Analysis of QTL for High Grain Protein Content in Canadian Durum Wheat

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Key Words: QTL, high grain protein content, durum wheat

Abstract

Durum wheat (*Triticum turgidum* L. var. *durum*) varieties with high grain protein concentration (GPC) produce pasta products with greater cooking firmness and increased tolerance to overcooking. However, the large environmental effect on expression of GPC and the negative correlation between GPC and grain yield slow breeding progress of durum wheat varieties with elevated GPC. Identification of molecular markers associated with high GPC would aid durum wheat breeders to select for this important trait earlier. The objectives of this study were to identify molecular markers associated with quantitative trait loci (QTL) for elevated GPC in durum wheat. A preliminary genetic map was constructed by screening polymorphic microsatellite markers on a set of 95 double haploid lines derived from the cross Strongfield (high GPC) X DT695 (low GPC). QTL analysis using single marker regression was performed on GPC data collected at Swift Current and Regina in 2002 and Swift Current, Regina and Saskatoon in 2003. To date, we have identified two QTL for GPC flanked by *Xgwm448* and *Xgwm558* on chromosome 2AS, and on chromosome 2BL at *wmc332*. No QTL for high GPC could be detected on chromosome 6BS, the location of a high GPC gene isolated previously from durum, wheat suggesting that Strongfield contains novel QTL for high GPC not previously reported in the literature. The molecular markers flanking the QTL identified in this study can be used by durum wheat breeders to enhance selection of high GPC in durum wheat.

Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is an allotetraploid (AABB, 2n = 4x = 28) with seven homoeologous chromosomes and is largely milled to produce semolina (coarse, granular particles of endosperm) for pasta production (Nachit et al., 2001). Among the world-leading durum wheat producing countries, Canada produces an average of 4.5 million tonnes of grain per year, accounting for 14% of world durum wheat production (CWB, 2004).

Adequate nitrogen fertilization rates and appropriate application timing can be used to elevate GPC (Feillet, 1988), but the continued use of fertilizers is becoming less economically appealing. Hence, development of varieties that are genetically predisposed to higher GPC may be a better

alternative. Genetic improvement of GPC has been the target of durum wheat breeding programs worldwide (Olmos et al., 2003). However, genetic improvement of GPC is difficult, largely because it is negatively correlated with grain yield (McNeal et al., 1972; Steiger et al., 1996) and genetic variation for protein content is small in comparison to variation between growing environments (Galova et al., 1999; Khan et al., 2000). The genotype x environment interaction effects on GPC indicates that the grain protein accumulation trait is polygenic and associated with quantitative trait loci (QTL) (Tanksley, 1993). Efforts to improve GPC can be accelerated by means of identification of the genes that affect GPC and direct selection of the alleles with positive effects (Olmos et al., 2003).

A source of high GPC for durum wheat was identified in an accession of tetraploid *Triticum turgidum* var *dicoccoides* (Acc. FA-15-3) (Avivi, 1978) and genetic analysis revealed that the high protein gene(s) were localized to chromosome 6B (Olmos et al., 2003). When chromosome 6B from *dicoccoides* was introduced into the durum wheat cultivar Langdon (LDN(DIC6B)), an increase in protein content (1.5%) with non-significant effects on protein quality, plant height, heading date, or yield was observed (Chee et al., 2001). Similar results were obtained by Mesfin et al. (1999) who found that introgression of the *dicoccoides* allele into common wheat varieties also increased GPC.

Compared to other Canadian durum wheat varieties, Strongfield (DT712) produces high GPC while maintaining high grain yield. Therefore, we are interested in determining if Strongfield possesses genes for GPC that are different from that currently identified in LDN(DIC6B). In addition, identification of molecular markers associated with high GPC would aid durum wheat breeders to select for this important trait earlier in the plant breeding program. Therefore, the objectives of this research are to identify QTL associated with high GPC from Strongfield durum wheat, and to determine if Strongfield possesses the QTL for high GPC previously reported on chromosome 6B.

Materials and Methods

Evaluation of GPC

Doubled haploid (DH) lines derived from the cross Strongfield (high GPC) / DT695 (low GPC), along with their parents, were evaluated for GPC in an alpha-lattice design with two replicates at Swift Current and Regina in 2002 and Swift Current, Regina and Saskatoon in 2003. Plots were harvested at maturity and dried to constant weight before grinding and protein analysis. Grain protein concentration was determined on individual plots using a Foss-6500 Near Infrared Reflectance Spectrophotometer calibrated with reference samples analyzed using a Leco-N Analyzer (LECO FP-528).

