Effect of the *Gpc-B1* region from *Triticum turgidum* ssp. *dicoccoides* on grain yield, thousand grain weight and protein yield

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ABSTRACT

We previously cloned the *Gpc-B1* gene and showed that wild emmer (*Triticum turgidum* ssp. *dicoccoides,* DIC hereafter) has a functional copy of this gene, whereas tetraploid and hexaploid commercial wheat varieties have a deletion or a non-functional copy as a result of a frame-shift mutation. The DIC allele accelerates senescence and increases protein, zinc and iron content in the grain relative to the non-functional allele. Here we describe the effect of the introgression of the DIC chromosome 6BS segment including *Gpc-B1* on grain yield, thousand grain weight (TGW) and protein yield (grain yield by grain protein content) in hexaploid and tetraploid wheat. We introgressed the DIC segment into six hexaploid and three durum varieties by six backcrosses and developed sister near isogenic lines (NIL) with and without the DIC segment. In 2006 and 2007, we grew the nine pairs of NILs in three locations in a split-plot design with five replications. The lines were maintained disease-free to avoid the confounding effect of the linked *Yr36* resistance gene. In 2006, durum lines carrying the DIC *Gpc-B1* allele showed average grain yield reductions between 6 and 17% relative to the recurrent parental lines. The same year, GPC durum lines showed an 8% reduction in TGW, explaining part of the decrease in grain yield. In 2007, however, durum lines with the DIC *Gpc-B1* region showed grain yield increases in two of the three locations tested, in spite of a consistent reduction in TGW. Hexaploid lines carrying the DIC *Gpc-B1* allele showed non-significant differences in grain yield compared to the corresponding recurrent parents in both years. The presence of significant gene by variety interactions indicate that the effect of the *Gpc-B1* region varies across genotypes. In general, grain protein concentration was significantly increased by the presence of the DIC allele, although the magnitude of the increase varied across locations and genetic backgrounds. Total protein yield was strongly affected by the variations in grain yield.

INTRODUCTION

Grain protein concentration (GPC) is a key factor to define wheat grain quality and, therefore, a frequent target of pasta and bread wheat breeding programs. However, selection for this trait is complicated by its quantitative nature, the high effect of the environment and the existence of negative correlations between GPC and grain yield in some segregating populations. Selection for GPC could be facilitated by the identification of the genes regulating this trait and by marker assisted selection for the favorable alleles.

We have recently cloned the *Gpc-B1* gene and showed that the functional allele from wild emmer (*Triticum turgidum* ssp. *dicoccoides,* DIC hereafter) increases protein, zinc and iron content in the grain and accelerates senescence relative to the non-functional allele present in cultivated wheat [1, 2]. Most current tetraploid and hexaploid commercial wheat cultivars have a deletion or a non-functional copy of *Gpc-B1*.

The accelerated senescence associated with the functional *Gpc-B1* DIC allele results in a shorter grain filling period and, therefore, has the potential to affect kernel size and grain yield. Here we describe the effect of the introgression of the DIC chromosome 6BS segment including *Gpc-B1* on grain yield (GYLD), thousand grain weight (TGW) and protein yield (PYLD; defined as GYLD by GPC) in hexaploid and tetraploid wheat.

MATERIALS AND METHODS

We introgressed the DIC segment into six hexaploid and three durum cultivars/breeding lines by six backcrosses and developed sister near isogenic lines (NILs) with and without the DIC segment, which will be referred hereafter as *Gpc-B1* and control lines respectively. In 2006 and 2007, we grew the nine pairs of NILs in three distinct locations in California: El Centro (32°48' N, 115°26' W), Davis (38° 32'N, 121° 46' W) and Tulelake (41°57' N, 121°28' W). Each experiment was arranged as a split-plot design with five randomized complete blocks with the exception of Davis, where ten blocks were used. Plot size averaged 3.4 (El Centro), 4.0 (Davis) and 7.4 m^2 (Tulelake). Fertilization and irrigation were applied according to common practices for high yield. Lines were maintained disease-free by applying fungicides when necessary, to avoid the confounding effect of the linked stripe rust resistance gene Yr36 [3]. At maturity and before machine-harvest, a sample of 0.5 m^2 from each plot (Davis 2006 and 2007) was hand-harvested to measure biomass and harvest index (HI). Plots were machine-harvested and grain was weighed to estimate GYLD. GPC was determined by near-infrared spectroscopy using an Infratec 1226 Grain Analyzer. TGW was estimated by manually counting 500 grains from each replication.

Analysis of variance was performed using SAS version 9.1 (SAS Institute Inc., USA). The general lineal model PROC GLM was used to analyze a three-way factorial ANOVA including gene (*Gpc-B1* DIC chromosome region present or absent), cultivars (3 durum and 6 common) and 6 environments (2 years and 3 locations). Environments and interactions including environment were considered random factors and blocks were nested within environments. LSMEANS were used to adjust means for the unbalanced design. Since exploratory models showed that the effect of the *Gpc-B1* chromosome region was different for tetraploid and hexaploid wheat, statistical analyses were also performed separately by wheat type.

