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Breeding for increased grain protein and micronutrient content in wheat: Ten years of the *GPC-B1* gene

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ABSTRACT

To provide food and nutrition security for a growing world population, continued improvements in the yield and nutritional quality of agricultural crops will be required. Wheat is an important source of calories, protein and micronutrients and is thus a priority to breed for improvements in these traits. The *GRAIN PROTEIN CONTENT-B1* (*GPC-B1*) gene is a positive regulator of nutrient translocation which increases protein, iron and zinc concentration in the wheat grain. In the ten years since it was cloned, the impacts of *GPC-B1* allelic variation on quality and yield traits have been extensively analyzed in diverse genetic backgrounds in field studies spanning forty environments and seven countries. In this review, we compile data from twenty-five studies to summarize the impact of *GPC-B1* allelic variation on fifty different traits. Taken together, the results demonstrate that the functional copy of the *GPC-B1* gene is associated with consistent positive effects on grain protein, Fe and Zn content with only marginally negative impacts on yield. We conclude that the *GPC-B1* gene has the potential to increase nutritional and end use quality in a wide range of modern cultivars and environments and discuss the possibilities for its application in wheat breeding.

Contents

Abbreviations: BAC, Bacterial Artificial Chromosome; DIC, *Triticum turgidum* var. *dicoccoides*; FYLD, flour yield; GPC, grain protein content; MAS, marker assisted selection; ns, not significant; QTL, quantitative trait locus; RNAi, RNA interference; TKW, thousand kernel weight; TW, test weight.

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1. Introduction

During the 20th century major advances in agronomy and plant breeding delivered significant improvements in yield, processing and end-use quality of the most widely-grown agricultural crops (Bradshaw, 2016; Moose and Mumm, 2008). Despite this progress, widespread food shortages persist, with an estimated 800 million undernourished people in the world today, predominantly in the developing world (FAO, 2015). Furthermore, it is estimated that agricultural production will need to increase by 60% to provide food security for a human population anticipated to reach 9 billion by 2050 (Alexandratos and Bruinsma, 2012). This challenge will likely be exacerbated by increased competition for land use, changing dietary habits and more volatile climatic patterns (Iizumi and Ramankutty, 2016; Peña, 2007).

In addition to undernourishment, malnourishment is highly prevalent and more than half of the world's population are estimated to suffer some form of micronutrient deficiency (Zhao and McGrath, 2009). Iron (Fe) deficiency alone affects 2.7 billion people worldwide (Hirschi, 2009) and an estimated 161 million children under the age of five are stunted due to chronic malnutrition (FAO, 2014). These nutritional shortfalls are directly responsible for increased mortality and morbidity, including higher rates of anemia, immune system deficiencies and stunted physical and neural development (Black, 2003; Das et al., 2016; Stein, 2010).

Wheat provides 20% of the calories and 25% of proteins consumed worldwide on a daily basis (http://www.wheatinitiative.org/; FAO, 2015) and is also an important source of micronutrients (Shewry and Hey, 2015), providing approximately 16% of Fe and 12% of zinc (Zn) in the average adult diet in the UK (Bates et al., 2014). Consequently, breeding for improvements in the yield and nutritional quality of wheat (such as increased protein and micronutrient levels in the grain) is a major goal of both public and private breeding programs to alleviate hunger and malnutrition (Lantican et al., 2016). Wild relatives of wheat exhibit a broader genetic diversity and higher concentrations of many grain nutrients than modern wheat cultivars, indicating that improvements in these traits in modern wheat cultivars are possible (Cakmak et al., 2004; Chatzav et al., 2010; Monasterio and Graham, 2000; Verma et al., 2016).

