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Effects of the *Gpc-B1* locus on high grain protein content introgressed into Argentinean wheat germplasm

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With 3 tables

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Abstract

Wheat grain protein content (GPC) is important for human nutrition and has a strong influence on the quality of pasta and bread. The objective of this study was to analyse the introduction of the *Gpc-B1* allele into two Argentinean bread wheat cultivars. Near-isogenic lines were developed in 'ProINTA Oasis' and 'ProINTA Granar' using marker-assisted selection. *Gpc-B1* lines showed a significant (P = 0.01) increase in GPC and a significant (P = 0.001) decrease in grain weight in comparison with control lines without *Gpc-B1*. Differences in yield were not significant (P = 0.49) between lines. *Gpc-B1* lines significantly reduced (P = 0.02) straw nitrogen concentration at maturity and significantly increased (P = 0.02) the nitrogen harvest index. When data were analysed by genotype and environment, differences in some analysed parameters were found, indicating that *Gpc-B1* expression may be affected by different genetic backgrounds and environmental conditions. These results suggest that the introgression of the *Gpc-B1* allele into Argentinean wheat germplasm could be a valuable resource for improving GPC with no detrimental effect on grain yield.

Key words: wheat — grain protein content — quality — nitrogen — *Gpc-B1* — *Triticum turgidum* var. *dicoccoides* — protein yield

Bread wheat (*Triticum aestivum* L.) is the most important winter crop in Argentina with a sown area between 4.5 and 6 million hectares in recent years (FAO 2011). This value could be increased through the development of novel wheat cultivars with an improved integral quality, as many Argentinean wheat cultivars do not meet the specific industrial demands of both local and foreign consumers.

Grain protein content (GPC) is one of the most important factors determining the quality of wheat in pasta and bread making, and it also contributes to human nutrition. In addition, high protein is one of the main targets for hard and durum wheat breeding programmes, as it determines premium prices in many markets around the world.

Despite its economic importance, the improvement in GPC by traditional breeding has slowed down because of the high influence of the environment on this trait and its negative correlation with yield (Austin et al. 1980, Masclaux et al. 2001, Lawlor 2002, Triboi and Triboi-Blondel 2002, Groos et al. 2003, Gonzalez-Hernandez et al. 2004, Barneix 2007).

Wild gene introgressions into cultivated modern genotypes have expanded the genetic diversity and have provided new alternatives to increasing GPC levels (Uauy et al. 2006a). The identification of genes with high GPC and the development of molecular markers for indirect selection are a convenient alternative, especially for those traits that exhibit high genotype–environment interactions and low heritability (Prasad et al. 2003).

A quantitative trait locus (QTL) for GPC was first mapped on chromosome arm 6BS in a population of recombinant inbred lines derived from the *Triticum turgidum* var. *durum* cultivar 'Langdon' (LDN) and the chromosome substitution line LDN (DIC6B) (Joppa et al. 1997). Chromosome 6B from this substitution line came from *T. turgidum* var. *dicoccoides* (Joppa and Cantrell 1990). The 6BS QTL was later mapped as a single Mendelian locus named *Gpc-B1*, within a 2.7-cM region (Olmos et al. 2003), and was associated with the GPC increase of 14 g/kg in both tetraploid and hexaploid lines across diverse environments (Joppa et al. 1997, Chee et al. 2001, Olmos et al. 2003). More recently, Brevis and Dubcovsky (2010) compared near-isogenic lines (NILs) with contrasting *Gpc-B1* alleles in tetraploid and hexaploid wheat and showed that the functional *Gpc-B1* is associated with increases in both protein content and total protein yield. These experiments were limited to hard spring cultivars adapted for California, and consequently, additional studies are necessary to expand their conclusions to other market classes and environments. Kumar et al. (2011) showed GPC increases in seven Indian bread wheat lines with *Gpc-B1,* while Carter et al. (2011) showed that differences in environmental conditions appear to impact the expression of the *Gpc-B1* gene, which may limit its utility as a breeding strategy for increasing GPC for spring wheat cultivars produced in regions with short growing seasons.

The objective of this study was to evaluate the *Gpc-B1* allele's effects on GPC, protein yield, yield and their components in two locally adapted bread wheat cultivars from Argentina, where the introgression of this allele is being reported for the first time.

