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Authors

Brevis, Juan Carlos
Morris, Craig F
Manthey, Frank
et al.

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Corresponding Author: Dr Jorge Dubcovsky,

Corresponding Author's Institution: University of California

First Author: Juan C Brevis, PhD

Order of Authors: Juan C Brevis, PhD; Craig F Morris, PhD; Frank Manthey, PhD; Jorge Dubcovsky

Abstract: Grain protein concentration (GPC) affects wheat nutritional value and several critical parameters for bread and pasta quality. A gene designated Gpc-B1, which is not functional in common and durum wheat cultivars, was recently identified in *Triticum turgidum* ssp. *dicoccoides*. The functional allele of Gpc-B1 improves nitrogen remobilization from the straw increasing GPC, but also shortens the grain filling period resulting in reduced grain weight in some genetic backgrounds. We developed isogenic lines for the Gpc-B1 introgression in six hexaploid and two tetraploid wheat genotypes to evaluate its effects on bread-making and pasta quality. In common wheat, the functional Gpc-B1 introgression was associated with significantly higher GPC, water absorption, mixing time and loaf volume, whereas in durum wheat, the introgression resulted in significant increases in GPC, wet gluten, mixing time, and spaghetti firmness, as well as a decrease in cooking loss. On the negative side, the functional Gpc-B1 introgression was associated in some varieties with a significant reduction in grain weight, test weight, and flour yield and significant increases in ash concentration. Significant gene \times environment and gene \times genotype interactions for most traits stress the need for evaluating the effect of this introgression in particular genotypes and environments.

Corresponding author's full postal address; Corresponding author's telephone and fax numbers and e-mail address;
Corresponding author requested information added

Short abstract/summary; 3-6 keywords (if relevant);
Keywords added

An alphabetical list of abbreviations;
Updated list in front page

In text references are cited in the accepted style; Complete alphabetical list of references in the accepted style**;
All tables cited in the text are supplied; All original figures are supplied; All figure legends are supplied; Tables,
figures and figure legends are supplied separately, not embedded in text.*

Checked

Reviewer #1: The authors are asked to attend the following observations:

Abbreviations: Include definition of SPC and NAC

Added to the Abbreviation list in the front page and the first time the abbreviation is mentioned in the text
NAC, domain present in NAM, ATAF and CUC genes
SPC, semolina protein concentration

Materials and Methods

Lanes 101-102. According to both, Payne 1987 and Pogna et al., 1992 (see reference below), Glu-B1 subunit 6+8 also contributes to weak gluten. You may want to consider indicating so in this paragraph. Actually, cv. 1041 did not show improvements in gluten strength -related parameters (Fig 1E and F).

(Pogna, N.E., Radaelli, R., Dackevitch, T., Curioni, A., and Dal Belin Perufo A. 1992. Benefits from genetics and molecular biology to improve the end use properties of cereals. In P. Feillet, ed. Cereal chemistry and technology: Along past and a bright future, p. 83-93. INRA, Montpellier, France.)

The *Glu-B1* 6+8 comment and the Pogna et al. (1992) reference were added as suggested: “The other hexaploid recurrent parents have *Glu-A1* and *Glu-D1* high-molecular weight glutenin alleles associated with strong gluten (Payne, 1987, Table 1). **According to both, Payne (1987) and Pogna et al., (1992) the *Glu-B1* subunits 6+8 present in UC1041 contributes to weak gluten.**”

Lane 176. Add I to AACC.

Corrected as requested

Results

Lane 231. The references Bloksma, 1972 and Souza et al., 2004 cited here, are not listed in the References Section
Bloksma 1972 reference was eliminated from the text

Souza et al. 2004 was added to the references: “Souza, E.J., Martin, J.M., Guttieri, M.J., O'Brien, K.M., Habernicht, D.K., Lanning, S.P., McLean, R., Carlson, G.R., Talbert, L.E., 2004. Influence of genotype, environment, and nitrogen management on spring wheat quality. *Crop Science* 44, 425-432.”

We apologize for this omission

Lane 257. In this paragraph the parameter indicated is SK weight (and SK size) and in Table 2 the parameter is indicated as grain weight (GWT). Change throughout the manuscript to SK S (or grain weight and grain size) for consistency. Differences in SK size are mentioned in the text but not presented in Table 2.

For consistency, the few references indicating SK weight were changes to grain weight. References to grain seize were eliminated from the text, and replaced by grain weight. For example:

3.1.2. “The *Gpc-B1* NILs showed highly significant ($P < 0.01$) decreases in **grain weight and test weight**, relative to the control NILs (Table 2).”

2.3.1. “The traits studied in common wheat were grouped in grain traits: test weight, **grain weight**,

Discussion

Lane 410-411. Is Walters in the reference Walters et al., 2009 misspelled (should it be Waters?)? Or this reference is not cited in the Reference Section?

It was corrected to Waters et al. 2009

Lane 427. Sorrels, in Breseghello and Sorrels, 2000, is spelled differently in the References Section.

It was corrected to “Breseghello and Sorrells” as in the references

References

Lane 554. Reference et al., 2006a listed here is not cited in the manuscript.

“Uauy et al. 2006a” was eliminated from the references and “Uauy et al. 2006b” was replaced in the text by “Uauy et al. 2006” throughout the text.

Effect of the grain protein content locus *Gpc-B1* on bread and pasta quality

Juan Carlos Brevis ^{a1}, Craig F. Morris ^b, Frank Manthey ^c and Jorge Dubcovsky ^{a*}

^a Department of Plant Sciences, University of California, Davis, CA 95616-8780, USA

^b USDA-ARS Western Wheat Quality Laboratory, Washington State University, Pullman, WA 99164-6394, USA

^c Department of Plant Sciences, North Dakota State University, Fargo, ND 58108-6050, USA

¹ Present address: Enza Zaden Research USA, P.O. Box 866, CA 95045, San Juan Bautista, CA, USA.

Corresponding author Jorge Dubcovsky
Dept. Plant Sciences, University of California,
Davis, CA 95616, USA
Office Phone: (530) 752-5159; FAX (530) 752-4361
Email: jdubcovsky@ucdavis.edu

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Abbreviations: ANOVA, analysis of variance; BAB, bread-baking water absorption; BC, backcross; BFY, break flour yield; DIC, *Triticum turgidum* ssp. *dicoccoides* accession FA-15-3; FASH, flour ash concentration; FPC, flour protein concentration; FY, flour yield; GASH, grain ash concentration; GPC, grain protein concentration; HRS, hard red spring; HWS, hard white spring; MAB, mixograph water absorption; NAC, domain present in NAM, ATAF and CUC genes; NIL, near-isogenic line; SASH, semolina ash concentration; SEM, standard error of the mean; SPC, semolina protein concentration; UC, University of California.

