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Effects of the Chromosome Region Including the *Gpc-B1* Locus on Wheat Grain and Protein Yield

Juan Carlos Brevis and Jorge Dubcovsky*

ABSTRACT

The recent cloning of the *Gpc-B1* gene from *Triticum turgidum* ssp. *dicoccoides* (Körn. ex Asch. and Graebn.) Thell. (DIC hereafter) chromosome 6BS revealed that modern wheat varieties have a nonfunctional allele. The DIC allele accelerates senescence and increases grain protein concentration (GPC) relative to the nonfunctional allele, but its effect on yield is not known. Here we describe the effect of *Gpc-B1* on grain yield, grain weight, protein yield (grain yield by GPC), and N harvest index (NHI) of common (*T. aestivum* L.) and durum [*T. turgidum* ssp. *durum* (Desf.) Husn.] wheat using BC_6F_3 near-isogenic lines (NILs, >99% identical) with and without the DIC *Gpc-B1* allele. Six hexaploid and three tetraploid pairs of NILs were tested in three California locations (2005–2007) and all showed higher GPC when the functional *Gpc-B1* allele was present ($P < 0.0001$). Hexaploid NILs with the DIC *Gpc-B1* allele showed a significant decrease in grain weight (2.8%, $P = 0.0004$) and a similar trend was observed between tetraploid NILs, although the differences were marginally not significant ($P = 0.08$). In spite of this reduction, the differences in grain yield between NILs with different *Gpc-B1* alleles across genotypes and environments were not significant. Protein yield was increased in the *Gpc-B1* NILs of both hexaploid ($P < 0.0001$) and tetraploid ($P = 0.06$) wheat. The *Gpc-B1* introgression in hexaploid NILs resulted in significantly ($P < 0.01$) lower straw N concentration at maturity and higher NHI, which suggests that the functional *Gpc-B1* allele improves N remobilization.

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Abbreviations: ANOVA, analysis of variance; BC, backcross; DIC, *Triticum turgidum* ssp. *dicoccoides* accession FA15-3; FLS, flag leaf senescence; GPC, grain protein concentration; GW, grain weight; GYLD, grain yield; HI, harvest index; HRS, hard red spring; LDN, durum cultivar Langdon; NHI, nitrogen harvest index; NIL, near-isogenic line; NIR, near-infrared; PYLD, protein yield; REC, Research and Extension Center; RSL, recombinant substitution line; SNC, straw nitrogen concentration; SPM, spike maturity; UC, University of California.

Wheat (*Triticum* spp.) has the highest grain protein concentration (GPC) among cereals, ranging from 8 to 15% (Johnson et al., 1985). Considering the average world production during the last decade (1998–2007; FAO, 2008) and a conservative GPC estimate of 10%, wheat provides approximately 60 million Mg of protein annually for human and livestock nutrition. Grain protein concentration is a critical trait that determines the nutritional value, the processing properties, and the market value of the grain. In many wheat growing regions, growers that produce pasta or bread wheat with high GPC receive premium prices.

Despite its importance, breeding success for high GPC has been relatively hindered by its complex inheritance and large variation due to environmental effects. As a result of a well-documented negative correlation between grain yield (GYLD) and GPC (Blanco et al., 2002; Feil, 1992; Gonzalez-Hernandez et al., 2004; Groos et al., 2003; Kibite and Evans, 1984; Levy and Feldman, 1987; Mesfin et al., 2000; Simmonds, 1995), selection for increased GYLD has probably countered gains in GPC during the past decades. Studies comparing wheat cultivars of

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different release periods have shown that modern cultivars have reduced GPC compared to older cultivars (Austin et al., 1980; Slafer et al., 1990; Fufa et al., 2005). The improvement of GPC in modern wheat cultivars without associated penalties on GYLD will require higher N use efficiency by increasing either N uptake or remobilization.

An additional constraint to GPC improvement is the limited range of genetic variation controlling protein quantity in modern wheat cultivars (Blanco and De Giovanni, 1995). Gene introgressions from wild relatives into cultivated genotypes have expanded the genetic diversity for this trait providing new alternatives to increase GPC. Wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides* (Körn. ex Asch. and Graebn.) Thell. (DIC hereafter), is a valuable source of genetic variation in GPC, since some accessions exhibit much higher GPC than most of the commercial wheat cultivars (Avivi, 1978).

A good example of the contribution of DIC to the improvement of GPC in commercial wheat varieties is the *Gpc-B1* gene. The positional cloning of *Gpc-B1* revealed that this gene is a NAC transcription factor (*NAM1*) closely related to the *Arabidopsis* NAC gene *NAP1* (Uauy et al., 2006b). Wild tetraploid wheat has a functional *Gpc-B1*, whereas commercial tetraploid and hexaploid wheat cultivars have a deletion at this locus or a nonfunctional copy as a result of a frame-shift mutation (Uauy et al., 2006b). The DIC allele accelerates senescence and increases protein, zinc, and iron concentration in the grain relative to the nonfunctional allele (Uauy et al., 2006a, 2006b). These effects make the DIC *Gpc-B1* introgression an interesting source to improve the nutritional value and the quality properties of the wheat grain.

However, the accelerated maturity associated with the functional *Gpc-B1* allele can shorten the grain filling period and result in potential GYLD penalties in certain genotype and environment combinations. Some preliminary studies have analyzed the effect of the functional *Gpc-B1* allele on yield components using single or two-row plots (Blanco et al., 2002; Chee et al., 2001; Joppa et al., 1997). However, it was later found that the stripe rust (caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriksson) resistance gene *Yr36* is tightly linked to the functional *Gpc-B1* allele (Uauy et al., 2005; Fu et al., 2009), which can affect the interpretation of the previous yield results. In this study we investigate the effect of the functional *Gpc-B1* allele on GYLD, GPC, protein yield (PYLD = GYLD × GPC), and N harvest index (NHI) in disease-free conditions, using larger yield plots and replicated field trials in multiple locations. Near-isogenic lines (NILs, >99% identical) of three tetraploid and six hexaploid wheat cultivars and breeding lines were analyzed to investigate possible gene by genotype interactions.

MATERIALS AND METHODS

Plant Materials

Near isogenic lines of DIC *Gpc-B1* were developed by six backcrosses to different hexaploid and tetraploid recurrent parents, self-pollination of BC_6 plant heterozygous for *Gpc-B1*, and selection of homozygous BC_6F_2 plants followed by a final cycle of self-pollination to increase seed and produce BC_6F_3 homozygous lines. The BC_6F_3 lines were 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 (LDN, CItr 13165), were the donors of the DIC *Gpc-B1* introgression for the hexaploid and tetraploid genotypes, respectively (Chicaiza et al., 2006). The original source of the DIC segment was the same for both donors (DIC accession FA15-3; Avivi, 1978), as reflected by identical alleles for all the molecular markers tested in this region (Khan et al., 2000). Both donors carry a segment of the DIC chromosome arm 6BS whose size was estimated as 15 to 30 cM (Mesfin et al., 1999; Khan et al., 2000), but this introgression could have been further reduced during the backcrossing process by recombination. Hereafter, the BC_6F_3 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.

Four hexaploid and two tetraploid pairs of sister NILs with and without the *Gpc-B1* gene were included in the 2005 field experiment. The hexaploid recurrent parents were the hard red spring (HRS) cultivars Anza (CItr 15284; Qualset et al., 1984) and Yecora Rojo (CItr 17414; Qualset et al., 1985), and the University of California (UC) breeding lines UC1037 (Solar/3/Cleo/I66//Anza) and UC1041 (Yecora Rojo/Tadinia). The tetraploid materials included the cv. Kofa (WestBred, LLC; Bozeman, MT) and the UC breeding line UC1113 (UC selection from CIMMYT cross CD52600 [Kifs//RSS/BD1419/3/Mexis-CP/4/Wahas/5/Yav79]). The *Gpc-B1* NILs of the cv. Yecora Rojo *Yr36–Gpc-B1* (PI 638740) and the durum [*T. turgidum* ssp. *durum* (Desf.) Husn.] line UC1113 *Yr36–Gpc-B1* (PI 638741) were deposited in the National Small Grains Collection, United States Department of Agriculture (Chicaiza et al., 2006).