Materials and Methods

Plant materials: Near-isogenic lines for *Gpc-B1* were developed in two spring hexaploid Argentinean wheat cultivars: 'ProINTA Granar' ('MARCOS-JUAREZ-INTA'//'PAK-3563'/'CHAP-70/3'/'DEI', 'P. Granar' hereafter) and 'ProINTA Oasis' ('OASIS'/'TORIM-73', 'P. Oasis' hereafter),

by six backcrosses, self-pollination of BC_6 plants heterozygous for *Gpc-B1* and selection of homozygous BC_6F_2 plants, followed by two cycles of multiplication to increase homozygous seeds. The common wheat cultivar 'Glupro' ('Columbus'/*T. turgidum* var. *dicoccoides*//'Len') (Khan et al. 2000) was the source of *Gpc-B1*. At the beginning of the germplasm development process, the selection of the positive plants for *Gpc-B1* was carried out by using the microsatellite markers *Xgwm508*, *Xgwm644* and *Xgwm193* (Röder et al. 1998, Olmos et al. 2003). *Xuhw89* (Distelfeld et al. 2006) was later used to confirm the genotypes in BC_6F_4 NILs.

Hereafter, the BC_6F_4 NILs carrying the *Gpc-B1* allele from *T. turgidum* var. *dicoccoides* will be referred to as *Gpc-B1* lines, whereas BC_6F_4 NILs without the *Gpc-B1* will be referred to as control lines.

Field experiments: The field experiments were organized in a completely randomized factorial design with five replications, throughout 2005, 2006 and 2008 at the Instituto de Recursos Biológicos (IRB), Instituto Nacional de Tecnología Agropecuaria (INTA), Hurlingham City, Province of Buenos Aires, Argentina (34°S, 58°W). Experimental units consisted of two rows of one-metre-long plots, spaced 0.25 m apart from one another. The main plot and subplots in each experiment corresponded with the genetic background and with the different *Gpc-B1* alleles to maximize the sensitivity of the comparison within isogenic pairs.

In 2009, the field experiments were hand-sown in two different locations, Hurlingham (IRB-INTA) and Balcarce (Province of Buenos Aires, 38°S, 58°W at Estación Experimental Agropecuaria Balcarce, INTA), under a factorial design with four replications in complete blocks and experimental units of 7 m^2. Temperature and precipitation data by month can be obtained from http://climayagua.inta.gob.ar and http://anterior.inta.gov.ar/balcarce/info/meteor.htm. When rain levels did not satisfy demands, the experiments were irrigated manually.

Biomass and yield evaluations: Grain protein content and thousand kernel weight (TKW) were evaluated in 2005, 2006 and 2008 experiments. After threshing, 1000 grains from each plot were manually cleaned, counted and dried to constant weight to obtain a value that was registered and informed.

To estimate yield components in the 2009 experiment, aboveground biomass was measured in a 1-m^2 sample from the centre rows of each plot, and samples were oven-dried at 50°C to a constant weight. Spikes per square metre (spikes/m^2) from each sample were counted, weighed and threshed, and the resultant grains were also weighed to calculate grain yield, aboveground biomass and the harvest index (HI, that is, the quotient between grain yield and aboveground biomass). Grains per square metre (grains/m^2) were calculated as the quotient for grain yield and TKW $\times$ 10^{-3}. A sample of 10 spikes was taken from the plot to measure the number of grains per spike and the grain yield per spike.

Protein and nitrogen determination: Grain protein content, nitrogen harvest index (NHI) and straw N concentration (SNC) were measured in 100 mg of straw and 100 mg of grain subsamples, previously ground in an experimental 1-mm screen mill. The samples were analysed for total N using micro-Kjeldahl distillation after wet digestion in concentrated H_2SO_4 and H_2O_2. The conversion factor from N to protein was 5.73. NHI was calculated as follows: grain N concentration $\times$ grain yield/total N in the plant (grain N + vegetative N). Protein yield was calculated by multiplying the grain yield and GPC.