Abstract

Grain protein concentration (GPC) affects wheat nutritional value and several critical parameters for bread and pasta quality. A gene designated *Gpc-B1*, which is not functional in common and durum wheat cultivars, was recently identified in *Triticum turgidum* ssp. *dicoccoides*. The functional allele of *Gpc-B1* improves nitrogen remobilization from the straw increasing GPC, but also shortens the grain filling period resulting in reduced grain weight in some genetic backgrounds. We developed isogenic lines for the *Gpc-B1* introgression in six hexaploid and two tetraploid wheat genotypes to evaluate its effects on bread-making and pasta quality. In common wheat, the functional *Gpc-B1* introgression was associated with significantly higher GPC, water absorption, mixing time and loaf volume, whereas in durum wheat, the introgression resulted in significant increases in GPC, wet gluten, mixing time, and spaghetti firmness, as well as a decrease in cooking loss. On the negative side, the functional *Gpc-B1* introgression was associated in some varieties with a significant reduction in grain weight, test weight, and flour yield and significant increases in ash concentration. Significant gene \times environment and gene \times genotype interactions for most traits stress the need for evaluating the effect of this introgression in particular genotypes and environments.

1. Introduction

Quality traits are becoming increasingly important in wheat breeding programs due to higher standards imposed by millers, bakers, and consumers. Increased urbanization and associated changes in dietary habits have resulted in an increasing demand for wheat with specific quality attributes (Peña, 2007). These food products include bread, cookies, and pastries made with common wheat (*Triticum aestivum* L.); and pasta made with durum wheat (*T. turgidum* ssp. *durum* L.). For both bread and pasta products, grain protein concentration (GPC) is a critical trait that affects both their nutritional value and several quality parameters (Dick and Youngs, 1988; Finney et al., 1987). GPC is also a key parameter for market grading and classification.

Wild emmer wheat, *T. turgidum* ssp. *dicoccoides* (DIC hereafter) is one of the most promising species for expanding the genetic variation in GPC (Avivi, 1978; Brevis and Dubcovsky, 2010; Gonzalez-Hernandez et al., 2004; Joppa et al., 1997; Levy and Feldman, 1987; Olmos et al., 2003). The DIC accession FA-15-3, collected in Israel, showed very high levels of GPC (Avivi, 1978) and was the source of the high grain protein content locus *Gpc-B1*, located on the short arm of chromosome 6B (Joppa et al., 1997; Olmos et al., 2003). The *Gpc-B1* gene encodes a NAC (domain present in NAM, ATAF and CUC genes) transcription factor designated NAM1 that is closely related to a group of three related Arabidopsis proteins including the No Apical Meristem (NAM) protein (Uauy et al., 2006). Modern tetraploid and hexaploid wheat cultivars have a deletion at this locus or a non-functional copy as a result of a frame-shift mutation, whereas DIC accessions have a functional *Gpc-B1* allele (Uauy et al., 2006).

Comparisons between near-isogenic lines (NILs) with contrasting *Gpc-B1* alleles in tetraploid and hexaploid wheat have been recently used to show that the functional *Gpc-B1* allele is associated with increases in both protein concentration and total protein yield (Brevis and Dubcovsky, 2010). The increased N accumulation in the grain was paralleled by a decrease of the residual N in the straw, suggesting a more efficient N remobilization (Brevis and Dubcovsky, 2010; Waters et al., 2009). The same set of NILs is used in this study to investigate the effect of the functional *Gpc-B1* allele on the major milling, bread-baking and pasta-making quality traits used for quality characterization of common and durum wheat. This set of NILs includes varieties with contrasting levels of GPC and high-molecular weight glutenin subunits

composition and therefore, are particularly valuable to test the effect of the *Gpc-B1* alleles on quality in different genetic backgrounds.

2. Experimental

2.1 Plant Materials

Near isogenic lines (NILs) of DIC *Gpc-B1* were developed by six backcrosses followed by two cycles of self-pollination to produce BC₆F₃ homozygous lines of six hexaploid and two tetraploid backgrounds (Brevis and Dubcovsky, 2010). The BC₆F₃ lines are expected to be more than 99% identical to the recurrent parent. The hexaploid cv. Glupro (Columbus/*T. turgidum* ssp. *dicoccoides*//Len) and the recombinant substitution line RSL65, derived from cv. Langdon (Citr 13165), were the donors of the DIC *Gpc-B1* introgression for the hexaploid and tetraploid genotypes, respectively (Chicaiza et al., 2006). The source of the chromosome segment present in both the tetraploid and hexaploid parental donors was the DIC accession FA-15-3 collected in Israel (Avivi, 1978). The size of the segment of the DIC chromosome arm 6BS introgressed into the parental donor lines was approximately 15-30 cM (Mesfin et al., 1999; Khan et al., 2000), but it could have been further reduced during the backcrossing process used to generate the NILs. Hereafter, the BC₆F₃ NILs carrying the *Gpc-B1* introgression will be referred to as *Gpc-B1* lines, whereas the recurrent parents will be referred to as control lines.

The hexaploid recurrent parents included the hard white spring (HWS) cv. Attila (PI 351590), and five hard red spring (HRS) genotypes, the cultivars Anza (Citr 15284), RSI5 (Resource Seeds, Inc.; Gilroy, CA), and Yecora Rojo (Citr 17414), and the University of California (UC) breeding lines UC1037 (Solar/3/Cleo/I66//Anza) and UC1041 (Yecora Rojo/Tadinia). Anza and RSI5 recurrent parents have low GPC, Attila has intermediate GPC levels, and the other three parental lines have high GPC (Table 1). Attila is the only genotype with the 1RS.1BL translocation, which is known to be associated with poor rheological properties (Dhaliwal et al., 1987). Anza, RSI5 and UC1037 have high-molecular weight glutenin alleles which are known to be associated with weak gluten (Payne, 1987): RSI5 has the null *Glu-A1c* allele, UC1037 has the *Glu-D1a* allele (2+12 subunits), and Anza has both the null *Glu-A1c* and *Glu-D1a* alleles (Table 1). The other hexaploid recurrent parents have *Glu-A1* and *Glu-D1* high-molecular weight glutenin alleles associated with strong gluten (Payne, 1987, Table 1). According to both, Payne

(1987) and Pogna et al., (1992) the *Glu-B1* subunits 6+8 present in UC1041 contributes to weak gluten.

The tetraploid recurrent parents used in this study included the cultivar Kronos (Arizona Plant Breeders; Arizona City, AZ), and the UC breeding line UC1113 (selection from CIMMYT cross CD52600 [Kifs//RSS/BD1419/3/Mexis-CP/4/Wahas/5/Yav79]). Kronos has high GPC and excellent pasta quality, whereas UC1113 shows intermediate levels of GPC and pasta quality (Table 1).

