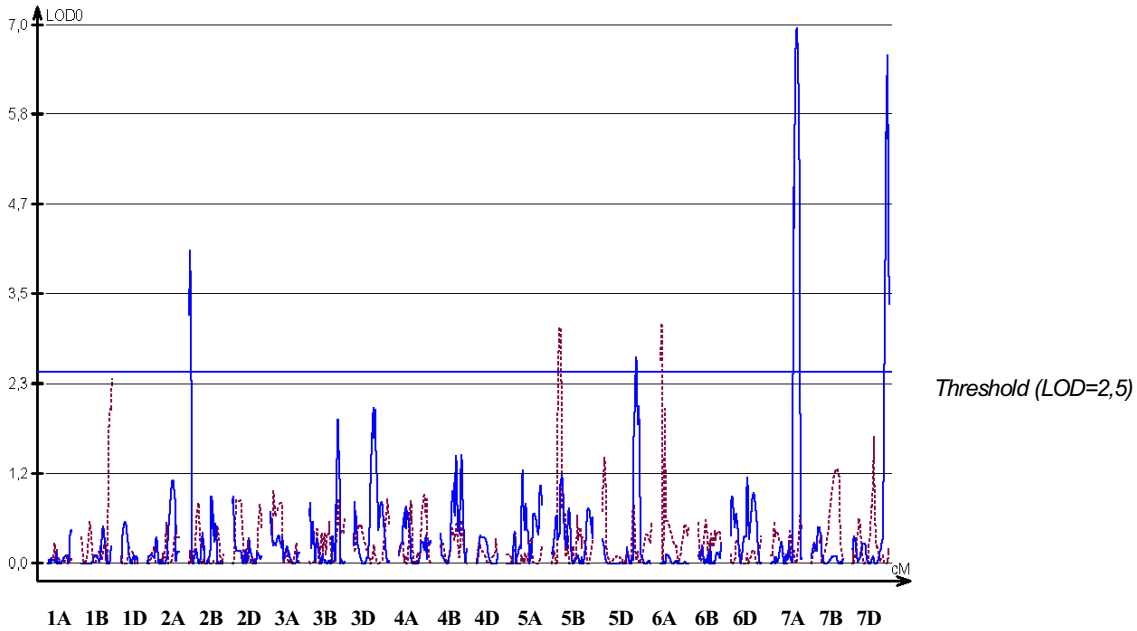

Fig. 3 QTLs for grain protein content on the genetic map of hexaploid wheat (*Triticum aestivum* L.) *Chinese Spring x SQ1* in rainfed conditions of 1999 (solid line) and 2005 (dotted line).

DH line 46 (24.7%) in 1999 and in DH line 31 (25.3%) in 2005. In irrigated sites the best GPCs were recorded in DH line 104 (25.1%) in 1999 and in DH line 45 (26.4%) in 2005.

Ten QTLs for GPC with a LOD score of at least 2.0 were identified on nine chromosomes (**Table 1**), with QTLs on 7AL chromosome were considered as the same one. Eight out of those 10 QTLs had a LOD score higher than 3.0 (**Figs. 2, 3**).

Three major QTLs designated as *QGpc.csdh-6AS*, *QGpc.csdh-7AL-1* and *QGpc.csdh-7DL* explained 18, 26, and 20% (P<0,001), respectively, of the phenotypic variance revealed in non-irrigated conditions (**Table 1**). For the irrigated site the only major *GPC* locus (R^2=0.23) was observed on chromosome 5AL (*QGpc.csdh-5AL*).

The QTLs *QGpc.csdh-5AL*, *QGpc.csdh-6BS*, and *QGpc.csdh-7DS* were expressed only in irrigated conditions while *QGpc.csdh-1BL*, *QGpc.csdh-2BS*, *QGpc.csdh-5BL*, *QGpc.csdh-5DL*, *QGpc.csdh-6A*, and *QGpc.csdh-7DL* were expressed only under rainfed conditions.

The *QGpc.csdh-7AL* was detected both in non-irrigated and irrigated conditions and explained from 14 to 26% of the total variation (**Table 1; Figs. 2, 3**). The increasing allele effect of this QTL came from CS (additive effect 0.64-0.82, **Table 1**). In fact, the CS allele increased GPC for the majority of QTLs. The SQ1 alleles increased GPC for only two QTLs, located on chromosomes 2BS and 7DS (**Table 1**).

