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ORIGINAL PAPER

Identification and validation of quantitative trait loci for grain protein concentration in adapted Canadian durum wheat populations

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Abstract Grain protein concentration (GPC) is one of the most important factors influencing pasta-making quality. Durum wheat (*Triticum turgidum* L. var *durum*) cultivars with high GPC produce pasta with increased tolerance to overcooking and greater cooked firmness. However, the large environmental effect on expression of GPC and the negative correlation with grain yield have slowed genetic improvement of this important trait. Understanding the genetics and identification of molecular markers associated with high GPC would aid durum wheat breeders in trait selection at earlier generations. The objectives of this study were to identify and validate molecular markers associated with quantitative trait loci (QTL) for elevated GPC in durum wheat. A genetic map was constructed using SSR and DArT® markers in an F_1-derived doubled haploid (DH) population derived from the cross DT695 × Strongfield. The GPC data were collected from replicated trials grown in six Canadian environments from 2002 to 2005. QTL associated with variation for GPC were identified on the group 1, 2, and 7 chromosomes and on 5B and 6B, but only *QGpc.usw-B3* on 2B and *QGpc.usw-A3* on 7A were expressed consistently in four and six environments, respectively. Positive alleles for GPC at these loci were contributed by the high-GPC parent Strongfield. The *QGpc.usw-A3* QTL was validated in a second DH population, and depending on environment, selection for the Strongfield allele at *barc108* resulted in +0.4% to +1.0% increase in GPC, with little effect on yield in most environments. Given the consistent expression pattern in multiple populations and environments, *barc108* could be useful for marker-assisted selection for high GPC.

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Introduction

Grain protein concentration (GPC) and gluten quality of durum wheat (*Triticum turgidum* L. var. *durum*) are the most important factors affecting pasta characteristics. In general, high GPC is associated with good pasta firmness and greater tolerance to over-cooking, particularly at high pasta drying temperatures (Autran et al. 1986; D'Egidio et al. 1990; Feillet and Dexter 1996). Semolina protein concentration alone accounts for 30–40% of the variability in pasta cooking quality (Dexter and Matsuo 1977). Given the importance of GPC, genetic improvement has been an objective of durum wheat breeding programs worldwide (Olmos et al. 2003). Response to selection for GPC is slow, largely because of the inverse correlation between GPC and grain yield (Cox et al. 1985; Steiger et al. 1996), and strong environmental influences on the expression of GPC (Blanco et al. 2006). Adequate quantity and appropriately timed nitrogen application can be used to elevate GPC (Feillet 1988), but with the increasing cost of nitrogen fertilizers, development of cultivars that are genetically superior for higher GPC is more economical for producers.

The inheritance of GPC is complex in hexaploid (*Triticum aestivum* L.) and durum wheat. Quantitative trait loci

(QTL) were reported on chromosomes 1B and 6A (Perretant et al. 2000), 3A (Prasad et al. 2003; Groos et al. 2003), 4A and 6B (Prasad et al. 2003) and 4D (Groos et al. 2003), and on the group 2 and 7 chromosomes (Dholakia et al. 2001; Prasad et al. 2003; Groos et al. 2003) of hexaploid wheat. In durum wheat, Blanco et al. (1996) reported QTL for GPC on 4BS, 5AL, 6AS, 6BS and 7BS. The high protein gene *Gpc-B1* derived from *T. turgidum* L. var. *dicoccoides* has been cloned (Uauy et al. 2006) and increases GPC by as much as 1.5% with non-significant effects on protein quality, plant height, heading date, or yield in near isogenic backgrounds (Chee et al. 2001). This gene is also associated with increased grain zinc and iron content, and is involved in earlier leaf senescence (Uauy et al. 2006). In tetraploid wheat, the majority of QTL have been identified from wild *T. turgidum* L. var. *dicoccoides* accessions (Joppa et al. 1997; Blanco et al. 2002, 2006; Olmos et al. 2003; Gonzalez-Hernandez et al. 2004).

The sources of high GPC QTL from the *T. turgidum* L. var. *dicoccoides* accessions are not used in Canadian breeding programs either because they do not improve protein concentration levels in adapted backgrounds (Kovacs et al. 1998) or because of negative effects on other important traits due to linkage drag (Colmer et al. 2006). Thus, identification of QTL associated with GPC in well-adapted genetic backgrounds would be useful. Strongfield, a Canadian durum wheat cultivar (Clarke et al. 2005), consistently displays high GPC coupled with high yield in Canadian environments and is used extensively in durum wheat-crossing programs worldwide. The objectives of this study were to identify and validate useful molecular markers associated with elevated GPC from Strongfield that could aid durum wheat breeders to select for this important trait at earlier generations.

Materials and methods

Plant material and trait evaluation

One hundred and eighty-five F_1-derived doubled haploid (DH) lines from the cross DT695 × Strongfield (Clarke et al. 2005) were grown along with their parents in two-replicate field trials in an alpha-lattice design at Regina (RG) and Swift Current (SC) in 2002; Saskatoon (SK), RG and SC in 2003, and SK in 2005. All locations are in Saskatchewan, Canada. DT695 is derived from the cross DT471/2*Kyle. Kyle is a Canadian durum wheat cultivar developed by Agriculture and Agri-Food Canada (Townley-Smith et al. 1987). The doubled haploid lines were generated using the maize pollen procedure described by Knox et al. (2000). At maturity, plots were harvested with a small-plot combine and dried to approximately 9% moisture. Yield was converted to a kg ha^{-1} based on the area harvested. Grain protein concentration (%) was determined on grain from individual plots using a FOSS-6500 Near-Infrared Reflectance Spectrophotometer (NIRS) calibrated with reference samples characterized for GPC using a Leco-N Analyzer (LECO FP-528). Prediction of GPC by NIRS was confirmed by analysis of 30 samples selected at random from each of the field trials using Leco-N analysis. The weight of 1,000 kernels (g) was determined for each plot.