2.2. Field Experiments

Wheat grain samples were obtained from field experiments conducted in 2006 and 2007 in Davis, CA at the UC Experimental Field Station (38° 32' N, 121° 46' W) and El Centro, CA at the UC Desert Research and Extension Center (32°48' N, 115°26' W), and previously described by Brevis and Dubcovsky (2010). Briefly, the experiments were arranged in a split-plot design with five (El Centro) and ten (Davis) randomized complete blocks. The main plot corresponded to the genetic background (cultivar or breeding line) and the subplots, to the presence/absence of the DIC *Gpc-B1* allele. In Davis, the experiments were sown in November and harvested in June, whereas in El Centro, the growing season was from December to May. Fertilization, irrigation, and disease control were applied to obtain high grain yield according to crop rotation and growing conditions and were described in detail by Brevis and Dubcovsky (2010). Plots were machine harvested at maturity.

For each experiment, four 600 g-grain samples of each hexaploid NIL and five 3 kg-grain samples of each tetraploid NIL were used for quality analyses. In Davis, composite grain samples were pooled from two blocks, and four samples for hexaploid wheat and five for tetraploid wheat were submitted for quality analyses. In El Centro, the first four blocks were sampled for hexaploid wheat, whereas all five blocks were used for durum wheat. Hexaploid wheat samples were evaluated at the USDA-ARS Western Wheat Quality Laboratory (Pullman, WA) for milling and bread-baking quality analysis. Durum wheat samples were assessed for pasta-making quality at the Durum Wheat Quality and Pasta Processing Laboratory at North Dakota State University (Fargo, ND).

2.3. Quality Analyses

Both wheat quality laboratories used analytical procedures standardized and described by the AACCI International (AACCI, 2000). The methodology used for the common wheat quality analyses was described before (Brevis et al., 2008) and is presented briefly below.

2.3.1. Hexaploid wheat

The traits studied in common wheat were grouped in *grain traits*: test weight, grain weight, grain hardness, and GPC; *flour and milling traits*: flour protein concentration (FPC), flour yield (FY), break flour yield (BFY), flour ash concentration (FASH), and milling score; and *bread-baking traits*: mixograph water absorption (MAB), bread-baking water absorption (BAB), bread dough mixing time, and loaf volume.

Grain weight, and hardness were the average of 300 grains obtained from an Single Kernel Characterization System Model 4100 (Perten Instruments, Springfield, IL, USA). GPC and FPC (expressed on a 12 and 14 percent moisture basis, respectively) were determined by near-infrared spectroscopy using an Inframatic grain and flour analyzers (Perten Instruments, Springfield, IL, USA), calibrated using Dumas combustion nitrogen (AACCI Approved Method 46-30). Grain samples were assessed for test weight and then scoured in a Cyclone Grain Scourer (model 6, Forster and Son, Ada, OK). A Brabender Quadrumat laboratory mill was used to estimate straight-grade white flour yield, break flour yield (the amount by weight of the total products recovered as flour off the break rolls), and milling score (Jeffers and Rubenthaler, 1977). FASH was measured from a 4-g flour sample ignited and heated at 550°C for 15 h (AACCI Approved Method 08-01). Straight-dough bread-baking analyses evaluated the optimum water absorption and mixing parameters in a 90-min fermentation method using 100 g flour (AACCI Approved Method 10-10B). Mixing time corresponded to the time required to mix the flour and other dough constituents to the optimum condition as judged by an experienced baker, whereas loaf volume was measured by canola seed displacement.

2.3.2. Tetraploid wheat

The traits assessed in durum wheat were grouped in *grain traits*: grain weight, test weight, grain ash concentration (GASH) and GPC; *semolina traits*: semolina protein concentration (SPC), wet

gluten, gluten index, semolina color, semolina ash concentration (SASH), and the mixogram parameters mixing time, peak height, peak width, and final height and width; and *pasta-making traits*: spaghetti color, cooked firmness and cooking loss.

Grain weight was estimated by counting the number of grains in 10 g of clean seed using an electronic seed counter. GPC and SPC were assessed as per hexaploid wheat based on AACCI Approved Method 46-30. SPC is presented on a 14 percent moisture basis. Milling and pasta processing procedures were described before (Carrera et al., 2007; Zhang et al., 2008). Briefly, grain samples were milled to semolina using a Bühler experimental mill fitted with two Miag laboratory scale purifiers (Bühler-Miag, Minneapolis, MN, USA). Hydrated semolina was extruded under vacuum as spaghetti using a DeMaCo semi-commercial laboratory extruder (DeFrancisci Machine Corp, Melbourne, FL, USA). Spaghetti was dried in a laboratory pasta drier (Standard Industries, Fargo, ND, USA) using a low temperature (40°C) drying cycle. Semolina ash concentration (SASH) and wet gluten were determined by AACCI Approved Methods 08-01 and 38-12, respectively. Gluten strength was evaluated by the gluten index method (AACCI Approved Method 38-12), and by using mixing curves from mixograph tests (AACCI Method 54-40A). Semolina and dry spaghetti color were measured as CIE *b*-values (yellowness) using a Minolta colorimeter Model CR310 (Minolta Corp., Ramsey, NJ). Spaghetti (10 g, 5 cm long) was cooked in 300 ml boiling distilled water for 12 min. The water was drained and the spaghetti was allowed to cool for 3 min. Cooked firmness was measured as the work required to shear five cooked strands of spaghetti at a right angle using a specially designed plexiglass tooth fitted to TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Cooking loss (CL) was the percent weight of solids lost after evaporating the cooking water to dryness at 110°C in a forced air oven.

2.4. Statistical Analyses

Analysis of variance was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC). The general linear model was used to test the effects of the *Gpc-B1* introgression within each isogenic pair and its interaction with genotype and environment (location by year combinations).

Exploratory models showed that the effect of the *Gpc-B1* introgression on quality parameters

was stronger in tetraploid than hexaploid wheat genotypes and, therefore, statistical analyses were performed by species.

Two types of analyses were performed. First, the data were analyzed across environments as a three-way factorial with two gene levels (with and without the DIC *Gpc-B1*), four environments, and six and two genotypes for hexaploid and tetraploid wheat, respectively. Environments were considered random factors (mixed model) to generalize conclusions across environments. This generalization uses the genotype x environment interaction as denominator in the F test, resulting in a more stringent test when the interactions are significant.

In the second set of analyses, the two locations were analyzed separately to describe the differences in the effect of the *Gpc-B1* introgression at Davis and El Centro across years. For the second model, the year (2006-2007) was used as a random factor. Data were transformed to meet the assumptions of the ANOVA model when necessary. If a transformation was applied, graphs and tables show untransformed least square means while the significance values correspond to the results of the analysis of the transformed data.