In 2006 and 2007, one tetraploid and two hexaploid cultivars were added to the previous six lines. The new tetraploid recurrent parent was the cv. Kronos (Arizona Plant Breeders; Arizona City, AZ), whereas the hexaploid materials were the hard white spring cv. Attila (PI 351590) and the HRS cv. RSI5 (Resource Seeds, Inc., Gilroy, CA).

Field Experiments

All the experiments reported here were arranged in a split-plot design with five randomized complete blocks, except for the 2006 and 2007 Davis experiments in which 10 blocks were used. In all the experiments, the main plot corresponded to the genetic background (cultivar or breeding line), and the subplots, to the two different *Gpc-B1* alleles. In this experimental design, the NILs with and without the functional *Gpc-B1* allele in the same genetic background are adjacent in the field, maximizing the sensitivity of the experiment to detect differences between *Gpc-B1* alleles.

In 2005, a preliminary field experiment was conducted at the UC Experimental Field Station in Davis, CA (38°32′ N,

121°46′ W), including the four hexaploid and two tetraploid accessions described above. This experiment consisted of 7-m^2 plots with five randomized complete blocks. In 2006 and 2007, the same experimental design was repeated in three California locations each year and included the six hexaploid and three tetraploid isogenic pairs described in the previous section. The three locations included the same Davis site as in 2005 and two UC Research and Extension Centers (RECs): El Centro (Desert REC, 32°48′ N, 115°26′ W) and Tulelake (Intermountain REC, 41°57′ N, 121°28′ W). Plot size averaged 3.4 m^2 (El Centro), 4.0 m^2 (Davis), and 7.4 m^2 (Tulelake).

All the experiments were machine sown. In Davis, sowing occurred in early November (fall planting) in a Yolo loam soil (fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvent) and the fertilization consisted of a preplanting application of 110 kg ha^{-1} N and 45 kg ha^{-1} P_2O_5, and a topdress application of 110 kg ha^{-1} N at tillering (GS25 on Zadoks scale; Zadoks et al., 1974). In El Centro, the lines were sown in December (fall planting) in a Holtville silty clay soil (clayey over loamy, smectitic over mixed, superactive, calcareous, hyperthermic Typic Torrifluvent) and the fertilization varied every year. In 2006, the experiment was planted after alfalfa (*Medicago sativa* L. subsp. *sativa*) and the fertilization consisted of a preplanting application of 70 kg ha^{-1} P_2O_5, and a topdress application of 350 kg ha^{-1} N at tillering (GS25). In 2007, the experiment was planted after Sudan grass [*Sorghum bicolor* (L.) Moench subsp. *drummondii* (Steud.) de Wet ex Davidse] and was fertilized with 110 kg ha^{-1} N and 90 kg ha^{-1} P_2O_5 at preplanting, and 380 kg ha^{-1} N applied in three irrigations. In Tulelake, the lines were sown in April (spring planting) in a Tulebasin mucky silty clay loam soil (fine, mixed, superactive, mesic Aquandic Endoaquoll) and were fertilized with a preplanting application of 70 kg ha^{-1} N and 90 kg ha^{-1} P_2O_5 each year. Irrigation was applied in all sites to supply water according to growth conditions. Plots were machine harvested at maturity and the seed was weighed to estimate GYLD. For the January to May growing periods mean temperatures were higher in El Centro (2006 = 18.0°C, 2007 = 18.2°C) than in Davis (2006 = 12.9°C, 2007 = 13.6°C), whereas total precipitation was higher in Davis (2006 = 372 mm, 2007 = 142 mm) than in El Centro (2006 = 6 mm, 2007 = 1 mm). Temperature and precipitation data by month were obtained from the UC Statewide Integrated Pest Management Program website (http://www.ipm.ucdavis.edu/WEATHER).

Disease Control

All *Gpc-B1* NILs carry both the *Gpc-B1* gene and the closely linked stripe rust resistance gene *Yr36* (Fu et al., 2009). As reported before, *Yr36* conferred partial resistance to stripe rust under field conditions (Uauy et al., 2005). To avoid bias by the differential susceptibility to stripe rust between the *Gpc-B1* and the control NILs the plots were maintained disease free. Fungicide was applied in Davis {propiconazole, 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl] methyl]-1*H*-1,2,4-triazole, 45 g ha^{-1} a.i. at GS30, and azoxystrobin, methyl (α*E*)-2-[[6-(2-cyanophenoxy)-4-pyrimidinyl] oxy]-α-(methoxymethylene)benzeneacetate, 180 g ha^{-1} a.i. at GS32 and GS58} and Tulelake (azoxystrobin, 180 g ha^{-1} a.i. at GS39). Fungicide applications were not necessary in El Centro. Disease severity was assessed at GS40 and GS60 by visually estimating the flag leaf area covered by the disease in a 0 to 100% scale to confirm the absence of stripe rust.

Phenology

Heading and maturity dates were assessed in Davis and El Centro (2006–2007). Heading date was recorded when 50% of the spikes in a plot were completely emerged. Maturity was assessed when 50% of the flag leaves (flag leaf senescence, FLS) or spikes (spike maturity, SPM) had lost their green color entirely. To quantify the *Gpc-B1* effects on maturity in different environments, the phenological data were analyzed by location. For the durum wheat NILs, the data included both 2006 and 2007 experiments. For the common wheat NILs, the data set from El Centro included both years, whereas for Davis, only the 2007 data set for phenological data was available (except Anza, for which both 2006 and 2007 were available).

Biomass and Grain Evaluations

To estimate yield components, aboveground biomass was measured in a 0.5-m^2 sample from the center rows of each plot. Biomass samples were taken in Davis and El Centro (2006). Samples were oven-dried at 50°C for 3 d before weighing. Spikes from each sample were counted, weighed, and threshed, and the resultant grain was weighed. This allowed estimating total aboveground biomass, number of spikes per square meter, and harvest index (HI). A sample of 10 spikes was taken from the plot to measure number of grains per spike.

Grain weight (GW) was determined on the basis of 500 grains. Grain protein concentration was determined for a 600-g grain sample by near-infrared (NIR) spectrometry using an Infratec 1226 grain analyzer (Tecator AB, Hoganas, Sweden) at the California Wheat Commission Laboratory (Woodland, CA). The Infratec 1226 was calibrated using standard samples measured by Dumas nitrogen combustion using a Leco TruSpec N Elemental Analyzer (Leco Corp., St. Joseph, MI). Protein yield was calculated by multiplying GYLD by GPC.

Straw Nitrogen Analyses and N Harvest Index

Total N concentration in the straw, hereafter referred to as straw N concentration (SNC), was analyzed from all hexaploid NILs tested in the 2005 and 2006 Davis experiments (except Yecora Rojo in 2006). Nitrogen measurements were based on five replicates in 2005 and six in 2006. Straw samples were taken from the biomass samples used for yield component analysis and preground in a model 4 Wiley mill (Thomas Scientific, Philadelphia, PA) using a 1-mm screen. Approximately 10 g of each sample was ground to a very fine powder using a ball mill. Finally, from the finely ground material, 0.35-g samples were analyzed for total N using a Leco FP-528 N gas analyzer (Leco Corp.) at the Agriculture and Natural Resources Analytical Lab of the University of California at Davis. This method quantitatively determines the amount of all forms of N (ammonium, nitrate, protein, and heterocyclic nitrogen) using an induction furnace and a thermal conductivity detector with a detection limit of 0.01% N (dry basis). The analysis method is further

described by the Association of Official Analytical Chemists (AOAC International, 1997).

To estimate NHI the following procedure was followed. The grain protein values obtained by NIR spectrometry were transformed to grain N concentration by dividing GPC values by the standard 5.7 conversion factor (AACC, 2000). Grain N concentration was then multiplied by GYLD to obtain the fraction of N present in the grain. Aboveground biomass and SNC were used to estimate the fraction of N present in the vegetative parts of the plant. Nitrogen harvest index was obtained by dividing the grain N fraction by the total N in the plant (grain N plus vegetative N).

Statistical Analyses

Analysis of variance was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC). The general lineal model (PROC GLM) was used to establish the significance of the effects of the *Gpc-B1* introgression within each isogenic pair, and the interactions of the gene segment with the genotype (cultivar or breeding line) and the environment (defined as the different location by year combinations). Exploratory models showed that the effect of the *Gpc-B1* introgression on most traits under study was different for hexaploid and tetraploid wheat. Therefore, the statistical analyses were performed separately for the two species.