Statistical analysis of phenotypic data

For each environment, the analysis of variance (ANOVA) was done separately using PROC MIXED of SAS (SAS Institute Inc. 2003) where genotypes were considered as fixed effects, and replications and blocks as random effects. The least square (LS) means from each environment were correlated using PROC CORR of SAS (SAS Institute Inc. 2003). Genetic variance of GPC (%) was estimated by performing the same analysis but with genotypes considered random. For each environment, phenotypic variance (σ_p^2) was estimated as the sum of genetic variance (σ_g^2) and average variance estimate of residual (σ_e^2), such that $\sigma_p^2 = (\sigma_g^2 + \sigma_e^2/r)$. Heritability was estimated as the proportion of genetic variance to phenotypic variance, such that $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2/r)$. Parental data were removed for heritability estimation. Confidence intervals for heritability estimates (h^2) were calculated according to Knapp et al. (1985).

SSR and DArT® marker analysis

For QTL analysis, 94 lines from the DH population were randomly selected. The genomic DNA was extracted from 2-week-old plants using the cetyl (hexadecyl) trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). Simple sequence repeat (SSR) markers polymorphic on parents were evaluated on the DH population. The SSR markers included gwm (Röder et al. 1998), *cfa-d* (Sourdille et al. 2003), *barc* (Song et al. 2005), *wmc* (Gupta et al. 2002; http://wheat.pw.usda.gov/ggpages/SSR/WMC), and *gdm* (Pestsova et al. 2000). The forward primer of each SSR marker pair was modified by incorporating the M13 sequence to the 5′ end during synthesis (Schuelke 2000). The universal M13 primer was labeled with either FAM, VIC, NED or PET fluorescent dyes. Reactions were performed in a 96-well polymerase chain reaction (PCR) plate each containing 25 µl of a reaction mixture of 2.5 µl 10× PCR buffer, 1.5 mM of $MgCl_2$, 0.1 mM of each dNTP, 0.04 µM of M13 sequence-modified forward SSR primer, 0.16 µM of reverse SSR primer, 0.16 µM of universal dye-labeled M13 primer, 1 U of *Taq* DNA polymerase (Invitrogen, Carlsbad, CA), and 50 ng of genomic DNA.

The PCR cycle after initial denaturation of 3 min at 94°C was 30 cycles of 30 s for denaturation at 94°C, 45 s annealing (temperature dependent on the individual primer), and 45 s extension at 72°C followed by seven cycles of 30 s at 94°C, 45 s at 53°C, 45 s at 72°C and final extension of 10 min at 72°C. Primer sequences and annealing temperature were those reported by Röder et al. (1998; http://wheat.pw.usda.gov). Amplification products (0.5 µl) were combined with 9.5 µl HiDi formamide (Applied Biosystems, Foster City, CA) and 0.05 µl ROX size standard, and run on a 36 cm electrophoretic capillary (3130 Genetic Analyzer, Applied Biosystems, Foster City, CA). The electrophorograms were analyzed with GeneMapper version 4.0. Markers with parental allele sizes differing by 8 base pairs (bp) were scored on 2% (w/v) agarose gel stained with 0.5 µg/ml ethidium bromide.

Diversity Array Technology (DArT®) markers shown to be cost-effective in generating genetic maps in a number of species (Wittenberg et al. 2005) including durum wheat (Pozniak et al. 2007) were applied to the DT695 × Strongfield population. The analysis was performed by Triticarte Pty Ltd (Canberra, Australia; http://www.triticarte.com.au) with the DH lines being scored for the presence or absence of hybridization based on fluorescence signal intensities. DArT markers are represented by their clone numbers, e.g. A106.

Genetic map and QTL analysis

A genetic linkage map of the DT695 × Strongfield population was constructed using the Haldane mapping function within the software JoinMap® 3.0 (Van Ooijen and Voorrips 2004). To improve map robustness, markers displaying unusually high frequencies of double crossover events and/or segregation distortion were also removed prior to final map construction. Final map construction consisted of SSR and DArT® markers joined at a LOD score of 3.0 using the "Second Order" mapping function in JoinMap® 3.0. Linkage groups were assigned to chromosomes by comparing markers on the generated map to previously published durum maps (Korzun et al. 1999; Nachit et al. 2001; Elouafi and Nachit 2004) and the hexaploid wheat SSR consensus map (Somers et al. 2004).

Least square means of GPC from the six environments were used in QTL analysis. Simple interval mapping (SIM) was used first to identify markers most significantly associated with variation in GPC. To enhance the power of QTL detection, the analyses were repeated using those markers identified by SIM as co-factors in a multiple QTL model (MQM) in MapQTL Version 5.0 (van Ooijen and Voorrips 2004). The genome-wide significance threshold ($P < 0.01$) of the LOD score was determined as described by van Ooijen (1999). For each QTL, the average QTL effect (one-half the difference between parental marker class means) was estimated by MapQTL. Single factor ANOVA was used to assess marker association with phenotypic variance for those markers not assigned to linkage groups. The MQM-identified QTL were designated as *QGpc.usw* as per the recommended rules for gene symbolization in wheat.