3. Results

3.1. Effects across environments in common wheat

3.1.1. Grain (GPC), flour protein concentration (FPC) and bread-baking quality traits

In the hexaploid recurrent parents GPC averaged 130.8 g kg^{-1} (Table 2), and ranged from 121.2 g kg^{-1} in the cv. Anza to 137.7 g kg^{-1} in the breeding line UC1041 (Fig. 1A). GPC was significantly ($P = 0.012$) higher in the lines carrying the DIC *Gpc-B1* introgression averaging 6.6 g kg^{-1} more protein than the control NILs (Table 2). Pair-wise comparisons by cultivar showed significant GPC increases in the *Gpc-B1* NILs of Anza ($P = 0.011$), RSI5 ($P = 0.019$), UC1041 ($P = 0.016$), and Yecora Rojo ($P = 0.015$) relative to their controls, whereas in Attila and UC1037 the differences were in the same direction but marginally not significant ($P = 0.06$; Fig. 1A). Average increases in GPC among the hexaploid NILs ranged from 4.1 g kg^{-1} in the cv. Yecora Rojo to 12.2 g kg^{-1} in the cv. RSI5.

FPC in the hexaploid recurrent parents averaged 114.2 g kg^{-1} (Table 2), and varied from 105.3 g kg^{-1} in the cv. Anza to 121.7 g kg^{-1} in the breeding line UC1041 (Fig. 1B). Protein losses due to

the milling process (GPC – FPC) were similar between *Gpc-B1* and control NILs, with average losses of 18.2 and 16.6 g kg⁻¹, respectively. The lines carrying the DIC *Gpc-B1* allele averaged 4.9 g kg⁻¹ more FPC than the control NILs (Table 2), but the differences were not significant ($P = 0.14$). When the data were analyzed by cultivar, all *Gpc-B1* genotypes showed higher FPC than their control NILs although only the cv. Anza showed a significant increase ($P = 0.014$; Fig. 1B). The *Gpc-B1* NIL of the cv. RSI5, which had the largest GPC increase among the hexaploid genotypes (Fig. 1A), also showed the largest increase in FPC relative to the control NIL, although this difference was marginally not significant ($P = 0.08$; Fig. 1B). This lack of significance is likely due to the higher coefficient of variation (CV) obtained for FPC (CV = 0.11) relative to that of GPC (CV = 0.06) in this pair of NILs.

The increase in GPC and FPC associated with the *Gpc-B1* introgression was paralleled by a significant increase in water absorption, a trait known to be highly correlated with GPC (Souza et al., 2004). Across genotypes, the *Gpc-B1* introgression was associated with an average increase in water absorption of 15 g kg⁻¹ that was highly significant ($P < 0.01$) for both MAB (amount of water absorbed by a standard amount of flour to make dough of proper consistency) and BAB (amount of water absorbed by a full bread formula to make dough of proper consistency) (Table 2).

The analyses by genotype showed consistent increases in water absorption associated with the *Gpc-B1* introgression in most genotypes (Fig. 1C). All the differences were significant ($P < 0.05$) with the exception of the cv. Attila that showed a marginally not significant increase ($P = 0.06$). Similarly, BAB was significantly ($P < 0.05$) higher in all *Gpc-B1* NILs except UC1037 and UC1041 ($P = 0.1$, Fig. 1D). The increases in MAB associated with the functional *Gpc-B1* allele ranged from 6.8 g kg⁻¹ in the breeding line UC1041 to 27.7 g kg⁻¹ in the cv. RSI5 (Fig. 1C), similar to those observed for BAB (Fig. 1D).

An additional benefit associated with the functional DIC *Gpc-B1* allele on baking quality was a significant increase in loaf volume ($P = 0.03$; Table 2). The average increase shown by the *Gpc-B1* NILs was of 79.1 cc (9.3% increase relative to the control NILs). When analyzed by cultivar, all *Gpc-B1* NILs showed higher loaf volume values relative to the control NILs, although these differences were significant ($P < 0.05$) only in three genotypes (Fig. 1E). Among the genotypes that exhibited significantly higher loaf volume in their *Gpc-B1* NILs, the cultivars Anza and

RSI5 averaged increases of 126 and 143 cc, respectively (>15% increase relative to the control NILs).

The *Gpc-B1* introgression was associated with a significant increase in mixing time ($P = 0.002$; Table 2) that was consistent across genotypes (Fig. 1F). Only the NILs for breeding line UC1041 did not show significant differences ($P = 0.2$).

3.1.2. Grain weight and milling quality traits

The *Gpc-B1* NILs showed highly significant ($P < 0.01$) decreases in grain weight and test weight, relative to the control NILs (Table 2). The genotypes that were most consistently affected by the *Gpc-B1* introgression were the breeding lines UC1037 and UC1041, and the cv. Yecora Rojo whose *Gpc-B1* NILs exhibited significantly ($P < 0.05$) lower values for all three traits (Fig. 1G; data not shown for test weight). The *Gpc-B1* introgression had no significant effect on grain hardness ($P = 0.76$, data not shown).

The reduction in grain weight associated with the functional *Gpc-B1* allele was paralleled by a 7 g kg⁻¹ reduction in FY ($P = 0.009$) relative to the control NILs. In spite of this 1% relative reduction, both *Gpc-B1* and control NILs averaged extraction rates above 70% (Table 2). The statistical analyses by genotype showed significant FY decreases for UC1037 ($P = 0.007$), UC1041 ($P = 0.04$), and Yecora Rojo ($P = 0.0003$) *Gpc-B1* NILs compared to that of the control NILs (Fig. 1H). These significant decreases varied from 9.2 g kg⁻¹ in the breeding line UC1041 to 19.5 g kg⁻¹ in the cv. Yecora Rojo (Fig. 1H).

Break flour yield across hexaploid genotypes was not significantly affected by the *Gpc-B1* introgression ($P = 0.13$). This result also showed more heterogeneous responses across genotypes (data not shown). The *Gpc-B1* NIL of the cv. Yecora Rojo showed a highly significant ($P = 0.01$) decrease in BFY that averaged 19.3 g kg⁻¹, paralleling its significant decrease in flour yield, whereas the differences in BFY for the other isogenic pairs were not significant.

Flour ash concentration (FASH), an additional important milling trait, showed no significant ($P = 0.2$) variation between *Gpc-B1* and control NILs across genotypes (Table 2). The analyses by genotype, showed a significant FASH increase ($P = 0.04$, 3.1%) for the *Gpc-B1* NIL of Yecora

Rojo and a significant FASH decrease ($P = 0.02$, 4.2% decrease) for the *Gpc-B1* NIL of RSI5 relative to their respective control NILs (Fig. 1I).

Overall, the milling score was significantly ($P = 0.007$) reduced in the *Gpc-B1* NILs (Table 2). The calculation of this parameter includes FY and FASH, therefore this negative result likely reflected the significantly lower FY associated with the *Gpc-B1* introgression. From the three genotypes that showed significant ($P < 0.05$) reductions in FY (Fig. 1H), two of them exhibited significantly ($P < 0.01$) lower milling scores in their *Gpc-B1* NILs relative to the control lines (Fig. 1J).