Obtained results suggested that SSR markers gwm292 and barc124 could be used for further genetic analyses of *QGpc.csdh-5DL* and *QGpc.csdh-2BS* (P<0.001), respectively. Both SSR markers were nearly coincided with the peaks of QTLs (**Fig. 4**). The map position of QTLs indicate that psr575.2 (P<0.001, 5AL), m51p65.7 (P<0.0001, 7AL), and wmc273 (P<0.001, 7DL) can be selected as a tightly linked DNA markers for *QGpc.csdh-5AL*, *QGpc.csdh-7AL-1*, and *QGpc.csdh-7DL*, respectively (**Table 1**). The detailed locations of all identified QTLs in both irrigated and rainfed conditions are shown in **Fig. 4**.

DISCUSSION

GPC is a very important quantitative trait that is directly related to the nutritional quality of wheat end products. It is also a very complex trait that is under the control of many genes spread out over the entire wheat genomes.

Eight GPC QTLs found in this work might be similar to those that have been identified in previous studies. For example, the chromosomal location of the QTL on chromosome 1BL (*QGpc.csdh-1BL*) is very similar to that found in Zanetti *et al.* (2001). The QTL for protein content (*QGpc.csdh-6BS*) that explained 9.8% of the phenotypic variation found in this work is likely to be related to the QTL detected in chromosome 6BS by Olmos *et al.* (2003) and Prasad *et al.* (2003). This gene, also known as *GPC-B1*, which was cloned, providing better understanding of GPC genetic control and also association with variation of zinc and iron content (Uauy *et al.* 2006). The locations of *QGpc.csdh-5BL*, *QGpc.csdh-7AL* and *QGpc.csdh-7DL* found under non-irrigated conditions were similar to QTLs that were mapped in the study of Groos *et al.* (2003). The QTL localized on chromosome 7DS (*QGpc.csdh-7DS*) in this work most probably is similar to that found in Prasad *et al.* (2003). Only two QTLs appeared not to have been reported previously. They were identified under rainfed conditions (*QGpc.csdh-2BS* and *QGpc.csdh-5DL*, **Table 1**).

Most of the revealed QTLs were specific to either non-irrigated or irrigated treatments, but the QTL for GPC on chromosome 7AL was common in both conditions. The identification of *QGpc.csdh-7AL* suggests the existence of a key gene that influences GPC under different water regime conditions. Environment greatly varied the expression of the gene.

A number of DNA markers that tightly linked with chromosome locations for the mapped QTLs for GPC were identified (**Table 1, Fig. 4**). The significant effects of major QTLs on GPC in the south-east of Kazakhstan reported in this study suggest that some marker-assisted selection (MAS) for these loci would be valuable for breeding programs.

In general, the complex genetic nature of GPC requires significant research efforts under a broad range of environmental conditions to identify favourable alleles that may help to improve grain quality in different regional breeding programs. In this study we have used a well-developed mapping population under two water regime conditions to identify DH lines with high levels of GPC in order to introduce them to local breeding program, to map new QTLs for GPC, and to reveal DNA markers for MAS-assisted breeding programs for the improvement of wheat grain quality in Kazakhstan.

CONCLUSIONS

This study is a further contribution towards better understanding of genetic complexity of GPC in wheat. Obtained results demonstrated significant level of variation in GPC content among doubled haploid lines of a mapping population under two water regime conditions. Two novel QTLs for GPC were identified and mapped in the population grown under rainfed conditions. Better understanding of the genetic control of GPC may provide necessary strengths to breeding programs related to GPC improvement.