Marker validation

One hundred and ten F_1-derived DH lines from the cross 9370-DJ**3 × Strongfield were used for marker validation. The parent 9370-DJ**3 is a breeding line developed at the Semiarid Prairie Agriculture Research Centre, Agriculture and Agri-Food Canada. The GPC data were obtained using NIRS on samples collected from each plot of two-replicate field trials grown in an alpha-lattice design at Regina and Swift Current in 2002, and at Regina, Swift Current and Saskatoon in 2003. Yield data was assessed on a plot basis and converted to kg ha^{-1} based on the plot area harvested. Data for each environment were analyzed separately to generate LS means using PROC MIXED of SAS (SAS Institute Inc. 2003) where genotypes were considered as fixed effects, and replications and blocks as random effects. The SSR markers linked to stable QTL identified in the DT695 × Strongfield population were analyzed against GPC LS means from the five environments using a single factor ANOVA (PROC MIXED of SAS) with each marker considered as a fixed effect.

Results

Environmental conditions

For this study, the two populations used for genetic analysis of GPC were evaluated in environments with very different environmental conditions (Table 1). In 2002, all test sites received above average precipitation, particularly in June when plants were tillering, and during grain fill in August. In 2003, below average precipitation coupled with above average temperatures in July and August resulted in extreme drought stress at all three environments. Above average precipitation at Saskatoon in June 2005 coupled with below average temperatures throughout the growing season delayed plant development and maturity compared to other environments.

Phenotypic data

Protein data for the DT695 × Strongfield mapping population was collected using NIRS following calibration with reference samples. The NIRS GPC data showed a high

Table 1 Growing season precipitation (mm) and average monthly temperatures (°C) in six environments used to evaluate the DT695 × Strongfield and validation mapping populations

Environment	Average rainfall (mm)						Average monthly temperatures (°C)				
	May	June	July	August	September	Total	May	June	July	August	September
SC 2002	12	123	73	102	59	369	8.7	15.7	19.5	15.3	12.0
RG 2002	9	129	29	113	38	318	8.0	16.3	20.0	16.2	11.9
RG 2003	31	31	42	12	25	141	11.6	16.0	19.8	20.9	11.5
SC 2003	41	78	8	20	31	178	10.7	15.1	19.7	21.3	11.8
SK 2003	14	31	64	31	25	165	11.8	15.9	18.2	20.6	11.3
SK 2005	31	193	53	54	74	405	10.2	14.4	17.5	15.4	11.3
30-Year average											
SC	44	66	52	40	28	230	11.1	15.6	18.1	17.9	11.8
RG	52	65	68	38	33	256	11.6	16.3	18.5	17.4	10.9
SK	42	61	57	35	29	224	11.8	16	18.3	17.6	11.5

RG Regina, *SC* Swift Current, *SK* Saskatoon

correlation (range 0.95–0.97; $P < 0.01$) with the LECO-generated GPC data. In the combined ANOVA (over all locations and years) the genotype × environment interaction was significant ($P < 0.01$), therefore data were interpreted separately for each environment. The ANOVA for GPC revealed significant differences ($P < 0.01$) in GPC among lines at all environments. Significant correlations of LS means among environments ranged from 0.23 to 0.68 (Table 2), consistent with strong environmental influence on phenotypic expression of GPC. Strongfield had significantly higher ($P < 0.05$) GPC than DT695 in all environments except at RG 2002 where GPC of Strongfield was only numerically higher (Table 3). Across environments, GPC ranged from 11.1 to 16.6% for DT695 and from 13.3 to 17.6% for Strongfield (Table 3). In all environments, bi-directional transgressive segregation was evident for GPC, with the lowest transgressive segregant being significantly lower than the low-GPC parent in three environments. The high transgressive segregant was significantly higher than Strongfield in all environments. The population range in GPC was lowest at SC 2003 (3.1%), and highest at RG 2002 (5.0%) (Table 3), and the average range across environments was 3.7%. The genetic variance of GPC across environments ranged from 0.20 to 0.53% and the estimated heritability ranged from 0.51 to 0.70 (Table 3).

Grain yields of Strongfield and DT695 were variable among environments, with Strongfield out-yielding DT695 only at SC 2003 and SK 2005 (Table 4). In contrast, DT695 produced statistically ($P < 0.05$) more grain than Strongfield at RG 2003 (Table 4). Average grain yields were less in 2003 because of drought conditions (Table 1). The ANOVA indicated significant differences in grain yield among DH lines in all environments (data not shown) and large transgressive segregation was evident in all

Table 2 Pearson's correlation coefficients among environments of grain protein concentration least square means of the DT695 × Strongfield doubled haploid mapping population

Environment	Pearson's correlation coefficient				
	RG 2002	SC 2002	SK 2003	RG 2003	SC 2003
SC 2002	0.58**				
SK 2003	0.43**	0.52**			
RG 2003	0.38**	0.56**	0.68**		
SC 2003	ns	0.23**	0.44**	0.28**	
SK 2005	0.39**	0.37**	0.54**	0.50**	0.24**

RG Regina, *SC* Swift Current, *SK* Saskatoon, *ns* not significant

** Significant at the level of 0.01 probability

environments. The greatest range in yield was at SK 2005 (2,149 kg ha^{-1}) and RG 2002 (2,523 kg ha^{-1}), but the latter site had higher residual variation compared to other sites (Table 4). Likewise, kernel weight was highly variable in the population and ranged from 31.0 to 51.4 g per thousand kernels for DT695 and from 30.1 to 47.9 g per thousand kernels for Strongfield (Table 4), depending on environment. Strongfield had lower kernel weights than DT695, but differences were only significant at SC 2002, RG 2003, and SK 2005. Large transgressive segregation for kernel weight was evident among the DH lines in all environments (Table 4). The range in kernel weight was lowest at SK 2005 (11.0 g/1,000 kernels) and highest at SK 2003 (16.3 g/1,000 kernels). Despite the large range in kernel weight, significant ($P < 0.01$) negative correlations were observed between GPC and 1,000-kernel weight only at SC 2002 and SK 2005 (Table 4). In contrast, grain yield was negatively correlated with GPC at all six environments, with r values ranging from −0.45 to −0.51 ($P < 0.01$; Table 4).