Through quantitative trait loci (QTL) analyses several genomic regions underlying sources of natural variation for quality traits have been identified and introduced into modern wheat cultivars using marker assisted selection (MAS) (Blanco et al., 2006; Peleg et al., 2009; Prasad et al., 2003; Pu et al., 2014; Sun et al., 2008; Uauy et al., 2006a). One major source of variation in grain protein content (GPC) was identified among a population of wild tetraploid emmer wheats (*Triticum turgidum* var. *dicoccoides*, accession FA-15-3, originated in Israel) (DIC) (Avivi, 1978). Using a population of disomic substitution lines of each DIC chromosome in the tetraploid durum cultivar Langdon, chromosome 6B from DIC was identified as the major source of the genetic variation conferring increased GPC (Cantrell and Joppa, 1991). This QTL was mapped to a 2.7 cM region on the short arm of chromosome 6B and characterized as a single Mendelian locus named *GPC-B1* (Chee et al., 2001; Joppa et al., 1997; Olmos et al., 2003). By comparing the colinearity of the 2.7 cM wheat region with the rice genome the *GPC-B1* locus was delimited to a 0.3 cM interval containing five candidate genes (Distelfeld et al., 2006). Using a *T. dicoccoides* Bacterial Artificial Chromosome (BAC) library, a physical map was produced (Cenci et al., 2003) which led to the identification of a single gene, *NO APICAL MERISTEM-B1* (*NAM-B1*, Genbank id: DQ869673 (Uauy et al., 2006a, b). For the purpose of this review this gene will be referred to as *GPC-B1* as it is more commonly known in the literature. *GPC-B1* encodes a NAC-domain transcription factor, a family of proteins which in other plant species play roles in regulating diverse developmental processes, including leaf senescence and defense and stress responses (Guo and Gan, 2006; Jukanti and Fischer, 2008; Olsen et al., 2005; Zhou et al., 2013). Reduced GPC in the variety Langdon is associated with a 1-bp insertion in the *GPC-B1* coding region which disrupts the open reading frame, resulting in the translation of a non-functional protein. Homoeologous copies of *GPC-B1* were identified on chromosomes 6A and 6D, and a paralogous gene *GPC2*, which shares approximately 90% sequence identity with *GPC1*, was identified on the homoeologous group 2 chromosomes (Uauy et al., 2006b). Both *GPC1* and *GPC2* are most highly expressed in flag leaves after anthesis (Uauy et al., 2006b).

Transgenic wheat lines expressing an RNA interference (RNAi) construct targeting all *GPC1* and *GPC2* homoeologous genes (*GPC*::RNAi) exhibited significant reductions in GPC, Fe and Zn levels (>30%) in the grain as well as a three-week delay in the onset of senescence when compared to control lines (Uauy et al., 2006b). The importance of *GPC1* to these phenotypes was confirmed using hexaploid and tetraploid TILLING mutants carrying non-functional mutations in all homoeologous copies of the *GPC1* gene, which exhibited comparable reductions in each of these traits (Avni et al., 2014; Pearce et al., 2014). These experiments demonstrate that *GPC1* is an important regulator of senescence and the subsequent translocation of protein and micronutrients to the developing wheat grain.

1.1. Distribution of GPC-B1 alleles

Surveys analyzing the distribution of the wild-type, functional *GPC-B1* allele among diverse wheat collections have found this allele to be widespread among ancestral wheats, but comparatively rarer among modern cultivars. In one survey, all 42 wild emmer accessions and 17 of 19 domesticated emmer accessions which were assayed carried the wild-type *GPC-B1* allele (Table 1). In contrast, among 57 durum (*T. turgidum* ssp. *durum*) and 34 bread wheat varieties (*T. aestivum* ssp. *aestivum*) all lines carried either the non-functional *GPC-B1* allele characterized in the variety Langdon, or a complete deletion of this gene (Uauy et al., 2006b). The wild-type *GPC-B1* allele was found in only two spelt wheats (*T. aestivum* ssp. *spelta*) and two bread wheats among the 62 wheat varieties displayed at the International Exhibition in London in 1862 (Asplund et al., 2010). Among the French National Institute for Agricultural Research (INRA) core collection of 367 global bread wheat varieties, just five carried the functional *GPC-B1* allele (Hagenblad et al., 2012).

Interestingly, the functional *GPC-B1* allele was relatively more common among a collection of spring wheat cultivars from northern latitudes and was present in 46 out of 138 accessions, all of which had Fennoscandian origin (Hagenblad et al., 2012). Similarly, among 22 spelt wheats provided by INRA and the Nordic Genetic Resource Center, five accessions carried the ancestral functional allele (Leino et al., 2009).

Taken together, these surveys show that despite being rare among wild ancestral wheat populations, the non-functional *GPC-B1* allele is highly prevalent among modern bread wheat varieties, with the exception of some cultivars with a northern origin. This pattern of distribution may be the result of the rapid fixation of this allele among modern cultivars following their domestication. Alternatively, it is possible that the non-functional *GPC-B1* allele was already present in the domesticated emmer wheats which gave rise to polyploid bread wheat. This latter possibility is consistent with the finding that the wild-type *GPC-B1* allele is common among Fennoscandian wheats, which share a common origin.