The 2005 data set was analyzed separately since it included a different number of NILs than the 2006–2007 experiments. The 2005 data set was analyzed using a two-way factorial analysis of variance (ANOVA) with two gene levels (with and without the DIC *Gpc-B1*) and six genotypes (cultivars or breeding lines). The GPC, GYLD, PYLD, and GW data from 2006 and 2007 were analyzed as a three-way factorial with two gene levels (with and without the DIC *Gpc-B1*), nine genotypes, and six environments (included as a random factor). The inclusion of environments as a random factor results in a more stringent criteria to declare significant differences than if treated as a fixed effect, because the interactions with the environment are used as denominators in the *F* test.

When necessary, data were transformed to meet the assumptions of homogeneity of variances and normality of residuals of the ANOVA model. 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. Correlations among traits were analyzed using SAS PROC CORR. To estimate the effect of *Gpc-B1* on these correlations we also calculated the differences between the two *Gpc-B1* alleles for the targeted traits and then established correlations between these differences.

RESULTS

2005 Experiment

In the 2005 experiment, the *Gpc-B1* introgression was associated with a significant ($P < 0.05$) increase of GPC in both hexaploid and tetraploid NILs (Fig. 1A and B). For the hexaploid genotypes (Anza, UC1037, UC1041, and Yecora Rojo), the NILs carrying the nonfunctional *Gpc-B1* allele (control lines) had an average GPC of 130.4 g kg^{-1}, whereas the isogenic lines with the functional DIC allele (*Gpc-B1* lines) had an average of 136.5 g kg^{-1} (4.7% increase, $P < 0.0001$, Fig. 1A). For the durum NILs (Kofa and UC1113), the control lines with the nonfunctional *Gpc-B1* allele had an average GPC of 132.1 g kg^{-1}, whereas their sister lines with the functional *Gpc-B1* allele had an average of 136.2 g kg^{-1} (3.1% increase, $P = 0.03$, Fig. 1A). When analyzed separately by genotype, all *Gpc-B1* NILs showed higher GPC relative to their control NILs, with increases ranging from 3.3 g kg^{-1} in Kofa to 9.2 g kg^{-1} in UC1041 (data not shown). However, the only differences that were significant were those for the NILs of Anza ($P = 0.008$), UC1037 ($P = 0.004$), and UC1041 ($P = 0.0006$), likely due to the limited number of replications of this single year, single location analysis.

In this experiment, the increase in GPC was not associated with GYLD penalties as shown in Fig. 1C and 1D. On the contrary, the *Gpc-B1* NILs showed a GYLD increase of 136 kg ha^{-1} (2.0% increase) for hexaploid and 369 kg ha^{-1} (4.6% increase) for tetraploid genotypes compared to the control lines, although these differences were not significant ($P = 0.28$ and $P = 0.22$, respectively). As a result of the simultaneous increases in GPC and GYLD, both hexaploid and tetraploid *Gpc-B1* NILs averaged greater PYLD than the recurrent parents (Fig. 1E, 1F), although the increase was significant ($P = 0.0009$) only for the hexaploid NILs. In spite of the absence of differences in GYLD the *Gpc-B1* introgression was associated with a highly significant ($P < 0.001$) decrease in GW in both wheat species (Fig. 1G and H). This suggests that the wheat plants were able to compensate the reduction in grain size by increasing other yield components (see the GW results section in the 2006–2007 experiment).

2006 and 2007 Experiments

Phenology

The presence of the DIC *Gpc-B1* segment was associated with significant ($P < 0.05$) differences in maturity dates as reflected by earlier dates of FLS and SPM in the *Gpc-B1* lines. The *Gpc-B1* NILs of all three durum lines showed highly significant ($P < 0.01$) differences in both FLS and SPM compared to the control lines (data not shown). In Davis, the durum *Gpc-B1* NILs matured on average 4.7 d earlier than the control NILs (FLS and SPM), whereas in El Centro the differences were 3.5 d for FLS and 2.1 d for SPM.

The common wheat lines showed similar results (data not shown). In El Centro (2006 and 2007), the hexaploid *Gpc-B1* NILs showed earlier ($P < 0.01$) maturity dates than the control lines, although the average difference was only 1 d. In this environment, the *Gpc-B1* NILs of Anza, RSI5, and Yecora Rojo showed significant differences relative to their recurrent parent, whereas NILs of Attila, UC1037, and UC1041 showed almost identical maturity dates. In Davis (2007), the hexaploid *Gpc-B1* NILs showed an average 2 d earlier maturity than the control

lines ($P < 0.0001$). In the ANOVA analyses by genotype, only the NILs of UC1041 and Yecora Rojo did not show significant difference in maturity in this environment.

Grain Protein Concentration

Grain protein concentration in the recurrent parents averaged 128.1 g kg^{-1} for hexaploid and 132.7 g kg^{-1} for tetraploid wheat (Table 1), and ranged from 117.9 g kg^{-1} in the HRS cv. Anza to 139.3 g kg^{-1} in the durum cv. Kofa (Fig. 2A and B). Grain protein concentration was consistently and significantly ($P < 0.0001$) higher in the lines carrying the DIC *Gpc-B1* introgression relative to the controls, both in the ANOVA across all genetic backgrounds (data not shown) and in the separate analyses by wheat species (Table 1).

Within the hexaploid group, the *Gpc-B1* lines exhibited an average increase in GPC of 7.4 g kg^{-1}, whereas in the tetraploid lines, the increase was 13.8 g kg^{-1} (Table 1), almost twofold higher than in the hexaploid *Gpc-B1* lines. The gene by environment and gene by genotype interactions were significant ($P < 0.05$) in the ANOVAs for both tetraploid and hexaploid genotypes, likely due to differences in the magnitude of the GPC increases. When tested by genotype, the *Gpc-B1* introgression was associated with significant ($P < 0.05$) GPC increases relative to the isogenic controls in all nine genetic backgrounds (Fig. 2A, 2B). These increases ranged from 4.5 to 15.2 g kg^{-1} in the hexaploid group and from 11.9 to 16.3 g kg^{-1} in the tetraploid group.

Across wheat species, the *Gpc-B1* introgression explained 23% of the variation in GPC, whereas genotype and environment accounted for 23 and 30% of the variation, respectively. When the ANOVA components were done separately by wheat species the *Gpc-B1* introgression explained 18% of the variation in GPC for the hexaploid NILs and 33% for the tetraploid NILs.

Grain Yield

Grain yield in the recurrent parents averaged 7.62 Mg ha^{-1} for hexaploid and 8.88 Mg ha^{-1} for tetraploid wheat (Table 1) and ranged from 7.08 Mg ha^{-1} in HRS breeding line UC1041 to 9.27 Mg ha^{-1} in the durum breeding line UC1113 (Fig. 2C and D). The durum genotypes had higher GYLD than the hexaploid lines across environments in agreement with previous studies (Dubcovsky and Dvorak, 2007).

Across wheat species, the *Gpc-B1* NILs showed nonsignificant ($P = 0.13$) differences in GYLD relative to their control lines (data not shown). A nonsignificant ($P = 0.17$) gene by environment interaction suggests that the effect of the DIC *Gpc-B1* introgression on GYLD was not significantly affected by the environment. However, the effect of the *Gpc-B1* region on GYLD was strongly affected by the genetic background as reflected by a highly significant ($P < 0.0001$) gene by genotype interaction. Across the