DNA isolation and Marker analysis

DNA was extracted from two-week old plants using the CTAB method. Microsatellite markers (*gwm*, *barc* and *wmc*) showing polymorphism after an initial PCR screening of the parents were selected to construct a framework map that was subsequently used for QTL analysis. All PCR reactions were performed in a 25-μL volume using a PTC-100 MJ Research Thermal Controller following Roder et al. (1998) with modifications. The PCR reaction mixture contained 2.5 μL 10x PCR buffer, final concentrations of 1.5 mM of $MgCl_2$, 1 unit of *Taq* Polymerase (*Invitrogen*), 0.2 mM of each dNTP, 100 ng genomic DNA, and 400 mM of each primer. The PCR cycle

includes steps of 3 minutes at 95^0C; followed by 40 cycles of 1 minute at 94^0C, 1 minute annealing (temperature dependant on the individual microsatellite), and 2 minutes at 72^0C; and final extension of 10 minutes at 72^0C. The PCR amplified fragments were separated in either 2% agarose gel stained with ethidium bromide or 6% polyacrylamide (19:1) gel with silver staining. Polymorphisms were scored according to the two parents. QTL analysis was performed using single marker linear regression analysis on least square means of protein concentration (Gupta, 2002).

Results and Discussion

The mean GPC of the two parents of the mapping population were significantly different in each of the testing environments with GPC values ranging from 11.1% to 16.6% for DT695 and from 13.3% to 17.7% for Strongfield (Table 1). The distribution of GPC in the DH lines averaged over all testing environments is presented in Fig. 1. Averaged over all environments, transgressive segregation was evident for high GPC (Figure 1), indicating that both parents possess positive alleles for GPC.

Table 1. Mean protein concentration (%) for Strongfield and DT695 over all testing environments.

	Swift Current 2002	Regina 2002	Mean 2002	Swift Current 2003	Regina 2003	Saskatoon 2003	Mean 2003	Mean 2002& 2003
Strongfield	14.9 a	13.3a	**14.1a**	14.6a	16.6a	17.7a	**17.2a**	**15.4a**
DT695	13.9 b	11.1b	**12.5b**	13.6b	15.9b	16.6b	**16.3b**	**14.2b**
LSD	0.5	0.5	**0.5**	0.5	0.5	0.5	**0.5**	**0.5**

Means in columns followed by the same letter are not significantly different at P≤0.05.

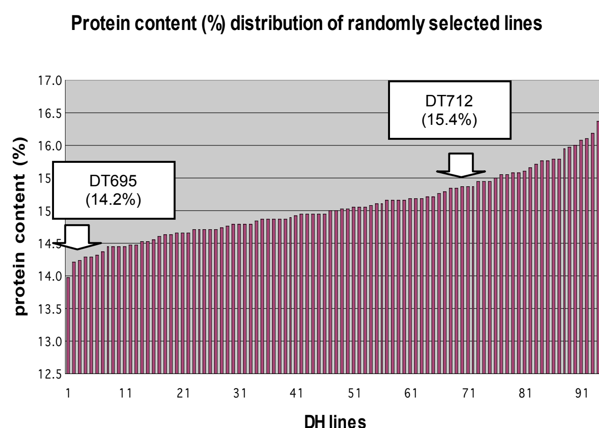

Figure 1. The range of average grain protein content of the 94 double haploid lines used in the preliminary QTL analysis. The average GPC of the parents are indicated by arrows.

Among the polymorphic markers detected on the DH population, single marker analysis revealed

that *gwm448*, *gwm558*, and *wmc332* were significantly associated with variation for GPC at most locations (Table 2). The *gwm448* and *gwm558* loci were found to be linked, and based on the wheat consensus map (Somers et al., 2004), are located on chromosome 2AS. The *wmc332* loci resides on chromosome 2BL (Somers et al., 2004). None of the markers associated with GPC were associated with kernel weight, indicating that the protein QTL identified in this study are not due to protein dilution by starch during grain fill.

Table 2. QTL analysis using single marker regression analysis. Values represent the regression coefficient, which is equal to two times the average effect of a marker allele substitution (QTL effect). A positive value indicates that Strongfield is contributing the positive allele at each marker loci.