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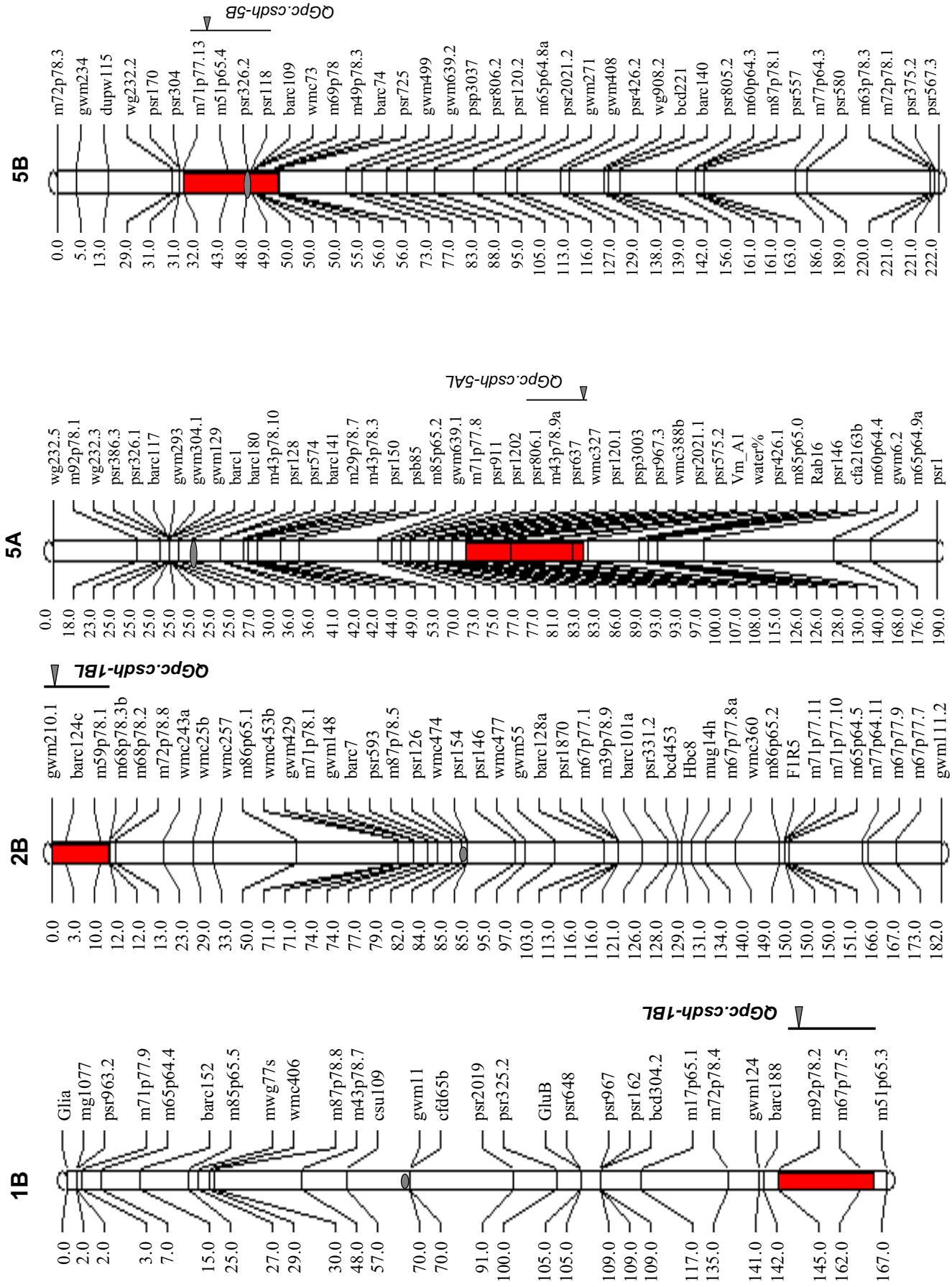
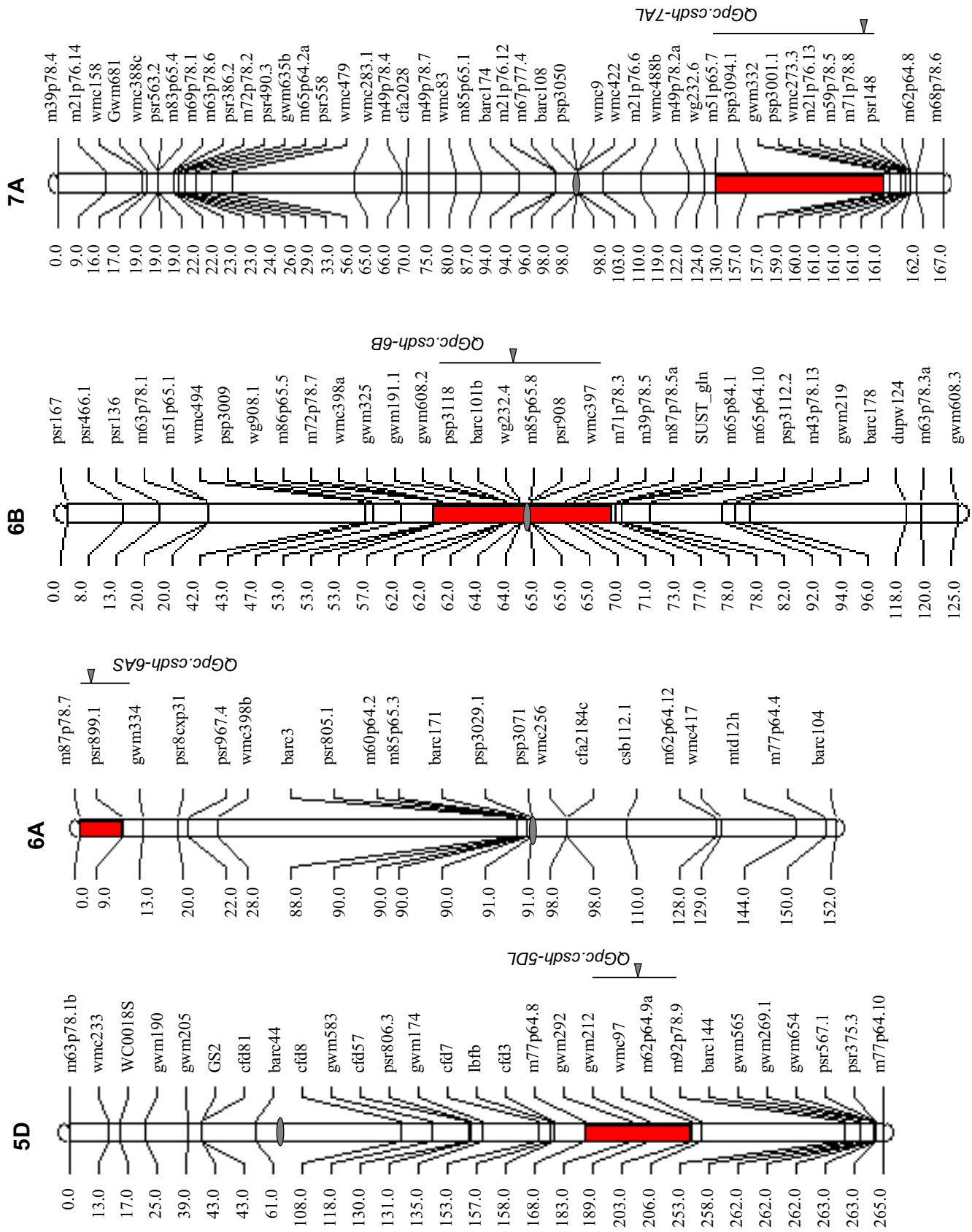