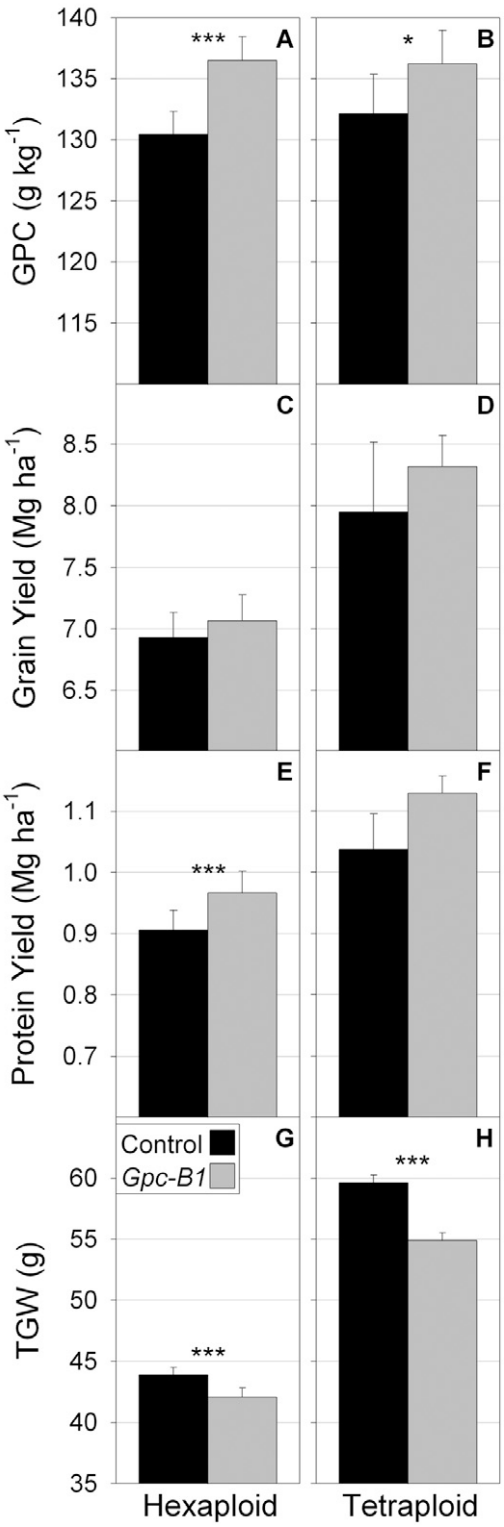

Figure 1. The 2005 experiment grown in Davis, CA. Effect of the *Gpc-B1* introgression on (A, B) grain protein concentration (GPC), (C, D) grain yield, (E, F) protein yield, and (G, H) grain weight (GWT) of four hexaploid (left) and two tetraploid (right) near-isogenic lines (NILs). Values are arithmetic means of five replications per NIL and error bars are SEMs. Asterisks indicate significant differences within cultivar or breeding line. *$P < 0.05$, ***$P < 0.001$.

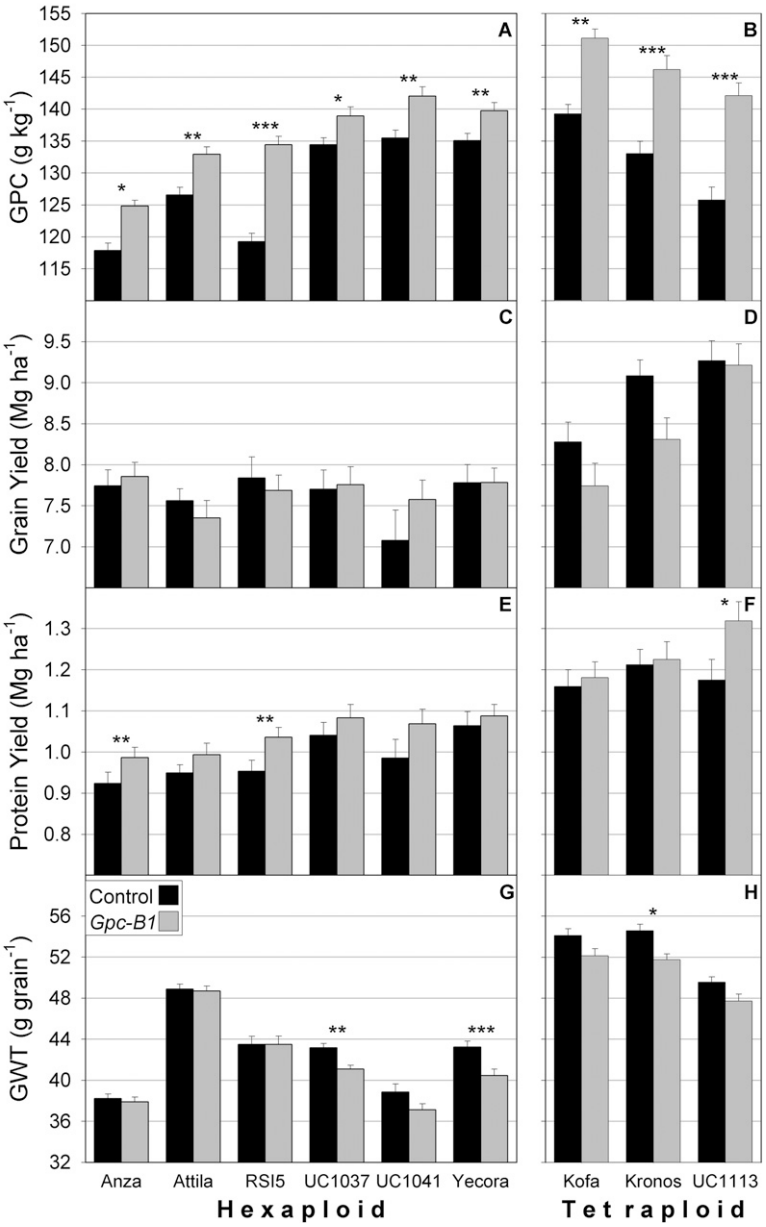

Figure 2. The 2006 and 2007 experiments grown in three California locations. Effect of the *Gpc-B1* introgression on (A, B) grain protein concentration (GPC), (C, D) grain yield, (E, F) protein yield, and (G, H) grain weight (GWT) of six hexaploid (left) and three tetraploid (right) near-isogenic lines (NILs). Values are arithmetic means and error bars are SEMs. Asterisks indicate significant differences within cultivar or breeding line. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

hexaploid genotypes, average GYLD was practically the same between *Gpc-B1* and control NILs ($P = 0.61$, Table 1). Within the tetraploid group, the presence of the *Gpc-B1* introgression was associated with an average GYLD decrease of 460 kg ha^{-1} (5.1% decrease) relative to the control NILs, although the difference was not significant ($P = 0.16$, Table 1).

When data were analyzed by wheat species, the gene by environment interaction became significant ($P < 0.05$), which indicates that the effect of the *Gpc-B1* segment on GYLD was affected by the environments. When the data were analyzed separately by genotype, none of them showed significant ($P < 0.05$) differences in GYLD associated with the different *Gpc-B1* alleles (Fig. 2C and D). The changes in GYLD associated with the *Gpc-B1* introgression were more variable among the hexaploid NILs, although none of the differences were significant (Fig. 2C). The *Gpc-B1* lines of the cultivars Attila and RSI5 had an average GYLD decrease of 209 and 151 kg ha^{-1}, respectively (Fig. 2C). The rest of the hexaploid *Gpc-B1* NILs had higher GYLD than their respective control NIL, and the differences varied from 2 kg ha^{-1} for Yecora Rojo to 499 kg ha^{-1} for UC1041 (Fig. 2C). Within the tetraploid genotypes, all three NILs showed decreases in GYLD associated with the presence of the DIC *Gpc-B1* allele (Fig. 2D). Compared to their control lines, the *Gpc-B1* NILs of durum cultivars Kronos and Kofa exhibited the greatest penalties in GYLD with average decreases of 776 and 537 kg ha^{-1}, respectively (Fig. 2D). The *Gpc-B1* NIL of the durum breeding line UC1113 exhibited the smallest decrease in GYLD among the tetraploid genotypes (56 kg ha^{-1}, Fig. 2D). When the data were analyzed individually by environment, some genotypes exhibited significant ($P < 0.05$) differences (increase and decrease) in GYLD between the *Gpc-B1* and control NILs (data not shown). These results highlight the importance of the environment (location and year) in modulating the effect of the DIC *Gpc-B1* allele.

The limited effect of the *Gpc-B1* allele on GYLD was also reflected in the analysis of variance components. Most of the variation in GYLD was explained by the environment and genotype factors and their interaction (75% of the variation), whereas the variance explained by the *Gpc-B1* introgression was negligible. Similarly, the *Gpc-B1* allele showed no significant effects on aboveground biomass, spike number, spikelets per spike, and grains per spike across environments (data not shown).