Table 1
Distribution of wild-type and non-functional *GPC-B1* alleles among worldwide Triticeae germplasm. *T. tu: Triticum turgidum; T. ae: Triticum aestivum.*

Reference	Species	*GPC-B1* allele		Notes
		Wild-type	Non-functional[a]	
Uauy et al., 2006b	*T. tu.* var. *dicoccoides*	42	0	Wild emmer accessions.
	T. tu. ssp. *dicoccum*	17	2	Domesticated emmer.
	T. tu. ssp. *durum*	0	57	Cultivated varieties.
	T. ae. ssp. *aestivum*	0	34	Accessions and varieties.
Distelfeld et al., 2006	*T. tu.* ssp. *durum*	0	39	Cultivated varieties.
	T. ae. ssp. *aestivum*	0	78	
Asplund et al., 2010	*T. ae.* ssp. *aestivum*	2	47	Historical wheat varieties displayed at the
	T. ae. ssp. *spelta*	2	5	International Exhibition in London (1862).
	T. ae. ssp. *compactum*	0	1	
	T. tu. ssp. *durum*	0	2	
	T. tu. ssp. *turgidum*	0	2	
	T. tu. ssp. *dicoccum*	0	1	
Hagenblad et al., 2012	*T. ae.* ssp. *aestivum*	5	362	Worldwide bread wheat accessions.
	T. ae. ssp. *aestivum*	46	92	Varieties and landraces released in the 19th
	T. ae. ssp. *spelta*	5	17	and 20th centuries with a northern origin (Russia, Canada, Mongolia, Japan and Fennoscandia).

[a] Non-functional frameshift mutation or complete deletion.

1.2. Survey of GPC-B1

Despite the high incidence of malnutrition globally, *GPC-B1* remains the only gene cloned in wheat with a characterized role in protein and nutrient remobilization. Because the functional *GPC-B1* allele is rare among modern wheat cultivars, the introgression of this allele represents a valuable breeding strategy to improve grain quality in diverse germplasm collections. However, these breeding efforts have been hampered by the strong impact of environmental variation on GPC and, in some instances, a negative correlation between yield and GPC (Barneix, 2007; Iqbal et al., 2016; Triboi and Triboi-Blondel, 2002).

It is now ten years since *GPC-B1* was cloned and the objective of this review is to summarize the data from all published studies which have described the impact of allelic variation at the *GPC-B1* locus on wheat production and quality traits. These studies range from the first reports of the DIC allele in 1978 (Avivi, 1978) to the most recently published study in 2016 (Kuhn et al., 2016). This body of research comprises 25 studies in both tetraploid durum wheat (11 studies) and hexaploid bread wheat (14 studies), encompassing a range of genetic backgrounds and 40 diverse environments in seven countries (Fig. 1). All described studies were conducted under field conditions except two (Kade et al., 2005; Maphosa et al., 2014). It is important to note that because each study applied different methodologies, experimental designs and statistical analyses, we have not attempted to perform any additional statistical meta-analyses for each trait. The data presented is a compilation of results from previously published studies and where possible, we have used common units for each parameter. The effects of *GPC-B1* on each trait are reported as percentages and ranges. Trends are represented either as significant increases (↑) or decreases (↓) ($P < 0.05$) or not significant (ns) for lines carrying the wild-type *GPC-B1* allele compared to the non-functional *GPC-B1* allele. The reported percentages were based on total sample size for each trait; each "*n*" signifies the comparison between a *GPC-B1* line *vs.* the respective control (e.g. $n = 2$ signifies that two biological replicates of lines with the functional *GPC-B1* allele were compared with their respective control lines with the non-functional allele). Full results from all studies are provided in Supplementary Table S1.