3.2. Effects across environments in durum wheat

3.2.1. Grain (GPC) and semolina protein concentration (SPC) and pasta quality traits

GPC in the tetraploid recurrent parents ranged from 124.8 g kg⁻¹ in the breeding line UC1113 to 133.8 g kg⁻¹ in the cv. Kronos (Fig. 2A). As in the hexaploid lines, GPC was significantly ($P = 0.005$) higher in the lines carrying the DIC *Gpc-B1* introgression with an average increase of 16.1 g kg⁻¹ (Table 3). The average increase in GPC associated with the *Gpc-B1* introgression in the tetraploid cultivars was more than twice the increase observed across the hexaploid genotypes (Tables 2-3). In the analysis by genotype, the two durum *Gpc-B1* NILs showed significant GPC increases ($P < 0.05$) relative to their respective controls (Fig. 2A). The *Gpc-B1* NIL of the breeding line UC1113 exhibited the highest average increase in GPC with an average increase of 17.7 g kg⁻¹ (14.2% increase relative to the control NIL).

SPC in the durum recurrent parents averaged 113.9 g kg⁻¹ (Table 3). The *Gpc-B1* introgression resulted in significant ($P = 0.007$) increases in SPC across genotypes that averaged 16.3 g kg⁻¹ (Table 3), and both tetraploid *Gpc-B1* NILs showed significantly higher SPC ($P < 0.05$) than their respective control NILs (Fig. 2B). On average, protein loss during milling was similar between *Gpc-B1* (15.2 g kg⁻¹) and control NILs (15.4 g kg⁻¹).

In the overall analyses across genotypes, the higher GPC and SPC in the *Gpc-B1* NILs resulted in positive effects on most quality traits. Wet gluten, a measure of gluten content, showed significant ($P = 0.023$) increases across genotypes. On average, the *Gpc-B1* NILs showed 18.9% higher wet gluten than the control NILs (Table 3). Although both Kronos and UC1113 *Gpc-B1*

NILs exhibited similar increases in wet gluten relative to their control lines, the difference was significant only for Kronos ($P = 0.013$, Fig. 2C), whereas for UC1113 the increase was marginally not significant ($P = 0.06$). Although gluten index showed a 20% increase in the *Gpc-BI* lines relative to the controls, this difference was not significant ($P = 0.15$; Table 3), and none of the genotypes showed significant changes in gluten index associated with the *Gpc-BI* introgression (Fig. 2D).

Across genotypes, the *Gpc-BI* NILs also exhibited improvements in the mixogram parameters. Mixing time and peak height were significantly ($P < 0.05$) higher in the *Gpc-BI* NILs (Table 3) relative to the control lines. The mixogram curves also showed significant ($P < 0.05$) increases in final height and width (data not shown) in the *Gpc-BI* NILs, confirming the enhanced rheological properties associated with the *Gpc-BI* introgression. In general, the mixogram parameters showed significant ($P < 0.05$) gene \times genotype interactions due to significant increases in the UC1113 *Gpc-BI* NIL and no significant changes for any of these traits in the cv. Kronos (Fig. 2E and F).

The spaghetti product made from semolina of the *Gpc-BI* NILs showed stronger cooked firmness ($P = 0.023$) and reduced cooking loss ($P = 0.006$) (Table 3). These positive results were consistent across genotypes. The *Gpc-BI* NILs of both Kronos and UC1113 had significant increases in cooked firmness (Fig. 2G) and decreases in cooking loss (Fig. 2H).

Semolina color (data not shown) was significantly ($P = 0.002$) higher in the *Gpc-BI* NIL of UC1113 but was not different between Kronos NILs ($P = 0.73$). The *Gpc-BI* introgression was not associated with significant changes in spaghetti color across genotypes ($P = 0.24$) or at the cultivar level (data not shown).

3.2.2. Grain weight and ash concentration

Average grain weight was not affected significantly across genotypes ($P = 0.10$, Table 3). However, in the analyses by genotype the *Gpc-BI* NIL of Kronos had significantly smaller grain weight relative to its control ($P = 0.01$, Fig. 2I). The effect of the *Gpc-BI* introgression on grain weight was paralleled by a significant decrease in test weight in the *Gpc-BI* NILs across genotypes ($P = 0.02$, Table 3). All pair-wise comparisons showed significant reductions in test

weight associated with the DIC *Gpc-B1* allele ($P < 0.05$; Fig. 2J). Average grain and semolina ash concentration were significantly ($P < 0.05$) higher in the lines carrying the *Gpc-B1* introgression relative to the controls, and this trend was consistent for the two tetraploid pairs of NILs (Fig. 2K and L).

3.3. Effects of the *Gpc-B1* introgression by location

An analysis by location was performed to compare the magnitude of the effects of the *Gpc-B1* introgression at the two locations using a selected group of traits (Table 4). The P values were calculated using a mixed model with year as a random factor. When the average changes between locations associated with the *Gpc-B1* introgression were compared, most of the differences between locations were not significant, although the magnitude of the changes tended to be larger in El Centro than in Davis.

For the tetraploid *Gpc-B1* NILs, only the increases in wet gluten and GI showed significant differences between locations, being larger in El Centro than in Davis (Table 4). For the hexaploid NILs, the increases in FPC and loaf volume shown by the *Gpc-B1* NILs were significantly higher in El Centro than in Davis as well (Table 4). The only trait that showed the opposite trend was flour yield, which showed significantly larger differences in Davis than in El Centro in the hexaploid NILs. The direction of the change (increase or decrease) for a given trait was the same at both locations.

In Davis, significant changes associated with the *Gpc-B1* introgression were observed only for tetraploid wheat. Mixing time and gluten index were significantly higher in the durum *Gpc-B1* NILs relative to the control lines (Table 4). None of the changes shown by the hexaploid *Gpc-B1* NILs were significant, although the increases in loaf volume and mixing time, as well as the decreases in grain weight and flour yield associated with the DIC *Gpc-B1* allele were close to the threshold of significant differences ($P = 0.06-0.08$, Table 4). In El Centro, significant changes were observed in FPC, loaf volume, and flour yield in the hexaploid *Gpc-B1* NILs, as well as in gluten index in the tetraploid *Gpc-B1* NILs (Table 4). At this location, GPC and MAB in the hexaploid NILs, and GPC, SPC, wet gluten, and grain weight in the tetraploid NILs showed marginally not significant changes associated with the *Gpc-B1* introgression (Table 4). The

analyses between locations and within location showed that the effect of the *Gpc-B1* introgression on the traits shown in Table 4 tended to be larger in El Centro than in Davis.

4. Discussion

4.1. Effects of *Gpc-B1* on baking and pasta quality traits

The presence of the *Gpc-B1* introgression was associated with a consistent increase on GPC across genotypes and environments, and with a positive effect on several bread-baking and pasta-making quality parameters. The increase in GPC was likely responsible for the significant increase in water absorption, mixing time, time to peak, and loaf volume in the hexaploid genotypes (Table 2, Fig. 1C-F), since these parameters are known to be correlated with GPC.