Protein Yield

Protein yield in the recurrent parents averaged 0.99 and 1.18 Mg ha^{-1} for hexaploid and tetraploid wheat, respectively (Table 1), and ranged from 0.92 Mg ha^{-1} in the HRS cv. Anza to 1.21 Mg ha^{-1} in the durum cv. Kronos (Fig. 2E and F). Hexaploid wheat lines carrying the DIC functional *Gpc-B1* allele yielded an average 56 kg ha^{-1} of protein more than the control lines ($P < 0.0001$, Table 1), as expected from the increase in GPC and the lack of differences in GYLD. Across tetraploid genotypes, the presence of the functional *Gpc-B1* allele was associated with an average increase in PYLD of 60 kg ha^{-1} but the difference was marginally not significant ($P = 0.06$, Table 1). The ANOVA for PYLD showed a significant gene by genotype

interaction, which was likely due to differences in the magnitude rather than in the direction of the effects. All the *Gpc-B1* NILs showed an increase in PYLD relative to the control but the differences were significant only for the hexaploid cultivars Anza ($P = 0.01$) and RSI5 ($P = 0.003$) and the durum breeding line UC1113 ($P = 0.02$, Fig. 2E and F). The highest increase in PYLD associated with the introgression of the functional *Gpc-B1* allele relative to their control lines corresponded to the cv. RSI5 (83 kg ha^{-1} or 8.7% increase) among hexaploid and the breeding line UC1113 (144 kg ha^{-1} or 12.3% increase) among tetraploid genotypes.

Grain Weight

The average GW in the recurrent parents was 42.6 mg grain^{-1} for hexaploid and 52.8 mg grain^{-1} for tetraploid genotypes (Table 1). The *Gpc-B1* introgression was consistently associated with a decrease in GW. The hexaploid *Gpc-B1* NILs showed a 2.8% decrease in GW on average relative to the control lines ($P = 0.0004$, Table 1). The *Gpc-B1* NILs of cultivars Anza, Attila, and RSI5 showed little change on GW (Fig. 2G), whereas the *Gpc-B1* NILs of UC1037, Yecora Rojo, and UC1041 showed a larger decrease, which ranged from 4 to 10% and was highly significant in the first two genotypes ($P < 0.01$, Fig. 2G). The tetraploid *Gpc-B1* NILs showed a 4.2% decrease in GW on average compared to the control lines, although this difference was marginally not significant ($P = 0.08$, Table 1). All three tetraploid genotypes with the *Gpc-B1* introgression showed a decrease in GW, although the differences were significant only for Kronos ($P = 0.01$, Fig. 2H).

Other yield components were measured to explain the larger effect of the *Gpc-B1* alleles on GW relative to its effect on GYLD. Analyses from 2006 at Davis and El Centro were not able to detect significant differences between the *Gpc-B1* and control NILs in number of spikes per square meter, number of grains per spike, HI, and aboveground biomass (data not shown).

Nitrogen Harvest Index

The significant increase in PYLD showed by the *Gpc-B1* lines suggests that *Gpc-B1* improves the N economy by favoring N remobilization to the grain. To test this hypothesis an analysis of the N balance was performed in the hexaploid NILs. Straw N concentration was analyzed in samples from the 2005 and 2006 Davis experiments. For both years, the *Gpc-B1* NILs showed a significantly ($P < 0.01$) lower SNC than the control lines, which on average represented a 15% decrease of the values observed in the control NILs (Table 2). The differences between isogenic pairs varied greatly among genotypes (2 to 27%, Fig. 3A, 3B), but the genotype by *Gpc-B1* allele interaction for SNC was not significant ($P > 0.10$). In the ANOVA by genotype, all hexaploid *Gpc-B1* NILs analyzed showed lower SNC than their respective control lines, but the differences were significant ($P < 0.01$) only for cv. RSI5 and breeding line UC1041 in the 2006 experiment (Fig. 3B). However, it is important to point out that the *Gpc-B1* NILs of Anza and UC1041 in 2005 and UC1037 in 2006 showed decreases in SNC of 15% or more compared to the control NILs (Fig. 3A and B) and were marginally not significant ($P = 0.07$–0.08).

The differences in NHI paralleled those in SNC. Across genotypes, NHI values were significantly ($P < 0.01$) higher in the *Gpc-B1* NILs than in the control lines both in 2005 and 2006 (Table 2). The NHI interaction between genotype and *Gpc-B1* alleles was highly significant in 2006 ($P = 0.0002$), probably due to the inconsistent decrease in NHI observed in Attila *Gpc-B1* NIL relative to the control line (5.4% decrease, $P = 0.23$), which was likely driven by a significant ($P = 0.0002$) decrease in GYLD in the Davis experiment (data not shown). All other genotypes showed an increase in NHI, which resulted in an average increase of 10.6% in 2005 and 10.9% in 2006 (Table 2) with a range from 5.5% in UC1037 (2005) to 39% in UC1041 (2006).

Table 1. Mean values, standard errors of the means (SEMs), and significance levels of grain protein concentration (GPC), grain yield (GYLD), protein yield (PYLD), and grain weight (GW) observed between the *Gpc-B1* and control near-isogenic lines (NILs) of six hexaploid and three tetraploid wheat genotypes grown in the 2006 and 2007 experiments in three California locations.

Hexaploid	N	Control	*Gpc-B1*	Δ (%)†	*P* value
		Mean ± SEM			
GPC, g kg^{-1}	420	128.12 ± 0.68	135.49 ± 0.64	+5.8	<0.0001
GYLD, Mg ha^{-1}	480	7.62 ± 0.10	7.67 ± 0.08	+0.7	0.61
PYLD, Mg ha^{-1}	420	0.99 ± 0.01	1.04 ± 0.01	+5.7	<0.0001
GW, mg grain^{-1}	480	42.64 ± 0.34	41.45 ± 0.34	–2.8	0.0004
Tetraploid	**N**	**Control**	***Gpc-B1***	**Δ (%)**	***P* value**
		Mean ± SEM			
GPC, g kg^{-1}	210	132.70 ± 1.17	146.49 ± 1.14	+10.4	<0.0001
GYLD, Mg ha^{-1}	240	8.88 ± 0.13	8.42 ± 0.16	–5.1	0.16
PYLD, Mg ha^{-1}	210	1.18 ± 0.02	1.24 ± 0.03	+5.1	0.06
GW, mg grain^{-1}	240	52.75 ± 0.40	50.54 ± 0.42	–4.2	0.08

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

Table 2. Mean values, standard errors of the means (SEMs), and significant levels of straw N concentration (SNC) and N harvest index (NHI) observed between the *Gpc-B1* and control near-isogenic lines (NILs) of four (2005 Davis) and five (2006 Davis) hexaploid genotypes.

2005 Davis	N	Control	*Gpc-B1*	Δ (%)†	*P* value
		Mean ± SEM			
SNC, g kg^{-1}	40	9.83 ± 0.46	8.35 ± 0.39	–15.1	0.0021
NHI, g kg^{-1}	40	531.6 ± 17.6	587.9 ± 15.0	+10.6	0.0017
2006 Davis	**N**	**Control**	***Gpc-B1***	**Δ (%)**	***P* value**
		Mean ± SEM			
SNC, g kg^{-1}	60	8.24 ± 0.38	7.02 ± 0.36	–14.8	<0.0001
NHI, g kg^{-1}	60	612.4 ± 22.2	679.4 ± 11.4	+10.9	<0.0001

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

In the analyses by genotype, most *Gpc-B1* NILs exhibited a nonsignificant increase in NHI with the exception of Anza in 2005 and RSI5 and UC1041 in 2006, which showed significantly ($P < 0.05$) higher NHI (Fig. 3C, 3D).

DISCUSSION

Phenology

The introgression of the DIC chromosome segment with the *Gpc-B1* functional allele was previously shown to accelerate senescence and grain maturity in both tetraploid and hexaploid wheat (Uauy et al., 2006a). The positional cloning of *Gpc-B1* revealed that this gene is a NAC transcription factor (*NAM1*, Uauy et al., 2006b) closely related to the *Arabidopsis* NAC gene *NAP1*, which has also been shown to be a central regulator of senescence (Guo and Gan, 2006). Experiments using wheat transgenic plants with reduced *Gpc-B1* transcript levels by RNA interference demonstrated that the differences in the *Gpc-B1* gene itself were the cause of the differences in senescence and grain maturity (Uauy et al., 2006b). Plant senescence is a key developmental process, and therefore it is not surprising that the phenological differences observed between the *Gpc-B1* and control NILs were also associated with pleiotropic effects on several other traits, including GPC, GYLD, grain size, and N utilization.