Statistical analysis: Analyses of variance (ANOVA) were performed using 'Infostat' software (Di Rienzo et al. 2011). LSD Fisher-test was used to compare means of *Gpc-B1* introgression within each isogenic pair and the interaction of the gene with genotype and environment when ANOVA revealed significant differences between means (Steel and Torrie 1989).

The 2005, 2006 and 2008 experiment results were analysed separately from those of 2009. All data were analysed using a three-way factorial with 2 gene levels (*Gpc-B1*/Control), 2 genotypes ('P. Oasis' and 'P. Granar') and 3 environments (years) for 2005, 2006 and 2008 experiments and 2 environments (Hurlingham and Balcarce) for 2009 experiment.

Results

Gpc-B1 lines consistently had greater GPC during 2005, 2006 and 2008 in Hurlingham (Table 1) in both backgrounds where *Gpc-B1* was introgressed. Over the three years of evaluation, GPC was 6.8% greater, on average (P = 0.004). No significant interactions were observed between gene by genotype and gene by year (P = 0.872 and P = 0.945, respectively). All *Gpc-B1* lines showed significant reduction in TKW when it was analysed by genotype. Grain size decreased significantly (P = 0.005) by 9.6% in 'P. Granar' background and by 7.1% in 'P. Oasis' (P = 0.0001). No significant interactions were observed between gene effect and year (P = 0.804 for 'P. Granar' and P = 0.293 for 'P. Oasis'); however, year effect was highly significant (P = 0.0001) for both cultivars.

During 2009, average GPC was 120.9 g/kg in control lines, while *Gpc-B1* lines showed a significant (P = 0.022) increase of 3.61 g/kg (Table 2) when data were analysed across all genotypes and locations. The analysis by genotype showed that *Gpc-B1* introgression was also associated with GPC increases (Table S1).

No significant effect of the gene introgression in aboveground biomass, grain yield and protein yield could be detected across all genotypes (P = 0.923, P = 0.491 and P = 0.126, respectively, Table 2). Nevertheless, when data were analysed by genotype, only 'P. Granar' *Gpc-B1* lines showed significant increases in grain yield (P = 0.020) and protein yield (P = 0.007) in comparison with control lines. Although grain yield was similar across all genotypes, there was a significant reduction (8.38%, P = 0.0001) in TKW in *Gpc-B1* lines (Table 2). In 'P. Granar' *Gpc-B1* lines, the reduction was 9.30% (P = 0.0005), whereas in 'P. Oasis' *Gpc-B1* lines, it was 7.5% (P = 0.003) relative to their respective control lines (Table S1).

The lower TKW did not affect grain yields because of a significant increase in grains/m^2 (P = 0.006) in *Gpc-B1* lines. This increase was explained by an increment in the spikes/m^2

Table 1: Mean values, standard errors of the means (SEMs) and significance levels of grain protein content (GPC, g/kg) of the two near-isogenic lines (NILs) grown in 2005, 2006 and 2008 experiments in Hurlingham location

	2005		2006		2008		
Cultivar	'P. Oasis'	'P. Granar'	'P. Oasis'	'P.Granar'	'P. Oasis'	'P. Granar'	Grand Mean
Control	119.4 ± 1.2	130.5 ± 0.4	93.5 ± 2.4	109.1 ± 1.1	111.9 ± 1.3	149.7 ± 7.4	118.9 ± 3.5
Gpc-B1	129.3 ± 1.8	135.6 ± 0.6	101.2 ± 1.9	116.6 ± 2.3	118.8 ± 3.9	160.6 ± 6.0	127.1 ± 3.6
Δ%[1]	8.3**	3.6**	8.3*	6.9*	7.1*	7.2*	6.8**

[1]Mean change between *Gpc-B1* and control (as percentage of the control).
*Significance at P = 0.05,
**Significance at P = 0.01.