Table 3 Least significant difference (LSD) and least square means of GPC (%) for the DT695 × Strongfield mapping population, parents and high and low transgressive segregants

Factor	Grain protein concentration (%)					
	RG 2002	SC 2002	SK 2003	RG 2003	SC 2003	SK 2005
DT695	13.9	11.1	16.6	13.3	15.9	13.2
Strongfield	14.9	13.3	17.6	14.8	17.1	14.4
High transgressive	17.8	14.4	18.8	16.3	18.5	15.4
Low transgressive	12.8	11.0	15.5	12.7	15.4	12.0
Population mean	14.7	12.8	17.0	14.2	16.5	14.2
LSD ($P < 0.05$)	1.1	1.0	0.8	1.0	0.9	0.9
σ_g^2	0.50	0.49	0.25	0.53	0.20	0.26
h^2	0.68	0.67	0.65	0.51	0.61	0.70
h^2 95% CI	0.55–0.77	0.56–0.75	0.53–0.74	0.35–0.63	0.48–0.71	0.59–0.77

The genetic variance (σ_g^2) and heritability estimates (h^2) of GPC and its 95% confident intervals for each environment are also presented

RG Regina, *SC* Swift Current, *SK* Saskatoon

Genetic map and QTL analysis

A total of 488 SSR markers were scored on the parents of the DT695 × Strongfield mapping population and 190 (40%) markers produced polymorphic fragments. Approximately 260 DArT® markers were polymorphic and scored in the DH population. The final genetic map was constructed based on second-order mapping function, which is a conservative test of linkage, and consisted of 140 SSR and 205 DArT® markers joined into 25 linkage groups. Twenty-four linkage groups were assigned to chromosomes based on previously published genetic maps. The remaining linkage group consisted of 13 tightly linked DArT® markers that spanned approximately 30 cM and could not be assigned to a chromosome. Chromosomes 1B and 3B were the only two chromosomes represented by a single linkage group, with the remaining chromosomes represented by two linkage groups, one for each chromosome arm. The SSR marker order was in good agreement with previously published wheat genetic maps (Groos et al. 2003; Elouafi and Nachit 2004; Somers et al. 2004; Blanco et al. 2006; Pozniak et al. 2007). The sum of linkage group lengths spanned 1,474 cM.

Using MQM, nine QTL for GPC were identified (Fig. 1), with average effects ranging from 0.16 to 0.46 (Table 5). No significant two-way interactions between QTL were identified. A QTL × environment interaction was evident for GPC in the DT695 × Strongfield population (Table 5) with six QTL significant in single environments (Table 5). The *QGPC.usw-A2* QTL on 2A was expressed in three environments. The two QTL *QGpc.usw-B3* on 2B and *QGpc.usw-A3* on 7A were consistently expressed and significant in four and six environments, respectively. The *QGpc.usw-B3* QTL spanned approximately 11 cM and its effect ranged from 0.20% at SC 2002 and SC 2003 to 0.26% at RG 2002. The *QGpc.usw-A3* QTL spanned approximately 10 cM (Fig. 1) with QTL effects ranging from 0.18% at SK 2005 to 0.46% at RG 2003. DT695 contributed the allele for elevated protein at four of the QTL (*QGpc.usw-B2*, *QGpc.usw-A2*, *QGpc.usw-B5* and *QGpc.usw-B6*; Fig. 1, Table 5), whereas Strongfield contributed alleles for elevated GPC at five of the QTL (*QGpc.usw-A1*, *QGpc.usw-B1*, *QGpc.usw-B3*, *QGpc.usw-B4*, *a*nd *QGpc.usw-A3*; Fig. 1). None of the markers excluded from the genetic linkage map were significantly associated with GPC as assessed using single marker analysis.

To determine if the GPC QTL identified were associated with variation in grain yield and kernel weight, single marker analysis was performed on those markers identified as being most associated with variation in GPC. Only markers linked to *QGpc.usw-B2*, *QGpc.usw-B4*, and *QGpc.usw-B5* were associated with kernel weight variation (Table 6). *QGpc.usw-B2* was also associated with variation in grain yield at SK in both 2003 and 2005. The *QGpc.usw-B4* QTL was associated with kernel weight in five ($P < 0.05$) of the six environments evaluated and the allele for reduced kernel weight was contributed by Strongfield, the high protein parent. The two major QTL *QGpc.usw-B3* and *QGpc.usw-A3* were not associated with kernel weight in any of the environments evaluated (Table 6). However, Strongfield alleles at *QGpc.usw-B3* and *QGpc.usw-A3* were associated with reduced yield, but each only in a single environment (Table 6).

Marker validation

In the 9370-DJ**3 × Strongfield validation population, significant differences in GPC between the parents were observed only at RG 2002 but significant ($P < 0.05$) bi-directional transgressive segregation was evident for GPC

Table 4 Least square means of grain yield (kg ha^{-1}) and 1,000-kernel weight (g) for the DT695 × Strongfield population, Strongfield and DT695, and high and low transgressive segregants across six environments