The tetraploid NILs showed larger differences in maturity, GPC, and TKW between *Gpc-B1* and control lines than the hexaploid NILs. This can be explained by a gene dosage effect due to the different number of additional *Gpc* functional genes present in each species (Fig. 4). Most tetraploid cultivars have only two functional *Gpc* copies (*Gpc-A1* and *Gpc-B2*), whereas most hexaploid genotypes have four (*Gpc-A1*, *Gpc-D1*, *Gpc-B2*, and *Gpc-D2*; Fig. 4). Therefore, the introgression of the active DIC allele of *Gpc-B1* had a relatively larger dosage effect on tetraploid (two functional genes in the control lines vs. three, in the *Gpc-B1* NILs) than on hexaploid wheat (four vs. five functional genes).

Differences among environments also played a critical role on the modulation of the effect of the *Gpc-B1* gene on senescence and grain maturity. A comparison of the two fall-planted locations showed larger differences in maturity in Davis than in El Centro. It is possible that the higher temperatures and arid conditions during grain filling in El Centro triggered a faster senescence in both *Gpc-B1* and control NILs, limiting the expression of differences in senescence, when compared to Davis (see the Field Experiments section of Material and Methods for mean temperature and precipitation data). However, the magnitude of the responses in GPC and grain size was not always proportional to the differences in senescence. For example, the average differences between durum *Gpc-B1* and control NILs in maturity were almost 70% larger in Davis (4.7 d) than in El Centro (2.8 d), but the corresponding differences in GPC were almost the same in both locations (2% larger in El Centro). Also, in the NILs of the hexaploid Anza the differences in senescence between the *Gpc-B1* alleles were almost the same in Davis (1.3 d) and El Centro (1.1 d), but the increase in GPC was more than twofold larger in Davis (7.7 g kg^{-1}) than in El Centro (3.5 g kg^{-1}). These results suggest that the magnitude of the effect on senescence and grain maturity cannot be used alone to predict the effect of the *Gpc-B1* introgression on GPC or grain size.

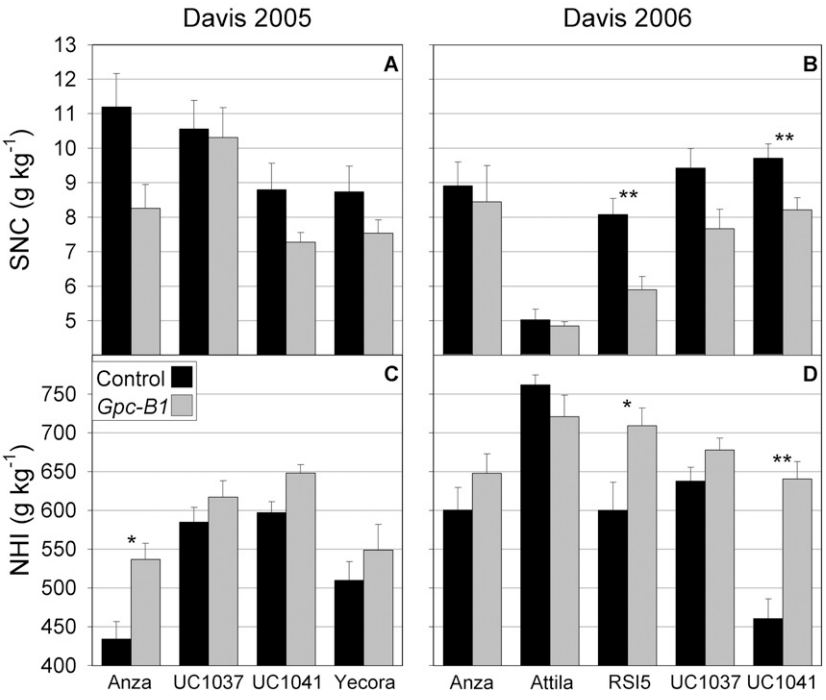

Figure 3. Effect of the *Gpc-B1* introgression on (A, B) straw N concentration (SNC) and (C, D) N harvest index (NHI) of hexaploid near-isogenic lines grown in Davis in 2005 and 2006. Values are averages of five replicates in 2005 and six in 2006, and error bars are SEMs. Asterisks indicate significant differences between NILs of the same cultivar or breeding line with different *Gpc-B1* alleles. *$P < 0.05$, **$P < 0.01$.

Grain Protein Concentration

The *Gpc-B1* introgression was consistently associated with significantly ($P < 0.05$) higher GPC across genotypes and environments in both tetraploid and hexaploid NILs. These results are consistent with those reported in previous field studies (Blanco et al., 2002; Chee et al., 2001; Joppa et al., 1997; Mesfin et al., 1999; Olmos et al., 2003). Comparisons among those studies also show a larger average effect of the *Gpc-B1* introgression on GPC in the tetraploid than in hexaploid genetic backgrounds, a similar trend to the one reported here. A field study in North Dakota using three hexaploid wheat

recombinant inbred populations showed that the presence of the functional *Gpc-B1* allele was associated with GPC increases ranging from 9.6 to 11.5 g kg^{-1} relative to the control lines (Mesfin et al., 1999). In comparison, a larger average increase in GPC (15 g kg^{-1}) was observed in a tetraploid wheat population segregating for the same DIC introgression (Chee et al., 2001). Similar values (13–15 g kg^{-1}) were observed by Blanco et al. (2002) in a tetraploid population from the cross between Messapia and DIC.

In spite of this general trend of larger GPC differences in tetraploid than hexaploid NILs, there was a larger variation within ploidy levels. Among the tetraploid lines, the increases in GPC varied from 11.9 to 16.3 g kg^{-1} in the 2006–2007 experiments (Fig. 2B). A wider range was found among the hexaploid NIL pairs included in this study. Five out of the six hexaploid genotypes with the *Gpc-B1* introgression showed increases in GPC from 4.5 to 7 g kg^{-1} relative to the corresponding control NIL (Fig. 2A). However, the *Gpc-B1* NIL of RSI5 showed an average GPC increase of 15.2 g kg^{-1} relative to the control (Fig. 2A), similar to the values observed in the tetraploid NILs.

Grain Weight and Grain Yield

The introgression of the functional DIC *Gpc-B1* allele accelerates senescence and grain maturity without affecting the time of anthesis and, therefore, results in a slightly shorter grain filling period that can affect grain size negatively. A decrease in GW was actually observed in the three tetraploid NILs and in three out of the six hexaploid lines included in this study (although the differences were significant only in half of them). Significant ($P < 0.05$) and opposite changes in GPC and GW were observed in three of the nine genotypes (UC1037, Yecora Rojo, and Kronos) although the average GPC percent increase was higher than the average GW percent decrease (Table 1). Responses in GW were not always directly proportional to the differences in GPC. For example, the durum breeding line UC1113 with the functional *Gpc-B1* allele showed the highest increase in GPC among the durum NILs (16.3 g kg^{-1}, 13% increase), but only a 3.7% decrease in GW (Fig. 2B and H), the lowest among tetraploid genotypes. Similarly, the *Gpc-B1* line of RSI5 exhibited the largest increase in GPC (15.2 g kg^{-1}, 12.7%) among the six hexaploid genotypes tested in 2006–2007, whereas the GW was unaffected by the *Gpc-B1* introgression (Fig. 2A and G). Similarly, the presence of the functional *Gpc-B1* allele in Anza and Attila was associated with a small decrease in GW (only 1%) but a larger increase in GPC (5%). A possible explanation for these results is the variation in the remobilization rate of N and carbohydrates to the grain among genotypes. The total amount of N and carbohydrates depends both on the duration of the grain filling period and the rate of remobilization. Although we can observe the differences in maturity, the role of the genetic background on the rate of remobilization is currently unknown.

For the 2006 and 2007 experiments, the average 4.2% decrease in GW observed in the durum *Gpc-B1* NILs likely accounts for most of the decrease in GYLD (average 5.1%, 456 kg ha^{-1}). This result was also reflected in a positive correlation between the differences in GW and the differences in GYLD between tetraploid NILs with and without the functional *Gpc-B1* allele ($r = 0.43$, $P < 0.0001$). The same correlation among the hexaploid NILs was lower ($r = 0.22$, $P = 0.0006$), as expected from the absence of differences in GYLD across the hexaploid genotypes in spite of a significant decrease in GW (Table 1). These results indicate a strong effect of genotype on the modulation of the effect of the *Gpc-B1* alleles on GYLD. This is also reflected in significant interactions between *Gpc-B1* alleles and genotype in the separate ANOVAs for tetraploid and hexaploid NILs. However, these correlations should be analyzed with caution since the ANOVAs for the individual tetraploid and hexaploid NILs showed no significant differences in GYLD between *Gpc-B1* and control NILs. The analysis of variance components showed that most of the variation in GYLD was determined by differences in genotype and environment, whereas the variation at the *Gpc-B1* locus had a negligible effect on this trait.