Locations	Chromosome 2AS				Chromosome 2BL	
	gwm448		*gwm558*		*Wmc332*	
	protein	kernel weight	protein	kernel weight	protein	kernel weight
Swift Current 2002	-0.65 **	0.20 ns	-0.44 **	0.49 ns	0.29 *	-0.90 ns
Regina 2002	-0.41 *	0.02 ns	-0.73 **	0.40 ns	0.42 *	-0.51 ns
Mean 2002	**-0.54 ****	**0.17 ns**	**-0.59 ****	**0.51 ns**	**0.36 ***	**-0.58 ns**
Regina 2003	-0.40 ns	-.43 ns	-0.4 **	-0.39 ns	0.32 **	-1.24 *
Swift Current 2003	-0.26 ns	-0.49 ns	-0.18 ns	-0.53 ns	0.15 ns	-0.83 ns
Saskatoon 2003	-0.49 **	-0.06 ns	-0.43 **	0.11 ns	0.28 *	-0.74 ns
Mean 2003	**-0.39 ****	**-0.29 ns**	**-0.36 ****	**-0.03 ns**	**0.26 ***	**-0.97 ns**
Mean 2002 and 2003	**-0.43 ****	**-0.04 ns**	**-0.43 ****	**0.13 ns**	**0.29 ***	**-0.84 ***

* = significant at P≤0.05, ** = significant at P≤0.01, ns = not significant

Analysis of the allelic effects of the GPC QTL indicated that DT695 was contributing the positive allele at the 2A QTL whereas Strongfield was contributing the positive allele at the QTL on chromosome 2BL (Table 2). In most environments, the average GPC of the DH lines containing the positive alleles was significantly higher in than those DH lines containing the negative alleles at the two QTL (Table 3). These results suggest that the markers associated with the identified QTL could be used to select breeding lines with elevated levels of GPC.

Table 3. Mean grain protein concentration (%)for those DH lines homozygous for alleles with a positive effect and lines homozygous for alleles with a negative effect on GPC at the two QTL identified in this study.

	Swift Current 2002	Regina 2002	Mean 2002	Swift Current 2003	Regina 2003	Saskatoon 2003	Mean 2003	Mean 2002& 2003
Positive alleles	15.2	13.1	**14.2**	14.7	16.9	17.4	**16.3**	**15.5**
Negative alleles	14.3	12.5	**13.4**	14.2	16.2	16.8	**15.8**	**14.8**
Difference	**0.9***	**0.6***	**0.8***	**0.5**	**0.7***	**0.6***	**0.5**	**0.7***

*Difference between means of DH lines with negative and positive alleles was significant at P≤0.05.

A QTL for GPC has been reported to be flanked by markers *gwm508*, *gwm518* and *gwm193* on chromosome 6B (Olmos et al., 2004). Therefore, QTL analysis was carried out to determine if Strongfield also possesses the QTL for GPC in the 6B region. None of the polymorphic markers (*gwm508*, *gwm518*, and *gwm193*) known to flank the QTL for GPC on chromosome 6B were associated with variation for GPC in our mapping DH population (Table 4), providing conclusive evidence that Strongfield does not possesses the high GPC QTL previously reported on chromosome 6B.

Table 4. QTL analysis for GPC using molecular markers reported to be associated with a previously reported QTL on chromosome 6B. Values represent the regression coefficient, which is equal to two times the average effect of a marker allele substitution (QTL effect).

Locations	*gwm508*	*gwm518*	*gwm193*
Swift Current 2002	0.02 ns	0.51 ns	-0.07 ns
Regina 2002	0.20 ns	0.24 ns	0.31 ns
Mean 2002	**0.12 ns**	**0.14 ns**	**0.12 ns**
Regina 2003	0.26 ns	0.25 ns	0.23 ns
Swift Current 2003	-0.01 ns	0.08 ns	-0.09 ns
Saskatoon 2003	-0.05 ns	-0.08 ns	-0.11 ns
Mean 2003	**0.07 ns**	**0.09 ns**	**0.01 ns**
Mean 2002 & 2003	**0.10 ns**	**0.11 ns**	**0.05 ns**

ns = not significant ($P \leq 0.05$)

Conclusions

We have identified two QTL for GPC flanked by *Xgwm448* and *Xgwm558* on chromosome 2AS, and on chromosome 2BL close to *wmc332*. No QTL for high GPC was detected on chromosome 6BS, suggesting that Strongfield contains novel QTL for high GPC not previously reported in the literature. The molecular markers flanking the QTL identified in this study can be used by durum wheat breeders to enhance selection high GPC in durum wheat.

Acknowledgements

Funding for this research was provided by NSERC and Western Grain Research Foundation, Canada. Scholarship support provided by the Technological and Professional Skill Development Project (TPSDP) of Ministry of National Education, Indonesia is gratefully acknowledged. Thanks to N. Hirji, C. Tang, A. Hahn, L. Zaworsky, A. Tomita, J. Ross and M. Olfert for their technical support on this project.

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