Factor	1,000-kernel weight (g)						Grain yield (kg ha^{-1})					
	RG 2002	SC 2002	SK 2003	RG 2003	SC 2003	SK 2005	RG 2002	SC 2002	SK 2003	RG 2003	SC 2003	SK 2005
DT695	37.7	44	47.3	51.4	31	46.8	4,651	3,285	2,397	3,321	1,759	6,055
Strongfield	35	39.7	46.7	47.9	30.1	42.2	5,013	3,329	2,246	2,932	2,008	6,757
High transgressive	42.2	48.3	54.4	56.3	37	50.7	5,362	4,047	2,819	3,723	2,365	6,928
Low transgressive	30.7	36	38.1	41.3	25.5	39.7	2,839	2,225	1,567	2,197	1,348	4,779
Population mean	36.2	41.7	46.7	48.9	31.5	43.6	4,313	3,265	2,355	2,889	1,856	6,007
LSD ($P < 0.05$)	4.2	3.1	3.6	2.6	2.2	3.7	928	487	470	380	339	510
GPC correlation (r)	−0.16*	ns	ns	ns	ns	−0.33*	−0.57**	−0.51**	−0.46**	−0.59**	−0.45**	−0.48**

The Pearson correlation coefficients between grain protein concentration, yield and 1,000-kernel weight for this population across environments are also presented

RG Regina, *SC* Swift Current, *SK* Saskatoon, *ns* not significant

* Significant at the level of 0.05 probability; **significant at 0.01 probability

across all environments, although no statistical difference between the low transgressive segregant and DT695 was detected at RG 2002 (Table 7). The range in GPC in this population was lowest at RG 2002 (3.4%) and highest at SC 2002 (5.4%) (Table 7) with an average range across environments of 4.3%. Correlations among LS means ranged from a low of 0.21 to high of 0.51, consistent with a strong genotype × environment interaction. Grain yield was statistically similar ($P > 0.05$) between parents, except at SC 2002 where Strongfield produced more grain (Table 7). However, yield was variable in the DH population with statistical differences ($P < 0.01$) between high and low transgressive segregants at all sites (Table 7). As in the DT695 × Strongfield population, there was a strong negative correlation between grain protein and grain yield with r values ranging from −0.33 to −0.75 (Table 8).

Given the consistent expression of *QGpc.usw-B3* and *QGpc.usw-A3* in the DT695 × Strongfield population, markers linked to these QTL were considered in the validation population. Of the markers linked to *QGpc.usw-A3* (Fig. 1), only *barc108* was polymorphic but consistently showed significant ($P < 0.01$) association with high GPC in the validation population (Table 8). The effect of the Strongfield allele at *barc108* ranged from +0.4% at RG 2002 to +1.0% at SK 2003 (Table 8). Despite the association with GPC in this population at all environments, *barc108* was associated with variation in grain yield only at RG 2003 and SK 2003 (Table 8). At those sites, DH lines carrying the Strongfield allele had 226 kg ha^{-1} (RG 2003) and 213 kg ha^{-1} (SK 2003) lower yield than lines carrying the 9370-DJ**3 allele (Table 8). Of the high GPC lines carrying the Strongfield allele, 58% had yield and GPC similar or greater than Strongfield at RG 2003. Similarly, 72% had similar or greater yield and GPC than Strongfield at SK 2003 (Table 8). None of the markers at *QGpc.usw-B3* (Fig. 1) were polymorphic in the validation population, and thus the QTL could not be verified.

Discussion

The present study was initiated to identify QTL within domesticated durum, *Triticum turgidum* L. var *durum,* associated with elevated GPC. The majority of QTL reported to date are derived from wild accessions of *T. turgidum* L. var. *dicoccoides.* Blanco et al. (2002) reported six QTL for elevated GPC from *T.diccocoides,* but most of these were associated with reduced grain yield (Blanco et al. 2002). The *Gpc-B1* locus from *T. diccocoides* (Olmos et al. 2003; Distelfeld et al. 2004, 2006; Uauy et al. 2006) is the most studied and has been suggested as an effective