Although increases in GPC usually improve quality, the magnitude of the effects depends on the individual proteins being increased. The consistent increase in water absorption and loaf volume observed in the *Gpc-B1* NILs indicates that the protein quality was not negatively affected. In tetraploid wheat, the increase in GPC was also associated with improved gluten quality. The mixogram parameters showed that semolina from the *Gpc-B1* NIL of UC1113 had better rheological properties than that of the recurrent parent (Fig. 2E and F). The cv. Kronos, on the other hand, showed no improvement in gluten quality as reflected in non-significant changes in gluten index, mixing time and time to peak between *Gpc-B1* and control NILs (Fig. 2D, E and F). These differences between Kronos and UC1113 might be associated to the fact that the Kronos recurrent parent already had stronger gluten than UC1113.

Our results expand those reported before by Kovacs et al. (1998), which showed higher GPC and better pasta disc viscoelasticity associated with the 6B introgression from DIC. The full pasta analyses included in our study showed that the *Gpc-B1* introgression was also associated with additional benefits in spaghetti firmness and cooking loss (Fig. 2G, H), two critical traits for pasta quality.

In hexaploid wheat, Mesfin et al. (2000) found significant increases in GPC and water absorption in recombinant inbred lines carrying the *Gpc-B1* introgression did not detect significant differences in loaf volume. The use of NILs in our study reduced the genetic variability, providing higher power to detect smaller differences in a wider range of quality traits. In

addition, the introgression of *Gpc-B1* into multiple genetic backgrounds of contrasting quality characteristics provides a broader picture of the expected effects of *Gpc-B1* introgression.

4.2. Effects of *Gpc-B1* on grain weight and milling traits

Although the favorable changes in GPC and quality described above likely outweigh the negative effects of the *Gpc-B1* introgression on several milling traits, these negative effects should be considered in decisions about the incorporation of *Gpc-B1* in different germplasm. Our results suggest that the lower flour yield in the hexaploid NILs and increased ash concentration in flour and semolina were driven by the negative effect of the *Gpc-B1* introgression on grain weight. Similar reductions in grain weight in the *Gpc-B1* lines were reported before by Brevis and Dubcovsky (2010) using a similar sets of NILs.

The negative effect on grain weight was more evident in the tetraploid than in the hexaploid NILs. Some hexaploid genotypes did not show any reduction in grain weight (Fig. 1G), and most of the other ones showed no significant reduction in total grain yield, suggesting that other yield components compensated the reductions in grain weight (Brevis and Dubcovsky, 2010). In spite of the reductions in grain weight both tetraploid and hexaploid NILs showed a significant increase in total grain protein (grain yield x protein concentration) due to better N remobilization from leaves to the grain (Brevis and Dubcovsky, 2010; Kade et al., 2005; Uauy et al., 2006; Waters et al., 2009).

The three hexaploid genotypes in which the presence of the *Gpc-B1* introgression was associated with lower flour yields (Fig. 1H) also showed a significant decrease in grain weight (Fig. 1G) and test weight, supporting the known relationship between grain weight and flour extraction. The contrasting results in flour ash showed by the *Gpc-B1* NILs of Yecora Rojo (3.1% increase) and RSI5 (4.2% decrease) mirrored the differences in grain weight observed in these two cultivars. A number of studies have shown that grain weight and morphology can affect milling yield and ash content (Baker et al., 1999; Berman et al., 1996; Wiersma et al., 2001).

Mesfin et al. (2000) also showed a significant decrease in test weight associated with the presence of the DIC 6B introgression in one population, but these changes were not associated with changes in flour extraction. The other population showed no changes in test weight but a

significant increase in flour extraction associated with the DIC 6B introgression (Mesfin et al. 2000). This inconsistent result may be explained by the higher genetic variability expected in the recombinant inbred lines used in their experiments. However, these results also highlight the fact that the relationship between grain weight and flour yield and flour ash is a complex one, and not always a decrease in grain weight results in lower extraction rates or increased ash content (Breseghello and Sorrells, 2000).

4.3. Differences of the *Gpc-B1* effects between hexaploid and tetraploid wheat

Hexaploid genotypes usually have four functional *Gpc* copies (*Gpc-A1*, *Gpc-D1*, *Gpc-B2*, and *Gpc-D2*), whereas most tetraploid cultivars have only two (*Gpc-A1* and *Gpc-B2*) (Uauy et al., 2006). Therefore, the addition of the functional DIC *Gpc-B1* allele had a relatively larger dosage effect on tetraploid (2 functional genes in the recurrent parents vs. 3 in the *Gpc-B1* NILs) than on hexaploid wheat (4 vs. 5 functional *Gpc* genes).

Previous studies have shown that the tetraploid genotypes showed larger differences than the hexaploid ones in maturity, GPC and grain weight associated with the *Gpc-B1* introgression (Brevis and Dubcovsky, 2010). The average increase in GPC in the tetraploid *Gpc-B1* lines was 2.5-fold higher than in the hexaploid *Gpc-B1* lines. A similar trend was observed for SPC-FPC (2.9-fold), grain weight (2.7-fold), test weight (2.0-fold), and SASH-FASH (10.0-fold). The only parameter that showed an opposite result was mixing time (2.3-fold larger increase in the hexaploid genotypes relative to the durum lines). This might be related to the fact that Kronos has strong gluten and that the addition of the *Gpc-B1* introgression did not increase that parameter further.

4.4. Differences of the effects by location

A challenging aspect of using an introgression or a gene for wheat improvement is to predict its effects in different environments. We initially hypothesized that environments resulting in larger differences in maturity would be associated with larger differences in quality traits. However, the quality data from Davis and El Centro contradicted this hypothesis. Whereas the differences in maturity between *Gpc-B1* and control NILs were smaller in El Centro (average 1-2 days) than in

Davis (average 2-5 days) (Brevis and Dubcovsky, 2010), a number of quality traits exhibited significantly larger differences in El Centro than in Davis (Table 4). This suggests that the effects of the *Gpc-B1* introgression on quality are not simply correlated with the changes in maturity or the duration of the grain filling period.

The deployment of the *Gpc-B1* allele from *T. turgidum* ssp. *dicoccoides* into wheat breeding programs has the potential of improving GPC in a wide range of germplasm due to the absence of the functional allele in most of the modern tetraploid and hexaploid commercial cultivars (Uauy et al. 2006). Overall, the increase of GPC in the *Gpc-B1* NILs was associated with beneficial effects on a number of bread- and pasta-making traits. However, the existence of gene \times genotype and gene \times environment interactions needs to be taken into consideration to decide which genotypes can benefit from the incorporation of the *Gpc-B1* allele.