RESULTS

Grain protein concentration (GPC)

The average GPC for the recurrent parents was 129.6 g kg^{-1}, and ranged from 117.9 g kg^{-1} (hard red spring cv. Anza) to 139.3 (durum cv. Kofa). Average values for the durum and hexaploid NILs are detailed in Table 1. GPC was consistently higher (9.6 g kg^{-1} or 7.4%, $P<0.001$) for the NILs carrying the DIC *Gpc-B1* segment than for the controls. Significant results were also observed in separate analyses for tetraploid and hexaploid NILs (Table 1).

Table 1. Effect of *Gpc-B1* introgression on GPC, TGW, GYLD and PYLD in six hexaploid (common) and three tetraploid (durum) near isogenic lines grown in six environments.

		GPR (g kg^{-1})		TGW (g)	
Wheat	**NIL**	**mean**	***P* value**	**mean**	***P* value**
Common	Control	128.1	**<.001**	42.64	**<.001**
	GPC	135.5		41.45	
Durum	Control	132.7	**<.001**	52.75	0.08
	GPC	146.5		50.54	
		GYLD (kg ha^{-1})		**PYLD (kg ha^{-1})**	
		mean	***P* value**	**mean**	***P* value**
Common	Control	7618	0.61	986	**<.001**
	GPC	7670		1042	
Durum	Control	8878	0.16	1182	0.06
	GPC	8421		1242	

The average increase in GPC associated with the *Gpc-B1* DIC allele was higher for the tetraploid NILs (13.8 g kg^{-1}) than for the hexaploid ones (7.4 g kg^{-1}). For both groups, the gene*environment and gene*cultivar interactions were significant ($P<0.05$), likely due to differences in magnitude of the GPC increase among cultivars and among environments. When tested by cultivar, the DIC *Gpc-B1* segment was associated with consistent GPC increases in all cultivars. The significant increases showed by the *Gpc-B1* lines ranged from 4.5 to 15.2 g kg^{-1} for the hexaploid NILs ($P<0.05$), and from 11.9 to 16.3 g kg^{-1} for the tetraploid NILs ($P<0.01$). Analysis of the variance components using VARCOMP showed that allelic variation at this locus explained 18 and 33% of the variation of GPC for hexaploid and tetraploid wheat, respectively. In the combined analysis including all cultivars, allelic variation at the *Gpc-B1* locus explained the same proportion of variation as cultivar (23%). Differences among environments contributed 30% of the variation. GPC was the only trait in this study for which the *Gpc-B1* segment explained a significant portion of variation.

Grain yield (GYLD)

The average GYLD for the recurrent parents across the six environments was 8,038 kg ha^{-1}, ranging from 9,269 kg ha^{-1} (durum breeding line UC1113) to 7,078 kg ha^{-1} (hexaploid breeding line UC1041). Notably, the durum control NILs showed higher GYLD across environments than the hexaploid NILs (Table 1). The NILs carrying the *Gpc-B1* DIC allele showed a non significant ($P=0.13$) decrease in GYLD of less than 2% relative to the control lines (118 kg ha^{-1}). The effect of the *Gpc-B1* region on GYLD was strongly affected by genetic background as shown by a highly significant ($P<0.001$) gene*cultivar interaction. Within the hexaploid cultivars, the average GYLD was practically the same for *Gpc-B1* and control NILs ($P=0.61$, Table 1). An average decrease of 5.1% (457 kg ha^{-1}) in GYLD was detected in the tetraploid *Gpc-B1* lines relative to the control, but the differences were not significant ($P=0.16$, Table 1). For both tetraploid and hexaploid NILs the gene*ENV and gene*cultivar interactions were significant, indicating a strong effect of genotype and environments on the *Gpc-B1* effect on GYLD.

When data were analyzed by cultivar, none of them showed significant ($P>0.05$) differences in GYLD between *Gpc-B1* alleles. The *Gpc-B1* durum NILs for Kronos and Kofa exhibited the greatest penalties in GYLD with an average decrease of 776 and 537 kg ha^{-1}, respectively, relative to the control NILs. The *Gpc-B1* UC1113 NIL had a slight decrease in GYLD equivalent to 56 kg ha^{-1}. The differences in GYLD between *Gpc-B1* alleles were more variable among the hexaploid genotypes. The *Gpc-B1* NILs for cultivars Attila and RSI5 showed average GYLD decreases of 209 and 151 kg ha^{-1}, respectively. The other hexaploid *Gpc-B1* NILs had slightly higher GYLD than their respective control line. The analysis of variance components showed that environments, cultivar and their interaction explain 75% of the variation in GYLD, whereas the variance explained by the *Gpc-B1* segment was negligible.