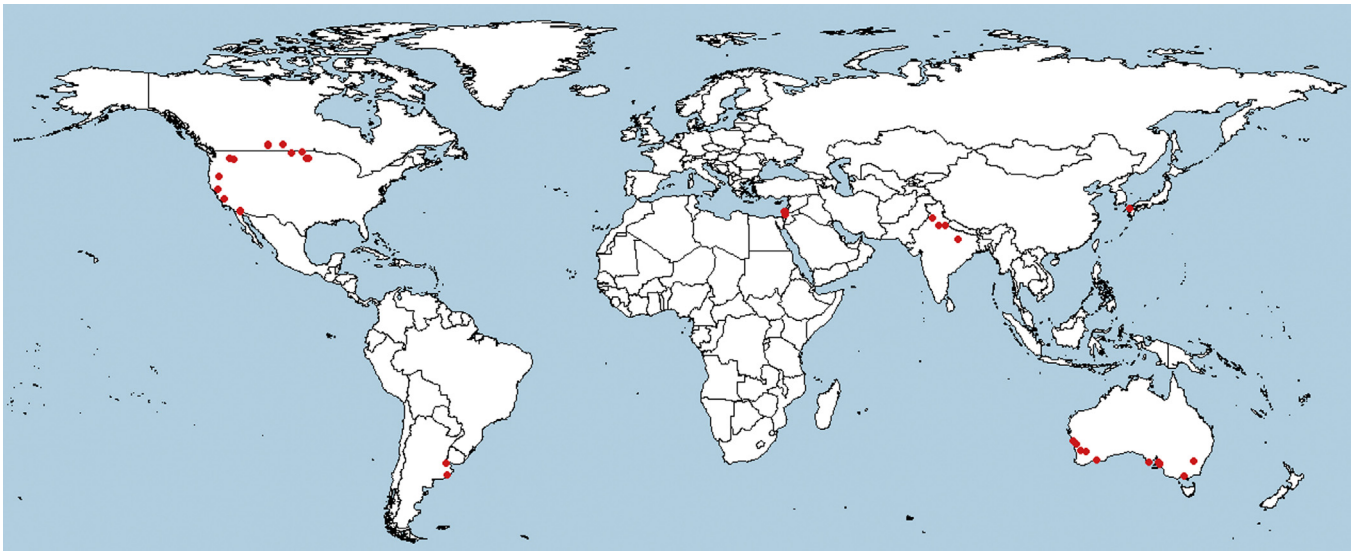

Fig. 1. Location of field trials testing the effects of *GPC-B1* allelic variation. Field trials have been performed in seven countries (Argentina, Australia, Canada, India, Israel, Japan and USA). Full details of locations and experimental designs are provided in Table S1.

2. Effects of *GPC-B1* on wheat grain nutritional content

The main motivation for the introgression of the wild-type *GPC-B1* allele into modern wheat cultivars is to improve grain nutritional content. GPC is a quantitative trait strongly influenced by genotype and environmental conditions, including water access and temperature during grain filling and fertilization (Abedi et al., 2011; Daniel and Triboi, 2000; López-Bellido and López-Bellido, 2001; Rharrabti et al., 2001; Tea et al., 2004). Of 25 studies analyzed (n = 127), 91% of lines carrying the wild-type *GPC-B1* allele averaged an increase of 21.8 g kg^{-1} in GPC compared to lines carrying the non-functional allele; the remaining 9% of comparisons showed no significant differences. Higher increases in GPC were found for bread wheat compared to durum wheat (Table 2), while across experiments Indian cultivars (which accounted for 48% of all comparisons for GPC) showed the highest increases (on average 31.2 g kg^{-1}, n = 61) associated with the wild-type allele (Kumar et al., 2011; Mishra et al., 2015; Vishwakarma et al., 2014, 2016). The gene-by-genotype interaction was significant in two of four studies and the gene-by-environment interaction was significant in three of eight studies where this was tested (Table S2). In all cases, this interaction was due to differences in the magnitude of the effect of GPC (Table S2).

Protein yield (derived from multiplying GPC by yield) was analyzed in seven studies (n = 37) and 30% of lines with the wild-type *GPC-B1* allele exhibited significant increases in the range of 22.9–144 kg ha^{-1} while 68% of the comparisons showed no significant differences (Table 2). The highest increases were observed in the durum cultivar UC1113 (144 kg ha^{-1}) and the bread wheat cultivar RSI5 (83 kg ha^{-1}), both derived from the UC Davis breeding program (Brevis and Dubcovsky, 2010). In one assay, protein yield was measured as grams per 1000 seeds and lines with the functional *GPC-B1* allele showed an average increase of 0.4 g/1000 seeds compared to their respective controls (Uauy et al., 2006a) (Table S1). Gene-by-genotype and gene-by-environment interactions for protein yield were calculated in two studies and one interaction was significant for each type. These significant interactions arose from differences in magnitude rather than in the direction of the effects (Table S2).