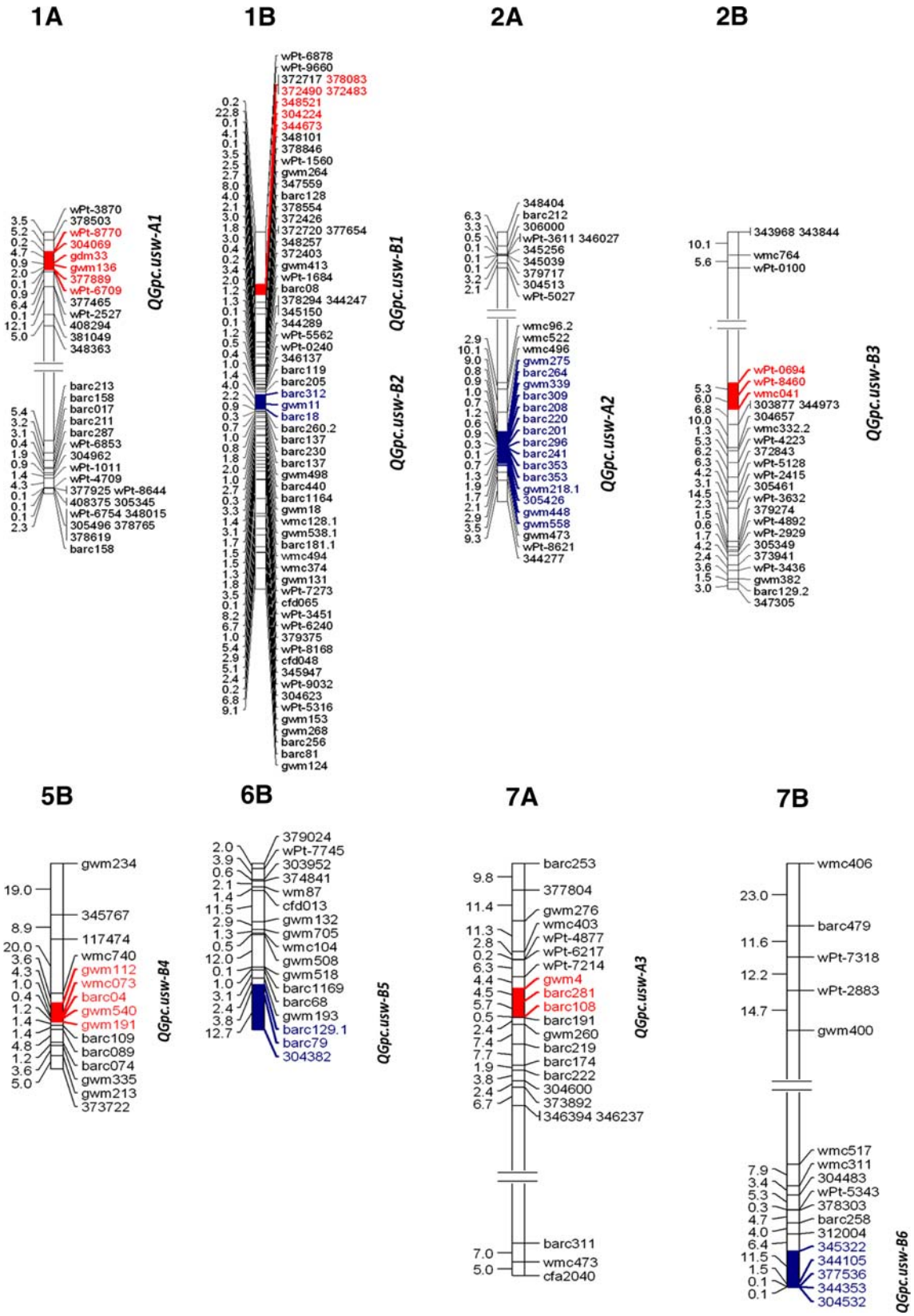

Fig. 1 QTL associated with grain protein concentration (GPC) in the DT695 × Strongfield doubled haploid mapping population. Elevated GPC loci contributed by Strongfield are indicated with *red* and by DT695 are indicated with *blue* (see also Table 5). Values to the *left* of the chromosomal bars indicate the genetic distance (cM) between markers

Table 5 The LOD scores and effect of QTL (%) associated with variation in grain protein concentration (GPC) in the DT695 × Strongfield doubled haploid mapping population

Chromosome/QTL		Positive allele	RG 2002		SC 2002		RG 2003		SC 2003		SK 2003		SK 2005	
			LOD	Effect	LOD	Effect	LOD	Effect	LOD	Effect	LOD	Effect	LOD	Effect
1A	*QGpc.usw-A1*	St	ns	–	ns	–	ns	–	3.2**	0.19	ns	–	ns	–
1B	*QGpc.usw-B1*	DT	ns	–	ns	–	ns	–	5.6**	0.27	ns	–	ns	–
1B	*QGpc.usw-B2*	St	ns	–	ns	–	3.8**	0.24	ns	–	ns	–	ns	–
2A	*QGpc.usw-A2*	DT	4.9**	0.35	4.1**	0.23	ns	–	5.1**	0.22	ns	–	ns	–
2B	*QGpc.usw-B3*	St	2.6**	0.26	3.4**	0.20	ns	–	3.7**	0.20	ns	–	4.8**	0.22
5B	*QGpc.usw-B4*	St	ns	–	ns	–	ns	–	ns	–	3.9**	0.19	ns	–
6B	*QGpc.usw-B5*	DT	ns	–	ns	–	ns	–	ns	–	3.6**	0.19	ns	–
7A	*QGpc.usw-A3*	St	4.9**	0.36	9.5**	0.37	10.5**	0.46	2.4*	0.16	8.5**	0.32	3.0**	0.18
7B	*QGpc.usw-B6*	DT	ns	–	ns	–	ns	–	ns	–	ns	–	3.1**	0.20

The QTL effect is presented for markers closest to the centre of the QTL where the parent contributing the allele for elevated GPC presented is St = Strongfield and DT = DT695

RG Regina, *SC* Swift Current, *SK* Saskatoon, *ns* not significant

* LOD significant at the level of 0.05 probability; **LOD score significant at the level of 0.01 probability

Table 6 Single factor ANOVA for association between yield (kg ha^{-1}) and 1,000-kernel weight (g) and markers significantly associated with grain protein concentration QTL in the DT695 × Strongfield doubled haploid mapping population (see Table 5)

GPC QTL:markers	Chromosome	Trait	*F* values					
			SC 2002	RG 2002	SK 2003	SC 2003	RG 2003	SK 2005
QGpc.usw-A1:*gdm33*	1A	Yield	ns	ns	ns	ns	ns	ns
		KWT	ns	ns	ns	ns	ns	ns
QGpc.usw-B2:*barc18*	1B	Yield	ns	ns	23.28**	ns	ns	18.06**
		KWT	9.13**	ns	9.05**	18.63**	ns	ns
QGpc.usw-A2:*barc201*	2A	Yield	6.11*	ns	ns	ns	7.50**	ns
		KWT	ns	ns	ns	ns	ns	ns
QGpc.usw-B3:*wmc41*	2B	Yield	ns	ns	ns	ns	ns	5.62*
		KWT	ns	ns	ns	ns	ns	ns
QGpc.usw-B4:*wmc73*	5B	Yield	ns	ns	ns	ns	ns	ns
		KWT	6.89*	5.49*	ns	10.13**	5.08*	8.52**
QGpc.usw-B5:*barc79*	6B	Yield	ns	ns	ns	ns	ns	ns
		KWT	8.14**	ns	6.08*	ns	5.07*	ns
QGpc.usw-A3:*barc108*	7A	Yield	ns	ns	ns	ns	15.4**	ns
		KWT	ns	ns	ns	ns	ns	ns

Only markers with significant *F* tests are presented

RG Regina, *SC* Swift Current, *SK* Saskatoon, *ns* not significant

* Significant at the level of 0.05 probability; **significant at the level of 0.01 probability

gene for elevation of GPC. The effect of this gene is independent of protein quality, plant height, heading date, and yield (Chee et al. 1998) and durum recombinant lines carrying this gene have shown improved GPC (Kovacs et al. 1998). However, in Canadian breeding programs, lines carrying this gene had reduced test weight and none showed GPC higher than the durum wheat cultivars included as experimental checks (DePauw et al. 1998; Kovacs et al. 1998). These results suggest that other effective genes for GPC exist in adapted backgrounds and effort to identify these genes is warranted.