The biomass analysis did not show significant differences in biomass production or harvest index between *Gpc-B1* and control NILs.

Thousand-grain weight (TGW)

Across all cultivars, the *Gpc-B1* NILs had a significantly ($P<0.01$) lower TGW than the control NILs (3.3% decrease). When analyzed by species, the durum *Gpc-B1* genotypes showed a non significant ($P=0.08$) decrease in

TGW (4.2%) relative to the control NILs (Table 1). This decrease ranged from 5.2% (cv. Kronos) to 3.7% (Kofa and UC1113). The hexaploid *Gpc-B1* NILs showed significantly (P<0.001) lower TGW (2.8%) relative to the control NILs (Table 1). However, *Gpc-B1* lines of cultivars Anza, Attila and RSI5 showed very similar TGW to their respective control NILs (P>0.40). UC1037 and Yecora Rojo *Gpc-B1* NILs showed significantly (P<0.01) lower TGW relative to the control NILs with an average decrease of 4.8% and 6.5%, respectively. The UC1041 *Gpc-B1* NIL showed a similar decrease in TGW (4.4%) but the difference was marginally non-significant (P=0.054).

The analysis of variance components showed that the largest proportion of variation in TGW was explained by differences among cultivar (65%), whereas the variance explained by allelic differences at the *Gpc-B1* locus was relatively small (2%).

Protein yield (PYLD)

PYLD was consistently higher in NILs carrying the *Gpc-B1* DIC allele than in those carrying the control allele. Across all cultivars, the *Gpc-B1* lines produced more protein (P<0.001) than the control NILs. The average increase was similar across hexaploid and tetraploid genotypes (56 and 59 kg ha^{-1}, respectively). However, different responses among cultivars were evident from a significant (P<0.001) gene*cultivar interaction. When analyzed by species, PYLD increase was significant only for the hexaploid group (Table 1). When analyzed by cultivar, the *Gpc-B1* lines of the hexaploid cultivars Anza and RSI5 and the durum breeding line UC1113 showed significant increase in PYLD (63, 83 and 114 kg ha^{-1}, respectively).
The analysis of variance components showed that environment, cultivar and their interaction explained 79% of the variation in PYLD, whereas the variance explained by the *Gpc-B1* introgression was less than 3%.

DISCUSSION

The presence of the functional *Gpc-B1* allele from DIC accelerates senescence [1, 2]. In greenhouse experiments using transgenic GPC::RNAi lines with highly delayed senescence and significant reductions of GPC, no significant differences in TGW were observed [2]. However, it was not known if the accelerated senescence and reduced grain filling period could have a negative impact in grain size and grain yield under field conditions. Our results suggest that only part of the GPC increases observed in the *Gpc-B1* NILs can be attributed to a concentration effect resulting from reduced TGW.

The effects of the DIC *Gpc-B1* introgression on GPC and TGW were in opposite directions and of different magnitude. The presence of the *Gpc-B1* DIC allele was associated with 6-10% increases in GPC and 3-4% decreases in TGW. The relative smaller effect of the *Gpc-B1* DIC allele on TGW than on GPC was also reflected in the overall significant increase in total protein yield.

The differences in GYLD were less consistent than the differences in TGW suggesting possible compensating mechanisms. Some *Gpc-B1* NILs showed no significant reductions in GYLD in spite of consistent decreases in TGW. It would be interesting to test if plants showing differences in TGW but not in GYLD differ in fertility.

The effect of the *Gpc-B1* introgression on durum NILs was larger than the effect on hexaploid NILs for most parameters. This is not surprising given the different number of functional *Gpc-B1* homoeologs and paralogs present in these species. Most tetraploid varieties have only two *Gpc* functional copies (6A and 2B), whereas most hexaploid genotypes have four (6A, 6D, 2B and 2D). Therefore, the introgression of the active DIC allele of *Gpc-B1* had a relatively larger dosage effect on tetraploid than on hexaploid wheat NILs.

For the four traits analyzed in this study the gene*environment and gene*cultivar interactions were generally significant. This suggests that the effect of the DIC *Gpc-B1* introgression on GYLD and grain protein yield will depend on the genotype where it is introgressed and the environment where it is used. This also suggests that part of the negative impact of the *Gpc-B1* DIC allele on TGW might be reduced by breeding. Examples of new *Gpc-B1* lines being released commercially are the common wheat cultivars ‘Lassik’ (UC Davis, CA) and ‘Farnum’ (Washington State Univ., WA), and the durum cultivar ‘Westmore’ (Arizona Plant Breeders, AZ). ‘Lassik’ showed a highly significant increase in GPC with no significant yield penalty relative to the recurrent parent Anza. These recent cultivar releases suggest that it should be possible to breed high-protein commercial varieties using the *Gpc-B1* DIC allele However, it should be kept in mind that this gene might be associated with a yield penalty in some genotypes and environments and that a dedicated breeding effort would be necessary for its successful incorporation into commercial varieties.

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