Four studies analyzed the effect of the *GPC-B1* allele on the relative distribution of N distribution in the straw and grain tissues (Brevis and Dubcovsky, 2010; Carter et al., 2012; Kade et al., 2005; Tabbita et al., 2013) (Table S1). None of these studies found differences in above-ground biomass (n = 11) or harvest index (n = 11) between lines. However, straw nitrogen concentration was significantly decreased (n = 9) and nitrogen harvest index was significantly increased (three studies, n = 7) in lines carrying the wild-type *GPC-B1* allele. This suggests that plants carrying the functional *GPC-B1* allele exhibit a higher rate of N remobilization from vegetative tissues to grains. This is consistent with physiological (Waters et al., 2009) and transcriptomic analyses of *GPC*::RNAi transgenic plants and *GPC1* TILLING mutants, which showed that *GPC1* regulates the expression of genes related to N metabolism (Cantu et al., 2011; Pearce et al., 2014). This effect of the *GPC-B1* allele may confer indirect environmental benefits since increased N remobilization efficiency from vegetative tissues to grains may reduce the need for N fertilizer applications, reducing costs and potential environmental damage resulting from excess fertilization (Brevis and Dubcovsky, 2010).

Significant increases in Fe and Zn concentrations were consistently found in lines carrying the wild-type *GPC-B1* allele (Table 2). Five studies quantified grain concentrations of these elements (n = 42 for each trait) and 95% of lines carrying the wild-type *GPC-B1* allele showed a significant increase in Fe content (on average 12.5 mg kg^{-1}), while 93% showed a significant increase in Zn content (on average 11.6 mg kg^{-1}, Carter et al., 2012; Distelfeld et al., 2007; Uauy et al., 2006b; Vishwakarma et al., 2016, 2014).

GPC and micronutrient content was positively correlated in all experiments and all lines carrying the functional *GPC-B1* allele that showed significant increases in Fe and Zn concentrations also

Table 2
Number of studies, mean values (x), ranges (in bold), sample size (n) and respective percentages represented by significant ($P < 0.05$) increases (↑), decreases (↓) or not significant effects (NS) of *GPC-B1* gene in 10 different agronomical traits across wheat species. Each unit of "n" represents a comparison between *GPC-B1* lines and respective control lines. For complete details see Table S1.

Quality traits	*T. aestivum*					*T. turgidum*				
	Nº studies		↑	↓	NS	Nº studies		↑	↓	NS
GPC (g kg^{-1})	14	x (range)	**22.9 (2.1–72)**			11	x (range)	**15.5 (6–40)**		
		n (%)	97 (90%)		11 (10%)		n (%)	18 (95%)		1 (5%)
PY (kg ha^{-1})[a]	5	x (range)	**49.7 (22.9–83)**	**−0.4**[d]		2	x (range)	**144**		
		n (%)	8 (27%)	1 (3%)	21 (70%)		n (%)	3 (43%)		4 (57%)
Fe (mg kg^{-1})	3	x (range)	**13.1 (2.3–24.2)**	**−0.2 (−0.2)**		2	x (range)	**6.6 (5–8.2)**		
		n (%)	38 (95%)	1 (3%)	1 (3%)		n (%)	2 (100%)		
Zn (mg kg^{-1})	3	x (range)	**11.8 (2.4–27.7)**			2	x (range)	**6.4 (5.5–7.4)**		
		n (%)	37 (93%)		3 (8%)		n (%)	2 (100%)		
Yield traits										
Yield (kg ha^{-1})[b]	10	x (range)	**399 (164–634)**	**−221 (−363–−166)**		5	x (range)			
		n (%)	17 (19%)	4 (4%)	70 (77%)		n (%)	0 (0%)		7 (100%)
TKW (g)	11	x (range)	**4.4 (0.8–7.3)**	**−2.2 (−5.7–−0.7)**		7	x (range)	**3.3 (3.3)**	**−2.3 (−3–−1.2)**	
		n (%)	44 (45%)	23 (23%)	31 (32%)		n (%)	1 (8%)	3 (23%)	9 (69%)
Senescence (days)	6	x (range)	**4.5 (2–8)**	**−3.1 (−9–−1.4)**		4	x (range)		**−3.9 (−4.5–−3)**	
		n (%)	13 (25%)	12 (23%)	28 (53%)		n (%)		7 (100%)	
Spike number (m^{-2})[c]	5	x (range)	**53.1 (53.1)**			1	x (range)			
		n (%)	20 (40%)		30 (60%)		n (%)			3 (100%)
Spikelets spike^{-1}	3	x (range)	**2.6 (1.5–4.5)**	**−2.1 (−5.5–−1)**		1	x (range)			
		n (%)	8 (18%)	17 (39%)	19 (43%)		n (%)			3 (100%)
Grains spike^{-1}	4	x (range)	**5.7 (3.2–8.5)**			1	x (range)			
		n (%)	9 (31%)		20 (69%)		n (%)			3 (100%)