The lack of proportionality between the responses in GW and GYLD in the hexaploid lines (Fig. 2C and G) may be explained by variation in the ability of different genotypes to compensate for the reductions in grain weight with increases in the number of grains per surface unit. However, in this study no significant difference in the number of spikes per square meter and number of

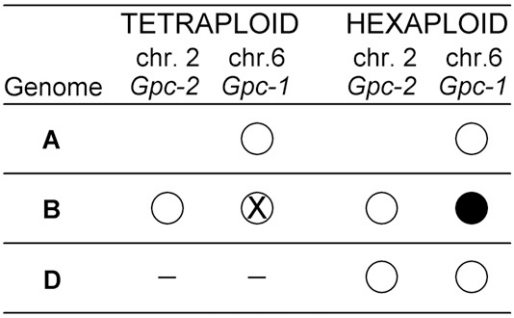

Figure 4. *Gpc* genes in modern tetraploid and hexaploid wheat cultivars. Open circles correspond to functional copies; a crossed circle corresponds to a +1-bp frameshift mutation, whereas a filled circle corresponds to either a complete deletion or the same +1-bp frameshift mutation. All 57 tetraploid genotypes characterized by Uauy et al. (2006b), including Kofa, Kronos, and UC1113, carried a +1-bp frameshift mutation at *Gpc-B1*. Among the 34 hexaploid genotypes characterized in the same study, the *Gpc-B1* deletion was the most frequent allele with (85%), whereas the +1-bp frameshift mutation was found in the other 15% of the lines. The nonfunctional *Gpc-B1* allele on chromosome 6B was replaced by a functional copy in the *Gpc-B1* NILs.

grains per spike was detected between the *Gpc-B1* and control NILs for the two hexaploid genotypes that showed a significant ($P < 0.01$) decrease in GW with no parallel changes in GYLD (data not shown). The number of grains per surface unit is a complex trait determined by several intercorrelated subcomponents (number of plants per surface unit, number of spikes per plant, and number of grains per spike) and the precise subcomponents responsible for the lack of proportionality between GW and GYLD in some genotypes are currently unknown.

The 2006–2007 data showed the usual negative relationship between GYLD and GPC. Within the tetraploid group, the relationship between GPC and GYLD was negative in all six environments ($r = -0.12$ to -0.56) and highly significant ($P < 0.001$) in four of them. Within the hexaploid NILs, significant ($P < 0.05$) negative correlations were detected only in two environments, whereas the other two showed a nonsignificant positive relationship between GPC and GYLD. The stronger negative relationship between GPC and GYLD in the tetraploid NILs relative to the hexaploid NILs parallels the stronger effects of the DIC *Gpc-B1* allele in the tetraploid lines.

Protein Yield

In the hexaploid lines, the absence of differences in GYLD between the NILs, combined with the significant increases in GPC, resulted in a significant increase in the total amount of protein produced per surface unit (PYLD increase = 56 kg ha^{-1}). In the tetraploid *Gpc-B1* lines, the increase in GPC (which was almost twofold higher than the one observed in the hexaploid NILs) was partially offset by the consistent decrease in GYLD. However, the net balance was similar to the one observed in the hexaploid NILs (PYLD increase = 60 kg ha^{-1}).

The positive PYLD balance indicates that, even though part of the increase in GPC in the durum *Gpc-B1* NILs may be due to the reduction in GW, there should be an additional source of N contributing to the amount of grain protein in the *Gpc-B1* lines.

Nitrogen Harvest Index

The hexaploid *Gpc-B1* lines showed higher GPC and lower SNC at maturity than the control NILs, resulting in a significant increase in NHI. These results agreed with those from Deckard et al. (1996) in tetraploid wheat, which showed that the differences in GPC between LDN (DIC-6B) chromosome substitution line relative to LDN were mostly associated with differences in the efficiency of N remobilization (although in one out of the three locations differences in N uptake were also found). Although most hexaploid *Gpc-B1* lines showed higher NHI values relative to the control NILs, the magnitude of the differences varied greatly among genotypes (Fig. 3C, 3D). The lower SNC and the increased NHI in the hexaploid *Gpc-B1* lines suggest that a higher proportion of the N stored in the vegetative structures was remobilized to the grain in these lines relative to the control NILs.

A similar association between increased N remobilization and the DIC *Gpc-B1* allele has previously been reported in greenhouse experiments. Kade et al. (2005) found increased NHI in a near-isogenic recombinant substitution line (RSL) carrying the DIC *Gpc-B1* allele in a LDN background relative to the LDN control. The *Gpc-B1* RSL showed both increased GPC and lower SNC suggesting higher N remobilization to the grain. The same study showed that, at anthesis, the concentration of soluble protein and amino acids in the flag leaf was higher in the recombinant *Gpc-B1* line than in LDN, suggesting that the accelerated senescence in the *Gpc-B1* RSL was likely associated with accelerated hydrolysis of proteins by proteolytic enzymes. Since GPC is related to the amount of soluble amino acids in the flag leaf (Barneix and Guitman, 1993) and the amount of amino acids exported to the phloem (Caputo et al., 2001), these differences in soluble amino acid concentrations in the flag leaf could explain part of the differences in N remobilization between NILs.

In a separate greenhouse study using RNA interference (RNAi), two transgenic wheat lines with reduced transcript levels of *Gpc1* and *Gpc2* showed a significant delay in senescence and a 30% decrease in GPC with no associated changes in GW (Uauy et al., 2006b). These changes were also associated with a significant increase in residual N in the flag leaves of the transgenic plants compared with the nontransgenic sister lines (Uauy et al., 2006b), which also supports the hypothesis of a more efficient N remobilization in plants with higher transcript levels of the functional copies of the *Gpc* genes. Since the described effects associated with the reduction of the *Gpc* transcript levels in the RNAi transgenic lines are the mirror image of the phenotypic effects observed in the NILs carrying the DIC chromosome segment, it is logical to assume that the effects on GPC and GW described in this study are also the result of the allelic differences at the *Gpc-B1* gene rather than at other linked genes within the introgressed chromosome 6BS segments from DIC.

CONCLUSIONS

In summary, the incorporation of the *Gpc-B1* gene into commercial wheat cultivars has the potential to improve PYLD by a more efficient remobilization of the N already present in the plant. This improved NHI explains the increase in GPC without a proportional reduction in GYLD observed across the hexaploid genotypes. The incorporation of the functional *Gpc-B1* allele could also be used to maintain levels of GPC similar to the control NILs with lower applications of N fertilizer. This strategy has the potential to reduce both the costs associated with high prices of N fertilizer and the

environmental problems resulting from excess fertilization. Since the active form of the *Gpc-B1* gene is absent in most of the modern tetraploid and hexaploid commercial varieties (Uauy et al., 2006b), the incorporation of this allele into wheat breeding programs has the potential to improve GPC in a wide range of germplasm.

However, this study shows that its incorporation may be associated with reduced grain weight and yield penalties, particularly in tetraploid cultivars. Therefore, a dedicated breeding effort is required to ameliorate this potential negative effect of the *Gpc-B1* DIC allele. For most traits analyzed here, the gene by environment and gene by genotype interactions were significant, indicating that there is a strong influence of genotype and environment on the effect of the *Gpc-B1* introgression. Therefore, appropriate genotype and environment combinations should be identified during the breeding process where the introgression of the functional *Gpc-B1* allele results in a positive cost–benefit balance.

The identification of lines such as RSI5, with very large increases in GPC and no negative effects on GW, suggests that the potential negative impact of the *Gpc-B1* DIC allele on grain size and GYLD might be limited by breeding. This seems to also be supported by the recent release of several commercial cultivars carrying the *Gpc-B1* DIC allele including the HRS cv. Lassik in California (backcross derivative of the cv. Anza with the *Gpc-B1* DIC allele, the *Yr17/Sr35/Lr36* translocation and *Glu-A1* and *Glu-D1* alleles for strong gluten); the HRS cv. Farnum in Washington (WA007869 *4/Glupro, PI 638535) and the durum cv. Westmore in Arizona and California (Arizona Plant Breeders, AZ, and University of California, Davis). Finally, it is important to note that these experiments were limited to hard spring cultivars adapted to California; therefore, additional studies might be necessary to expand the conclusions presented here to other market classes and environments.