Table 2: Mean values, standard errors of the means (SEMs) and significance levels of the different analysed parameters of the two near-isogenic lines grown in the 2009 experiment in Hurlingham and Balcarce locations

Hurlingham/ Balcarce	Control	*Gpc-B1*	
	Mean ± SEM		Δ(%)[1]
GPC (g/kg)	120.9 ± 1.6	124.5 ± 2.1	2.9*
Aboveground biomass (g/m)	1731.2 ± 211.9	1724.3 ± 219.8	0.4
Grain yield (Mg/ha)	5.4 ± 0.3	5.6 ± 0.3	2.9
Protein yield (kg/ha)	654.3 ± 32.1	693.1 ± 37.8	5.9
TKW (g)	30.1 ± 0.4	27.6 ± 0.4	−8.3***
Grains/m^2	16904.1 ± 1090.1	19217.4 ± 1529.1	13.6**
Spikes/m^2	438.44 ± 30.06	491.5 ± 41.0	12.1**
Grains per spike	41.2 ± 1.3	42.5 ± 1.4	3.2
Grain yield per spike (g)	1.2 ± 0.1	1.1 ± 0.1	−5.6*
HI (%)	35.9 ± 2.9	35.9 ± 2.6	−0.1
NHI (g/kg)	691.8 ± 45.8	740.6 ± 36.3	7.1*
SNC (g/kg)	6.8 ± 1.4	5.3 ± 1.1	−21.7*

GPC, grain protein content; TKW, thousand kernel weight; NHI, nitrogen harvest index; SNC, straw nitrogen concentration; HI, harvest index.
[1]Mean change between *Gpc-B1* and control (as percentage of the control).
*Significance at P = 0.05,
**Significance at P = 0.01,
***Significance at P = 0.001.

(P = 0.008) against a not significant (P = 0.199) grains per spike number between *Gpc-B1* and control lines. In the analysis by genotype, only the 'P. Granar' *Gpc-B1* lines showed significant increases in grains/m^2 (P = 0.001) and spikes/m^2 (P = 0.024) relative to the control lines (Table S1).

HI was not affected by the gene introgression (P = 0.968), but there was an increase in NHI (P = 0.019) and a decrease in SNC (P = 0.019) when data were analysed across all genotypes (Table 2). When data were analysed by genotype, only the 'P. Granar' *Gpc-B1* lines showed significant (P = 0.012) increases in HI and NHI (P = 0.008) and a significant (P = 0.010) decrease in SNC (Table S1).

The gene introgression did not show any interaction with genotype in most of the measured parameters, except for HI (P = 0.006). Only TKW and SNC showed significant (P = 0.035 and P = 0.044, respectively) gene by location interaction (Table 3). When the main factors of the ANOVA were evaluated across all genotypes, all parameters showed significant effects of genotype and environment, expect for GPC and TKW where the environment effect was not significant (P = 0.069 and P = 0.508, respectively, Table 3).

Table 3: Significance (P-values) of genotype and location principal effects, gene by genotype and gene by location interactions of ANOVA for the 2009 experiment grown in Hurlingham and Balcarce

Variable	Genotypes	Locations	Gene × Genotype	Gene × Location
GPC (g/kg)	0.0001	0.069	0.713	0.925
Aboveground biomass (g/m^2)	0.140	0.0001	0.086	0.947
Grain yield (Mg/ha)	0.0001	0.0001	0.207	0.487
Protein yield (kg/ha)	0.029	0.0001	0.109	0.398
TKW (g)	0.0001	0.508	0.648	0.035
Grains/m^2	0.013	0.0001	0.234	0.056
Spikes/m^2	0.012	0.0001	0.050	0.050
Grains per spike	0.0001	0.003	0.840	0.973
Grain yield per spike (g)	0.0001	0.004	0.768	0.123
HI (%)	0.0001	0.0001	0.006	0.736
NHI (g/kg)	0.001	0.0001	0.225	0.196
SNC (g/kg)	0.002	0.0001	0.166	0.044

GPC, grain protein content; TKW, thousand kernel weight; NHI, nitrogen harvest index; SNC, straw nitrogen concentration; HI, harvest index.

Discussion

Gpc-B1 has been described as a gene that increases GPC in tetraploid and hexaploid wheat (Brevis and Dubcovsky 2010). Our results showed that when the *Gpc-B1* gene was introgressed into Argentinean germplasm, it consistently increased GPC across environments, ranging from 3.6 to 9.9 g/kg. The data analysed by genotype showed that the presence of *Gpc-B1* never diminished GPC and that this increase was significant across all years included in the study (Table 1) except for 'P. Oasis' in Hurlingham and 'P. Granar' in Balcarce for 2009 experiments (Table S1).