GPC: Grain protein content; PY: Protein yield; TKW: Thousand kernel weight.
[a] In Kade et al., 2005; Distelfeld et al., 2007 and Vishwakarma et al., 2014 yield was measured in g per plant.
[b] In Uauy et al., 2006a PY was measured in g per 1000 seeds.
[c] In Vishwakarma et al., 2014 and Vishwakarma et al., 2016 spikes were measured per plant.
[d] Value expressed in g of proteins/1000 seeds.

showed significant increases in GPC. This correlation has previously been observed in bread wheat (Peterson et al., 1986; Raboy et al., 1991), maize (Feil and Bänziger, 1993) and triticale (Feil and Fossati, 1995). A positive correlation between Fe and Zn levels has also been shown in different DIC accessions, suggesting the existence of shared transport mechanisms regulating the translocation of these nutrients to the developing grains (Cakmak et al., 2004). Transcriptomic analysis showed that *GPC1* is associated with changes in the expression levels of several classes of Fe and Zn transporters (Pearce et al., 2014). Further studies will be required to determine whether *GPC1* regulates the expression of these genes directly or indirectly and whether the observed changes in expression are important determinants of grain micronutrient content.

The wild-type *GPC-B1* allele is also associated with increased grain concentrations of other elements (Table S1). Langdon recombinant substitution lines carrying chromosome 6B from DIC exhibited increased Mn grain content of 10.9 mg kg^{-1} compared to the control (Distelfeld et al., 2007) while the hexaploid cultivar Scarlet, which carries the functional *GPC-B1* allele showed increased Ca grain concentration of 0.1 mg kg^{-1} compared to control line (Carter et al., 2012). An analysis of twelve Swedish spring cultivars released during the 20th century showed that the five lines carrying the wild-type *GPC-B1* allele were associated with increased Mg, P and S grain concentrations compared to those carrying the non-functional allele (Asplund et al., 2013). Both GPC::RNAi transgenic lines and *GPC1* TILLING mutants showed significant decreases in Cu levels and significant increases in K and B in their grain (Waters et al., 2009; Pearce et al., 2014).

3. Effects of *GPC-B1* on yield and yield components

When introgressing a new gene or QTL for quality into adapted cultivars, a critical consideration is the effect of the new variant on grain yield. Of the 15 studies ($n = 98$) which analyzed the effects of *GPC-B1* on yield, 79% of comparisons showed no significant differences; 17% of comparisons found the wild-type *GPC-B1* allele to have a positive effect on yield of up to 634 kg ha^{-1}, while 4% showed a reduction in yield of up to −363 kg ha^{-1} (Table S1). Within wheat species 77% of the bread wheat lines showed no differences while 19% of lines with the functional *GPC-B1* allele exhibited significant increases in yield (Table 2). For durum wheat, all seven comparisons showed the *GPC-B1* allele had no significant effect on yield. In three studies ($n = 25$), yield was quantified per plant (Table S1); 56% (all bread wheat varieties with Indian origin) showed the functional *GPC-B1* allele to be associated with a significant increase in yield (Vishwakarma et al., 2014), while the remaining comparisons (all durum wheat varieties) showed no significant differences for this trait (Distelfeld et al., 2007; Kade et al., 2005). Three studies reported the gene-by-genotype interaction and two were significant, due to differences in the direction of the effects. A total of eight studies reported gene-by-environment interaction and only one was significant (Table S2). These results emphasize that the largest source of variation in grain yield was determined by the genotype, the environment and the interaction between these components, whereas the variation explained by the *GPC-B1* introgression was negligible (Brevis and Dubcovsky, 2010). Despite the lack of significant negative effects on yield, three out of seven studies which analyzed the association between GPC and yield found these traits to be negatively correlated. Full details of the results of these interactions are provided in Table S2.

As well as overall yield, several studies analyzed individual yield components (Table 2). Thousand kernel weight (TKW) was quantified in 18 studies ($n = 111$) and 36% of the comparisons showed no significant differences; 23% of the lines with the *GPC-B1* functional allele showed significant decreases of 2.2 g while the remaining 41% of comparisons had significant increases of 4.4 g because of the wild-type *GPC-B1* allele (43 of the 45 lines showing increases in TKW were from India, Table S1). The only gene-by-genotype interaction reported for this trait was not significant. Four of six gene-by-environment interactions were significant, resulting from differences in the magnitude of the effect size. Four studies analyzed the correlation between GPC and TKW and one study found a significant negative correlation (Table S2).