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References

American Association of Cereal Chemists. 2000. Approved methods of the American Association of Cereal Chemists. 10th ed. AACC, St. Paul, MN.

AOAC International. 1997. AOAC official method 990.03 Protein (crude) in animal feed. Official methods of analysis of AOAC. 16th ed. AOAC International, Arlington, VA.

Austin, R.B., J. Bingham, R.D. Blackwell, L.T. Evans, M.A. Ford, C.L. Morgan, and M. Taylor. 1980. Genetic improvements in winter wheat yields since 1900 and associated physiological changes. J. Agric. Sci. 94:675–689.

Avivi, L. 1978. High grain protein content in wild tetraploid wheat *Triticum dicoccoides* Korn. p. 372–380. *In* S. Ramanujam (ed.) Proc. 5th. Int. Wheat Genetic Symp., New Delhi, India. 23–28 Feb. 1987. Indian Soc. Genet. and Plant Breeding, Indian Agric. Res. Inst., New Delhi, India.

Barneix, A.J., and M.R. Guitman. 1993. Leaf regulation of the nitrogen concentration in the grain of wheat plants. J. Exp. Bot. 44:1607–1612.

Blanco, A., and C. De Giovanni. 1995. *Triticum dicoccoides* for qualitative improvement of durum wheat: Associations of protein loci to grain traits in recombinant inbred lines. p. 149–159. *In* N. di Fonzo et al. (ed.) Durum wheat quality in the Mediterranean region. CIHEAM/ICARDA/CIMMYT, Zaragoza, Spain. 17–19 Nov. 1993. Mediterranean Agronomic Inst., Chania, Greece.

Blanco, A., A. Pasqualone, A. Troccoli, N. Di Fonzo, and R. Simeone. 2002. Detection of grain protein content QTLs across environments in tetraploid wheats. Plant Mol. Biol. 48:615–623.

Caputo, C., N. Fatta, and A.J. Barneix. 2001. The export of amino acid in the phloem is altered in wheat plants lacking the short arm of chromosome 7B. J. Exp. Bot. 52:1761–1768.

Chee, P.W., E.M. Elias, J.A. Anderson, and S.F. Kianian. 2001. Evaluation of a high grain protein QTL from *Triticum turgidum* L. var. *dicoccoides* in an adapted durum wheat background. Crop Sci. 41:295–301.

Chicaiza, O., I.A. Khan, X. Zhang, J.C. Brevis, L. Jackson, X. Chen, and J. Dubcovsky. 2006. Registration of five wheat isogenic lines for leaf rust and stripe rust resistance genes. Crop Sci. 46:485–487.

Deckard, E.L., L.R. Joppa, J.J. Hammond, and G.A. Hareland. 1996. Grain protein determinants of the Langdon durum-*dicoccoides* chromosome substitution lines. Crop Sci. 36:1513–1516.

Dubcovsky, J., and J. Dvorak. 2007. Genome plasticity a key factor in the success of polyploid wheat under domestication. Science 316:1862–1866.

FAO. 2008. FAOSTAT home page. Available at http://faostat.fao.org/site/567/default.aspx#ancor (verified 10 Nov. 2008). FAOSTAT, Food and Agriculture Organization of the United Nations, Rome.

Feil, B. 1992. Breeding progress in small grain cereals—A comparison of old and modern cultivars. Plant Breed. 108:1–11.

Fu, D., C. Uauy, A. Distelfeld, A. Blechl, L. Epstein, X. Chen, H. Sela, T. Fahima, and J. Dubcovsky. 2009. A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science 323:1357–1360.

Fufa, H., P.S. Baenziger, B.S. Beecher, R.A. Graybosch, K.M. Eskridge, and L.A. Nelson. 2005. Genetic improvement trends in agronomic performances and end-use quality characteristics among hard red winter wheat cultivars in Nebraska. Euphytica 144:187–198.

Gonzalez-Hernandez, J.L., E.M. Elias, and S.F. Kianian. 2004. Mapping genes for grain protein concentration and grain yield on chromosome 5B of *Triticum turgidum* (L.) var. *dicoccoides*. Euphytica 139:217–225.

Groos, C., N. Robert, E. Bervas, and G. Charmet. 2003. Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. Theor. Appl. Genet. 106:1032–1040.

Guo, Y., and S. Gan. 2006. AtNAP, a NAC family transcription factor, has an important role in leaf senescence. Plant J. 46:601–612.

Johnson, V.A., P.J. Mattern, C.J. Peterson, and S.L. Kuhr. 1985. Improvement of wheat protein by traditional breeding and

genetic techniques. Cereal Chem. 62:350–355.

Joppa, L.R., C. Du, G.E. Hart, and G.A. Hareland. 1997. Mapping gene(s) for grain protein in tetraploid wheat (*Triticum turgidum* L.) using a population of recombinant inbred chromosome lines. Crop Sci. 37:1586–1589.

Kade, M., A.J. Barneix, S. Olmos, and J. Dubcovsky. 2005. Nitrogen uptake and remobilization in tetraploid 'Langdon' durum wheat and a recombinant substitution line with the high grain protein gene *Gpc-B1*. Plant Breed. 124:343–349.

Khan, I.A., J.D. Procunier, D.G. Humphreys, G. Tranquilli, A.R. Schlatter, S. Marcucci-Poltri, R. Frohberg, and J. Dubcovsky. 2000. Development of PCR-based markers for a high grain protein content gene from *Triticum turgidum* ssp. *dicoccoides* transferred to bread wheat. Crop Sci. 40:518–524.

Kibite, S., and L.E. Evans. 1984. Causes of negative correlations between grain yield and grain protein concentration in common wheat. Euphytica 33:801–810.

Levy, A.A., and M. Feldman. 1987. Increase in grain protein percentage in high-yielding common wheat breeding lines by genes from wild tetraploid wheat. Euphytica 36:353–359.

Mesfin, A., R.C. Frohberg, and J.A. Anderson. 1999. RFLP markers associated with high grain protein from *Triticum turgidum* L. var. *dicoccoides* introgressed into hard red spring wheat. Crop Sci. 39:508–513.

Mesfin, A., R.C. Frohberg, K. Khan, and T.C. Olson. 2000. Increased grain protein content and its association with agronomic and end-use quality in two hard red spring wheat populations derived from *Triticum turgidum* L. var. *dicoccoides*. Euphytica 116:237–242.

Olmos, S., A. Distelfeld, O. Chicaiza, A.R. Schlatter, T. Fahima, V. Echenique, and J. Dubcovsky. 2003. Precise mapping of a locus affecting grain protein content in durum wheat. Theor. Appl. Genet. 107:1243–1251.

Qualset, C.O., H.E. Vogt, and N.E. Borlaug. 1984. Registration of Anza wheat. Crop Sci. 24:827–828.

Qualset, C.O., H.E. Vogt, and N.E. Borlaug. 1985. Registration of Yecora Rojo wheat. Crop Sci. 25:1130.

Simmonds, N.W. 1995. The relation between yield and protein in cereal grain. J. Sci. Food Agric. 67:309–315.

Slafer, G.A., F.H. Andrade, and S.E. Feingold. 1990. Genetic improvement of bread wheat (*Triticum aestivum* L.) in Argentina: Relationships between nitrogen and dry matter. Euphytica 50:63–71.

Uauy, C., J.C. Brevis, X. Chen, I. Khan, L. Jackson, O. Chicaiza, A. Distelfeld, T. Fahima, and J. Dubcovsky. 2005. High-temperature adult-plant (HTAP) stripe rust resistance gene *Yr36* from *Triticum turgidum* ssp. *dicoccoides* is closely linked to the grain protein content locus *Gpc-B1*. Theor. Appl. Genet. 112:97–105.

Uauy, C., J.C. Brevis, and J. Dubcovsky. 2006a. The high grain protein content gene *Gpc-B1* accelerates senescence and has pleiotropic effects on protein content in wheat. J. Exp. Bot. 57:2785–2794.

Uauy, C., A. Distelfeld, T. Fahima, A. Blechl, and J. Dubcovsky. 2006b. A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science 314:1298–1301.

Zadoks, J.C., T.T. Chang, and C.F. Konzac. 1974. A decimal code for the growth stages of cereals. Weed Res. 14:415–421.