The negative effects on grain weight associated with the functional *Gpc-B1* allele were more evident in the tetraploid than in the hexaploid genotypes, likely due to the higher plasticity of the hexaploid lines (Dubcovsky and Dvorak, 2007). It is possible that a dedicated breeding effort to combine the functional *Gpc-B1* allele with other maturity alleles may ameliorate some of the negative effects on grain weight observed in the near isogenic lines. In this study the functional *Gpc-B1* allele was introgressed by backcrossing without any additional selection, which may have resulted in a suboptimal maturity time.

The beneficial effects of the functional *Gpc-B1* allele were particularly evident in the genotypes with low GPC such as the hexaploid cultivars Anza and RSI5, and the durum breeding line UC1113. However, some beneficial effects were also observed in genotypes with high GPC. Finally, it should be noted that this study was limited to spring wheat genotypes and that therefore, the extrapolation of this conclusions to winter wheat cultivars would require further studies.

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7. Tables

Table 1. Description of the hexaploid and tetraploid recurrent parent lines including market class, source, grain protein concentration (GPC), high molecular weight (HMW) glutenin subunits, and presence of the 1RS.1BL translocation.

Recurrent Parent	Market		GPC [†] g kg ⁻¹	HMW glutenins			1RS.1BL transloc.
	Class	Source		<i>Glu-A1</i>	<i>Glu-B1</i>	<i>Glu-D1</i>	
Anza	HRS	UCD	117.9	Null	7+8	2+12	No
Attila	HWS	CIMMYT	126.6	2*	7+8	5+10	Yes
RSI5	HRS	RSI	119.3	Null	17+18	5+10	No
UC1037	HRS	UCD	134.4	2*	13+16	2+12	No
UC1041	HRS	UCD	135.5	1	6+8	5+10	No
Yecora	HRS	UCD	135.1	1	17+18	5+10	No
Kronos	Durum	APB	133.0	Null	7+8	-	No
UC1113	Durum	UCD	125.8	Null	7+8	-	No

[†] Average of two years and three locations (Brevis and Dubcovsky, 2010). HRS, hard red spring; HWS, hard white spring; UCD, University of California, Davis; RSI, Resource Seed, Inc.; APB, Arizona Plant Breeders.

Table 2. Hexaploid wheat (6 genotypes grown in 2006 and 2007 at two California locations). Overall effects of the *Gpc-B1* introgression on *grain traits*: grain weight (GWT), test weight (TWT), grain protein concentration (GPC); *milling quality traits*: flour protein concentration (FPC), flour yield (FY), break flour yield (BFY), flour ash concentration (FASH), and milling score; and *bread-baking quality traits*: , mixograph water absorption (MAB), bread-baking water absorption (BAB), mixing time, and loaf volume.

Trait	Control	<i>Gpc-B1</i>	Δ^* (%)	P value
	Mean \pm SEM			
<i>Grain traits</i>				
GWT (mg)	2.9 \pm 0.01	2.8 \pm 0.01	-1.4	0.008
TWT (kg hL ⁻¹)	82.0 \pm 0.1	81.0 \pm 0.1	-1.1	0.0001
GPC (g kg ⁻¹)	130.8 \pm 0.7	137.4 \pm 0.6	+5.0	0.012
<i>Milling traits</i>				
FPC (g kg ⁻¹)	114.2 \pm 0.6	119.1 \pm 0.8	+4.9	0.14
FY (g kg ⁻¹)	718.4 \pm 0.1	711.4 \pm 0.1	-1.0	0.009
BFY (g kg ⁻¹)	380.2 \pm 0.2	375.9 \pm 0.2	-1.1	0.13
FASH (g kg ⁻¹)	4.2 \pm 0.004	4.2 \pm 0.004	+0.5	0.2
Milling score	85.3 \pm 0.2	84.5 \pm 0.2	-1.0	0.007
<i>Baking traits</i>				
MAB (g kg ⁻¹)	614.1 \pm 1.9	629.4 \pm 1.7	+2.5	0.009
BAB (g kg ⁻¹)	646.1 \pm 2.0	661.9 \pm 1.9	+2.4	0.009
Mixing time (min)	2.58 \pm 0.08	3.27 \pm 0.09	+27.1	0.002
Loaf volume (cc)	849.5 \pm 7.9	928.6 \pm 6.7	+9.3	0.03

* Δ , mean change between *Gpc-B1* and control NILs (as percent of the control).

Table 3. Tetraploid wheat (2 genotypes grown in 2006 and 2007 at two California locations). Overall effects of the *Gpc-B1* introgression on *grain traits*: grain weight (GWT), test weight (TWT), grain ash concentration (GASH), and grain protein concentration (GPC); *semolina traits*: semolina protein concentration (SPC), semolina ash concentration (GASH), wet gluten (WG), gluten index (GI), mixing time, mixogram peak height; and *pasta-making quality traits*: spaghetti cooking loss (CKL), and cooked spaghetti firmness (FIRM).

Trait	Control	<i>Gpc-B1</i>	Δ^* (%)	P value
	Mean \pm SEM			
<i>Grain traits</i>				
GWT (mg)	50.1 \pm 0.6	48.2 \pm 0.5	-3.8	0.10
TWT (kg hL ⁻¹)	81.7 \pm 0.2	79.9 \pm 0.3	-2.2	0.019
GASH (g kg ⁻¹)	15.4 \pm 0.2	16.3 \pm 0.2	+5.8	0.006
GPC (g kg ⁻¹)	129.3 \pm 1.4	145.3 \pm 1.5	+12.4	0.005
<i>Semolina traits</i>				
SPC (g kg ⁻¹)	113.9 \pm 1.3	130.2 \pm 1.4	+14.3	0.007
SASH (g kg ⁻¹)	6.51 \pm 0.1	6.85 \pm 0.1	+5.2	0.018
WG (g kg ⁻¹)	306.1 \pm 5.0	364.0 \pm 7.2	+18.9	0.023
GI (g kg ⁻¹)	594.0 \pm 53.0	719.1 \pm 30.9	+21.1	0.15
Mixing time (min)	2.67 \pm 0.11	2.98 \pm 0.13	+11.6	0.0012
Peak height (cm)	5.86 \pm 0.15	6.60 \pm 0.11	+12.6	0.016
<i>Pasta quality traits</i>				
FIRM (g cm)	5.10 \pm 0.11	6.04 \pm 0.12	+18.4	0.023
CKL (g kg ⁻¹)	69.4 \pm 0.6	63.8 \pm 0.7	-8.1	0.006

* Δ , mean change between *Gpc-B1* and control NILs (as percent of the control).

Table 4. Average effects of the *Gpc-B1* introgression on selected quality traits of hexaploid and tetraploid wheat genotypes by location. P_{DA} and P_{EC} represent the significance level of the difference between *Gpc-B1* and control NILs within location (DA = Davis, EC = El Centro). P_{LOC} indicates the significance of the differences between the two locations.