Validation of the use of NIRS was confirmed by the high correlation of GPC of the 30 random plot samples chosen from each environment and measured with both the NIRS and Leco-N Analyzer. The high correlation between NIRS and LECO generated GPC confirmed NIRS data were reliable for predicting GPC for the mapping study consistent with earlier studies (Long et al. 2008).

Table 7 Least square means of grain protein concentration (%) and grain yield (kg ha^{-1}) in the 9370-DJ**3 × Strongfield validation population, its parents, and high and low transgressive segregants

	Grain protein concentration (%)					Yield (kg ha^{-1})				
	RG 2002	SC 2002	RG 2003	SC 2003	SK 2003	RG 2002	SC 2002	RG 2003	SC 2003	SK 2003
Strongfield	14.5	13.1	16.3	17.0	17.8	4,573	3,558	2,794	1,986	2,620
9370-DJ**3	12.8	12.3	16.5	18.0	17.8	5,134	2,894	2,981	1,857	2,510
High transgressive	15.8	16.4	19.1	19.8	19.9	5,711	4,227	3,748	2,504	3,201
Low transgressive	12.4	11	14.2	16	15.9	2,847	1,783	1,739	1,323	1,784
Population mean	13.9	12.8	16.6	17.7	17.8	4,538	3,094	2,797	1,906	2,512
LSD ($P < 0.05$)	0.8	1	0.9	1.2	0.6	647	660	301	238	379

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Table 8 Single factor ANOVA for *barc108* and grain protein concentration in the 9370-DJ**3 × Strongfield validation population

	RG 2002	SC 2002	RG 2003	SC 2003	SK 2003
barc108 F test-GPC	5.65**	9.16**	12.76**	5.51*	22.56**
barc108 F test-yield	ns	ns	10.58**	ns	13.63**
Yield–GPC correlation (*r*)	−0.33**	−0.75**	−0.75**	−0.71**	−0.68**
Barc108 class least square means					
Strongfield allele					
GPC (%)	14.0	13.0	17.0	17.8	18.2
Yield (kg ha^{-1})	4,512	3,110	2,680	1,870	2,400
Proportion	81%	79%	58%	81%	72%
9370-DJ**3 allele					
GPC (%)	13.6	12.4	16.1	17.3	17.2
Yield (kg ha^{-1})	4,614	3,220	2,906	1,927	2,613
GPC SED (%)	0.14	0.19	0.23	0.18	0.20
Yield SED (kg ha^{-1})	149	76	118	41	68

Least square means for genotypes homozygous for *barc108* (*QGpc.usw-A3*) and standard error of the difference (SED) between the two marker classes are presented

RG Regina, *SC* Swift Current, *SK* Saskatoon

* Significant at the level of 0.05 probability; **significant at the level of 0.01 probability

The effect of genotype by environment (G × E) interactions was high for GPC as shown by the low correlation of LS means among environments (Table 3), the moderate heritability estimate (0.51–0.70) and by the variable expression of QTL in different environments (Table 5). This was not surprising given the dramatically different environmental conditions observed over the 3 years of testing (Table 1). Significant negative correlations between GPC and kernel weight at SC 2002 and SK 2005 (Table 4) indicated the presence of genetic factors that are likely pleiotropic on GPC. Despite these negative correlations, only three GPC QTL were associated with kernel weight (Table 6). Of these three, *QGpc.usw-B4* was associated with kernel weight in nearly all environments tested (Table 6) and the allele for reduced kernel weight was contributed by Strongfield, which also contributed the high protein allele at this locus (Fig. 1). Thus *QGpc.usw-B4* is probably not associated with GPC per se, but is likely associated with grain protein dilution by the reduced starch content in smaller kernels from Strongfield.

Strongfield showed significantly higher GPC compared to DT695 (Table 3), an average of 1.4%, and is consistent with earlier reports of Strongfield expressing high GPC when grown in Canadian environments (Clarke et al. 2005). In the DT695 × Strongfield population, the transgressive segregation for high GPC (Table 3) indicated that both Strongfield and DT695 possess desirable alleles for elevated GPC. This was confirmed by the QTL analysis showing QTL contributions to elevated GPC from both parents (Fig. 1). Interestingly, only QTL with positive effects from Strongfield were identified at RG 2003 (Fig. 1; Table 5) despite a greater than 3% range in GPC in that environment (Table 3). This implies that additional smaller effect QTL may be segregating in this population that were

not identified either because of the variability in phenotypic data due to environmental influences or lack of marker coverage in some genomic regions (Fig. 1). Only 1B and 3B had adequate marker coverage to form a single linkage group, with the remaining chromosomes represented by at least two linkage groups. Addition of molecular markers in the regions not adequately covered would resolve this hypothesis. The high GPC gene Gpc-B1 on chromosome 6BS derived from the wild tetraploid accession Acc. FA-15-3 (*T. turgidum* var *dicoccoides)* (Avivi 1978; Joppa et al. 1997; Olmos et al. 2003) is located within an approximately 0.3-cM interval of *gwm508* and *gwm193* (Khan et al. 2000; Olmos et al. 2003; Distelfeld et al. 2004), or within a 245-kb physical contig interval of *Xuhw89* and *Xucw71* (Distelfeld et al. 2006). Our *QGpc.usw-B5* QTL was localized very close to *Gpc-B1* on chromosome 6BS (Fig. 1), but was associated with kernel weight in half the environments (Table 6) and only associated with GPC QTL in one of the six environments. Thus this QTL is likely different from *Gpc-B1*.