Test weight (TW) was quantified in seven studies ($n = 32$). In 63% of the comparisons, no significant differences were observed, but in 34% of comparisons, the functional *GPC-B1* allele was associated with a significant decrease averaging 15.6 kg m^{-3} (Table S1).

One study described a negative correlation between GPC and TKW hypothesizing that the reduction in grain size could, in part, contribute to increases in GPC due to a higher proportion of protein in the grain relative to carbohydrates (Joppa et al., 1997). However, in the studies reviewed here, decreases in TKW were not always proportional with GPC. In one study, lines with the *GPC-B1* functional allele showed significant increases in GPC but mild decreases in TKW (Brevis and Dubcovsky, 2010). One possible explanation for this correlation is that the rate of N and carbohydrate uptake to the grains is influenced by differences in the genetic background.

Spike number was analyzed in six studies ($n = 53$); 38% of the comparisons showed significant increases in lines carrying the functional *GPC-B1* allele while the remaining 62% of comparisons showed no significant differences between genotypes (Table S1). One experiment quantified spike density and found the wild-type *GPC-B1* allele to be associated with increases of 53.1 spikes m^{-2} (Tabbita et al., 2013). In two studies, spike number was quantified per plant and lines carrying the wild-type *GPC-B1* allele exhibited an average increase of 4.5 spikes per plant (Vishwakarma et al., 2016, 2014). Spikelets per spike were analyzed in four studies ($n = 47$), 47% of the comparisons showed no significant differences while the functional *GPC-B1* allele was associated with significant increases of 4.5 spikelets per spike in 17% of comparisons and significant decreases of 5.5 spikelets per spike in 36% of the comparisons (Table S1). All significant increases or decreases reported were derived from two studies of Indian cultivars (Vishwakarma et al., 2016, 2014). Finally, grains $spike^{-1}$ was measured in five studies ($n = 32$, Table S1); 72% of the comparisons between lines carrying the functional and non-functional *GPC-B1* allele showed no significant differences; the remaining 28% showed an average increase of 5.7 grains $spike^{-1}$ associated with the wild-type *GPC-B1* allele (Mishra et al., 2015). These observations of individual yield components suggest that the overall neutral effect of the wild-type *GPC-B1* allele on yield may arise from increases in spike number compensating for decreases in TKW.

4. Effects of *GPC-B1* on phenology and physiology

Yield and quality components can also be affected by phenology, particularly by heading date and the onset of senescence. Because *GPC1* expression is low before anthesis (Uauy et al., 2006b), *GPC-B1* allelic variation is expected to have minimal impact during pre-anthesis phases of development. Consistent with this hypothesis, *GPC-B1* allelic variation had no significant effect on time to anthesis in 88% of the comparisons between genotypes (eleven studies, $n = 51$, Table 2).

Senescence was accelerated in 32% of the lines carrying the wild-type *GPC-B1* allele (ten studies, $n = 60$) while 47% of comparisons showed no significant effect. Is important to note that senescence values correspond to a mixture of protocols for measuring this trait, namely onset of visual senescence, 50% senescence and complete peduncle yellowing. Unexpectedly, 21% of

comparisons showed a significant delay in the onset of senescence by an average of 4.5 days associated with the wild-type *GPC-B1* allele (all from Indian origin, Vishwakarma et al., 2014, Table S1). These results are inconsistent with experiments using *GPC*::RNAi transgenic lines and *GPC1* TILLING mutants to characterize the role of this gene, which demonstrated that *GPC1* plays a role in promoting the onset of senescence (Avni et al., 2014; Pearce et al., 2014; Uauy et al., 2006b).

Within wheat species, the *GPC-B1* allele was associated with accelerated senescence in all durum wheat lines, while only one-quarter of bread wheat lines carrying the wild-type *GPC-B1* allele exhibited accelerated senescence (Table 2).

Height was quantified in five studies in bread wheat ($n = 63$): 33% of lines carrying the functional *GPC-B1* allele exhibited an increase of 3.8 cm, while 29% of lines with this allele exhibited a decrease of 7.6 cm (Table S1).

The more rapid onset of senescence associated with the functional *GPC-B1* allele is expected to shorten the grain filling period which may be indirectly responsible for reduced TKW. In two studies, reduced TKW was associated with accelerated senescence; six lines showed an average reduction in TKW of 3 g and an acceleration in the onset of senescence of an average 2.6 days (Carter et al., 2012; Uauy et al., 2006a) (Table S1). It is also possible that an increased number of grains per surface area resulting from a higher number of tillers may contribute to the negative effect of *GPC-B1* on TKW. If the number of grains increase, the same amount of assimilates will be distributed in more sinks, causing an overall reduction in carbohydrate distribution in each grain, reducing the grain size in lines carrying the wild-type *GPC-B1* allele (Fischer, 2011). Many environmental factors affect tillering, including nitrogen supply, which can impact the plant's cytokinin levels (Liu et al., 2011; Sakakibara, 2006; Wang and Below, 1996). Because *GPC-B1* affects N metabolism, this may indirectly influence tiller production (Tabbita et al., 2013).