Fig. 4 The locations of QTLs for GPC in 95 doubled-haploid lines of SQ1 x Chinese Spring mapping population grown in South-East of Kazakhstan. QTL regions are shown in bars. The peaks of QTL are indicated by arrows.



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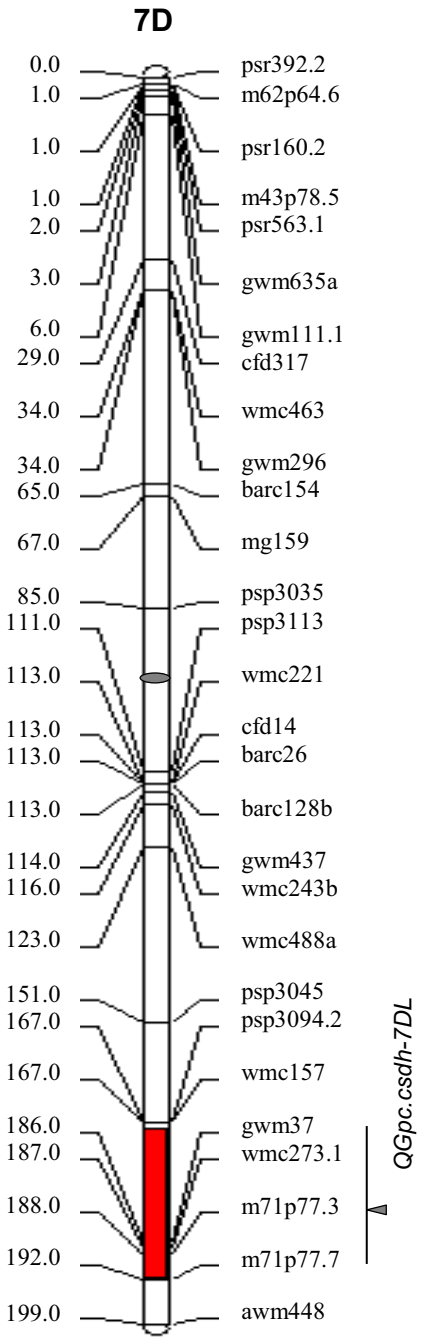
7D
0.0 psr392.2
1.0 m62p64.6
1.0 psr160.2
1.0 m43p78.5
2.0 psr563.1
3.0 gwm635a
6.0 gwm111.1
29.0 cfd317
34.0 wmc463
34.0 gwm296
65.0 barc154
67.0 mg159
85.0 psp3035
111.0 psp3113
113.0 wmc221
113.0 cfd14
113.0 barc26
113.0 barc128b
114.0 gwm437
116.0 wmc243b
123.0 wmc488a
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187.0 wmc273.1
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192.0 m71p77.7
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QGpc.csdh-7DL