At a physiological level, GPC is influenced by a number of factors including nitrogen uptake, assimilation, and remobilization to the grain during grain filling. The early steps of nitrate and ammonia assimilation and nitrogen remobilization involve three gene families, those coding for nitrate reductase (*NAR*), nitrite reductase (*NIR*), and glutamate/glutamine synthase (Boisson et al. 2005). The wheat glutamine synthetase (*GS2*) gene has been mapped to the telomeric regions of the group 2 chromosomes (Habash et al. 2007) and is likely not associated with the *QGpc.usw-B3* QTL identified in our study, positioned near the centromere of 2B (Fig. 1). Using Chinese Spring deletion lines, homeologous genes of Fd-glutamate synthase have been localized to the group 2 chromosomes near the centromere (Boisson et al. 2005) and may be associated with the *QGpc.usw-B3* QTL. A nitrate reductase (*NAR*) gene has been reported on the short arm of chromosome 7A (Habash et al. 2007), and thus would not be associated with *QGpc.usw-A3* which mapped to the long arm of 7A.

The GPC QTL on the group 1 chromosomes localized to regions that house known gliadin and glutenin genes. The gliadin locus *Gli-A1* identified by Elouafi and Nachit (2004) was linked to *gwm136*, as was the *QGpc.usw-A1QTL* identified in our study. We did not measure protein quality in our study, but the influence of the 1A region on gluten strength needs to be assessed prior to recommending this QTL for marker assisted selection. *QGpc.usw-B2* localized distal from the centromere on 1B and may be associated with the *Gli-B1/Glu-B3 loci* which have been mapped to this region (Blanco et al. 1998). However, this QTL was associated with kernel weight and yield in some environments (Table 6), and thus it is more probable that genetic factors influencing kernel size and/or yield are located in this region.

The most conservative approach to justify QTL useful for marker-assisted selection is by selecting those QTL that are identified in multiple environments and/or in multiple populations and are not negatively associated with pleiotropic effects on other important traits. In our study, several QTL were identified in the DT695 × Strongfield population. The two most stable QTL *QGpc.usw-B3* and *QGpc.usw-A3* are the most promising targets for marker-assisted breeding because these were consistently expressed in a range of environments representing dramatically different climatic conditions (Table 1). Individually, the *QGpc.usw-B3* and *QGpc.usw-A3* QTL result in an average GPC effect of 0.23% and 0.30%, respectively, with no negative pleiotropic effect on kernel weight. However, these two QTL were associated with a small reduction in yield in some environments. In the DT695 × Strongfield population *QGpc.usw-A3* was significantly associated with GPC in all environments (Table 5), but the high GPC allele at this QTL was only associated with reduced yield at RG 2003 (Table 6). Similarly, in the validation population, this QTL was associated with GPC in all five environments (Table 8), but only associated with reduced yield in two environments. Despite the association with reduced yield at these sites, a high proportion of lines (>58%) had yield equal to or greater than Strongfield coupled with high GPC equivalent to Strongfield at these two sites (Table 8). This, together with the strong negative correlation between yield and GPC suggests that this QTL is only weakly associated with grain yield.

The *QGpc.usw-A3* has yet to be identified in durum wheat, but is likely the same as that reported in the hexaploid wheat Avalon × Hobbit RIL population, because both are linked to *barc108* (Turner et al. 2004). The presence of a common QTL in durum and bread wheat confirms the importance of this QTL and suggests a common genetic mechanism for grain protein accumulation in the two species. The *QGpc.usw-B3* QTL marked by *wmc41* has yet to be reported, but it appears to be homeologous to a QTL identified on chromosome 2D which is also associated with *wmc41* (Prasad et al. 1999). The *wmc41* marker has been validated to be useful for selection of high GPC in a bread wheat population (Harjit-Singh et al. 2001). In our validation population *wmc41* was not polymorphic and thus could not be validated. However, 9370-DJ**3 had similar GPC to Strongfield in most environments (Table 5), perhaps because this line is already fixed for this major QTL.

The discovery of molecular markers linked to phenotypic variation is only a preliminary step in establishing a marker-assisted selection program for genetic improvement because QTL may be population-specific and their effects on phenotypic expression may be overestimated, particularly for complex traits like GPC. The *QGpc.usw-A3* QTL was

expressed in the DT695 × Strongfield population in all evaluated environments, and was significant in the validation population in all environments. Our results confirm that selection of this locus, at least in crosses involving Strongfield, can be an effective means to improve or maintain GPC levels in durum wheat breeding programs. However, because Strongfield was a common parent in both populations, validation of this QTL in diverse genetic backgrounds is still required to confirm that this marker would be useful in other breeding populations. Production of near isogenic lines (NILs) for the *QGpc.usw-A3* QTL in multiple genetic backgrounds should be examined to confirm the expression of this QTL in those backgrounds. Such a confirmation would warrant further efforts to pursue finer mapping and positional cloning using the NILs to elucidate the gene(s) associated with elevated GPC at this QTL. In addition, these NILs could be used to better understand the physiological mechanisms associated with elevated GPC as the result of this QTL. In conclusion, we identified a major QTL for high protein concentration originating in domesticated durum from the cultivar Strongfield that could aid durum wheat breeders in selecting for this important trait at earlier generations.

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