Trait*	Davis, CA		El Centro, CA		
Hexaploid	Δ^{**}	P_{DA}	Δ	P_{EC}	P_{LOC}
GPC (g kg ⁻¹)	+5.7	0.27	+7.5	0.06	0.16
FPC (g kg ⁻¹)	+1.7	0.73	+8.1	0.04	0.003
MAB (g kg ⁻¹)	+12.5	0.21	+18.0	0.07	0.12
LVOL (cc)	+44.1	0.06	+114.2	0.01	0.0002
Mixing time	+0.77	0.08	+0.62	0.10	0.19
GWT (mg)	-1.5	0.06	-1.4	0.13	0.78
FYLD (g kg ⁻¹)	-8.9	0.08	-5.2	0.04	0.025
FASH (g kg ⁻¹)	+0.02	0.56	+0.02	0.41	0.95
Tetraploid					
GPC (g kg ⁻¹)	+14.2	0.18	+17.9	0.07	0.077
SPC (g kg ⁻¹)	+14.4	0.21	+18.1	0.05	0.079
WG (g kg ⁻¹)	+41.0	0.28	+75.0	0.09	0.003
GI (g kg ⁻¹)	+10.9	0.02	+239.1	0.01	<0.0001
Mixing time	+0.35	0.03	+0.28	0.10	0.62
GWT (mg)	-2.2	0.46	-1.6	0.09	0.42
SASH (g kg ⁻¹)	+0.32	0.25	+0.35	0.19	0.82

*GPC, grain protein concentration; FPC, flour protein concentration; MAB, mixograph water absorption; LVOL, loaf volume; GWT, grain weight; FASH, flour ash concentration; FY, flour yield; SPC, semolina protein concentration; WG, wet gluten, GI, gluten index, SASH, semolina ash concentration.

** Δ , mean change between *Gpc-B1* and control NILs (in trait units). ‘+’ indicates an average increase in the *Gpc-B1* NIL, whereas ‘-’ indicates an average decrease in the *Gpc-B1* NIL relative to the control NIL.

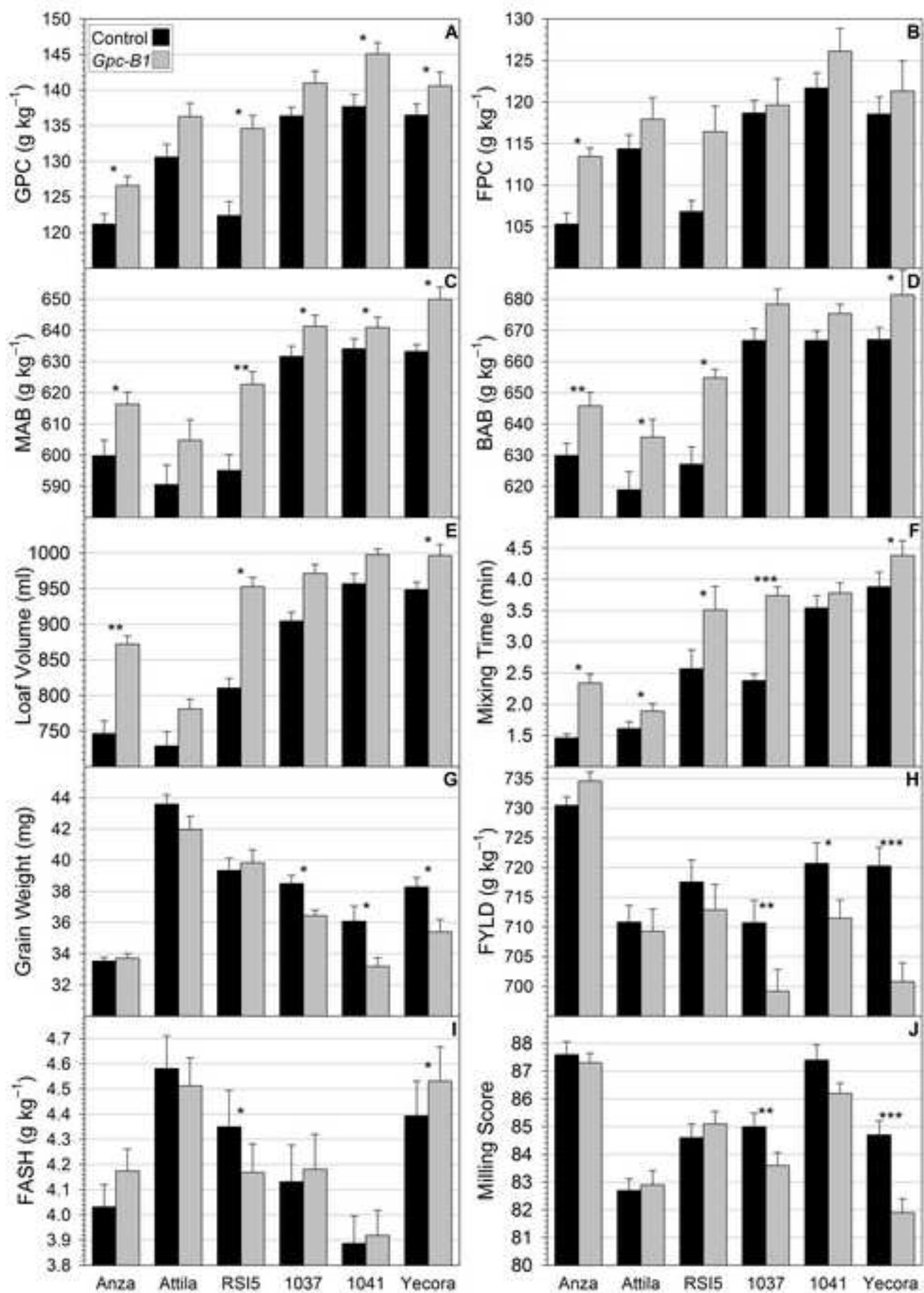
8. FIGURE LEGENDS

Figure 1. Effect of the *Gpc-B1* introgression in six hexaploid near isogenic lines (NILs) grown at two California locations. (A) Grain protein concentration (GPC), (B) flour protein concentration (FPC), (C) mixograph water absorption (MAB), (D) bread-baking water absorption (BAB), (E) loaf volume, (F) mixing time, (G) grain weight, (H) flour yield (FY), (I) flour ash concentration (FASH) and (J) milling score. Values are arithmetic means of 16 replications and error bars are SEMs. Asterisks indicate significant differences within cultivar or breeding line. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 2. Effect of the *Gpc-B1* introgression in two tetraploid near isogenic lines (NILs) grown at two California locations. (A) Grain protein concentration (GPC), (B) semolina protein concentration (SPC), (C) wet gluten, (D) gluten index, (E) mixing time, (F) mixogram peak height, (G) spaghetti cooked firmness, (H) spaghetti cooking loss, (I) grain weight, (J) test weight, (K) grain ash concentration (GASH) and (L) semolina ash concentration (SASH). Values are arithmetic means of 20 replications and error bars are SEMs. Asterisks indicate significant differences within cultivar or breeding line. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure

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Figure

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