These results are similar to those reported in previous studies. Mesfin et al. (2000) showed GPC increases ranging from 9.6 to 11.5 g/kg using three hexaploid wheat recombinant inbred populations. Chee et al. (2001) showed an increase in GPC of 15 g/kg in a tetraploid wheat population. In addition, in a population derived from a cross between Messapia and *T. turgidum* var. *dicoccoides,* Blanco et al. (2002) showed increases ranging from 13 to 15 g/kg. In 2010, Brevis and Dubcovsky showed increases in GPC ranging from 11.9 to 16.3 g/kg in three tetraploid lines and from 4.5 to 15.2 g/kg in six hexaploid genotypes. Kumar et al. (2011) showed GPC increases from 14.8% to 17.8% in seven BC_3F_6 lines for *Gpc-B1* compared with their control lines.

When the agronomic characters were studied in NILs derived from Argentinean germplasm, no yield reduction was observed in any case. However, there was a consistent decrease in grain size in both Argentinean backgrounds, where *Gpc-B1* was introgressed. This decrease in grain size has been observed previously by Uauy et al. (2006b), Brevis and Dubcovsky (2010) and Kumar et al. (2011). In our experiments, the reduction in kernel weight was compensated by an increase in grains/m^2 due to an increase in the spikes/m^2. Consequently, *Gpc-B1* lines did not exhibit a reduction in grain yield.

These results show that GPC in both Argentinean cultivars, especially in 'P. Granar', could be raised without any yield penalty.

HI was not affected by the gene introgression (Table 2), but there was an increase in NHI and a decrease in SNC, which would explain a major GPC in the *Gpc-B1* lines due to a higher N remobilization. Brevis and Dubcovsky (2010) observed similar increases in NHI and decreases in SNC.

Carter et al. (2011) suggested that differences in environmental conditions appeared to impact the expression of the *Gpc-B1* gene, which might limit its utility as a breeding strategy to increase GPC in spring wheat cultivars produced in regions with short growing seasons. In this work, we observed that the effect of *Gpc-B1* was sufficiently consistent across years and environments to be implemented in breeding programmes for improving GPC.

Most of the traits analysed here did not show gene by genotype and gene by location interactions (Table 3). However, individual effects of genotype and location were significant (Table S1). These results emphasize the importance of these factors in modulating the effect of the *Gpc-B1* allele and suggest that the breeder should select the appropriate genotype with *Gpc-B1* in the appropriate location to have a positive cost-benefit balance.

The lines compared in these experiments are near-isogenic, so the differences in grain size and year number should be attributed to a specific effect of *Gpc-B1* or genetic factor linked to the introgressed gene. When the grain number is increased, the final grain weight usually decreases, as the same amount of assimilates should be allocated to more sinks (Fischer 2011), which could explain the observed decrease in grain size. However, we can only speculate about the cause of the *Gpc-B1* introgression on the increase in spikes/m^2. The number of tillers produced by cereals is affected by many environmental factors, including nitrogen supply, and is related to cytokinin levels (Wang and Below 1996, Liu et al. 2011). It has been observed that the main effect of *Gpc-B1* is related to an acceleration of leaf senescence and more efficient N remobilization (Kade et al. 2005, Uauy et al. 2006b, Waters et al. 2009). Cytokinin concentration is partly modulated by the amount of available nitrogen (Sakakibara et al. 2006). Therefore, it is possible that by affecting N metabolism, *Gpc-B1* is indirectly affecting tiller production.

As the active form of the *Gpc-B1* gene is absent in most commercially grown varieties (Uauy et al. 2006a), the incorporation of this allele has the potential to improve the grain and industrial quality. The use of MAS for wheat breeding programmes is a useful tool for developing wheat genotypes with high GPC. The present results provide the first evidence that the incorporation of *Gpc-B1* into Argentinean wheat genotypes allow the breeding of cultivars with higher GPC without adversely affecting grain yield.

At present, we are developing new Argentinean lines with *Gpc-B1* to evaluate all the possible differential responses of this allele in the various wheat-growing regions of the country.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Values, standard errors of the means (SEMs) and significance levels of the different analyzed parameters of the two near-isogenic lines grown in the 2009 experiment for the two locations under study.