Contrary to these findings, a more recent study has shown that starch content in the grain is limited by grain filling capacity rather than the duration of photosynthesis (Borrill et al., 2015). Despite their longer periods of photosynthesis, *GPC*::RNAi plants accumulated the same levels of starch in their grain and had similar grain weight to control lines. The authors conclude that for starch content in the wheat grain, sink capacity is a more likely limiting factor than duration of photosynthesis (Borrill et al., 2015).

Expression studies consistently show that *GPC-B1* is expressed only after anthesis (Avni et al., 2014; Borrill et al., 2016; Cantu et al., 2011; Pearce et al., 2015, 2014; Uauy et al., 2006a, b). Therefore, the effect of *GPC-B1* allelic variation on spike and tiller number, two traits which are determined before the onset of anthesis (González et al., 2011; Slafer et al., 2009), is unexpected. It is possible that these effects may result from the expression of *GPC-B1* in tissues and/or developmental timepoints which have yet to be studied. Further investigation into the effect of *GPC-B1* on these traits is warranted.

5. Effects of *GPC-B1* on bread-making and pasta quality

Several reports have described beneficial effects of the wild-type *GPC-B1* allele on bread- and pasta-making quality (Brevis and Dubcovsky, 2010; Brevis et al., 2010; Kovacs et al., 1998; Kuhn et al., 2016; Mesfin et al., 2000). In hexaploid bread wheat (Table S3), flour protein concentration was analyzed in one study ($n = 6$) and one line carrying the wild-type *GPC-B1* allele showed a significant increase of 7.5 g kg^{-1} for this trait compared to the non-functional allele. Mixograph water absorption was analyzed in two studies ($n = 8$) and 88% of lines carrying the wild-type *GPC-B1* allele showed significant increases of an average 15.1 g kg^{-1}. Increases in bread-baking water absorption ($n = 6$) were found in 67% of the lines with the wild-type *GPC-B1* allele, averaging an increase of 19 g kg^{-1}. Loaf volume was analyzed in three studies ($n = 12$) and 25% of the lines with the wild-type *GPC-B1* allele showed a significant increase of 98.3 ml compared with lines carrying the non-functional allele. The functional *GPC-B1* allele also had a positive effect on mixing time in two studies; 70% of the lines with the functional *GPC-B1* allele ($n = 10$) showed a significant increase of 0.7 min. These general increases may arise from the positive correlation between GPC and water absorption and between GPC and mixing properties which were previously reported (Brevis et al., 2010; Juhász et al., 2015; Souza et al., 2004). In three studies, flour yield (FYLD) showed a more heterogeneous response across genotypes ($n = 12$). The wild-type *GPC-B1* allele was associated with reductions of 13.3 g kg^{-1} in 25% of the comparisons and with increases of 1.3 g kg^{-1} in 17% of the comparisons. Two studies showed that the functional *GPC-B1* allele had only minimal effects on flour ash concentration, milling score, grain hardness and break flour yield (Table S3).

In tetraploid durum wheat the effect of *GPC-B1* on pasta quality has been reported (Table S4). The wild-type *GPC-B1* allele was associated with significant increases in sedimentation volume, mixograph total energy and cooked pasta disc viscoelasticity (Kovacs et al., 1998). In another study, thirteen different pasta quality traits were measured in the durum variety UC1113 and Kronos NILs for the *GPC-B1* allele in two different environments (Davis and El Centro, California) (Brevis et al., 2010). Overall, the wild-type *GPC-B1* allele had a positive impact in semolina protein content (increased 16 g kg^{-1}), wet gluten (increased 50 g kg^{-1}), mixing time and peak height (increased 0.65 min and 1.3 cm respectively), cooked firmness (increased 0.9 g cm) and cooking loss (decreased 5 g kg^{-1}). Mixogram height, mixogram width and semolina color also increased because of the functional *GPC-B1* allele. Two traits, gluten index and spaghetti color, were not significantly affected by variation at the *GPC-B1* locus. The wild-type *GPC-B1* allele was associated with negative effects on grain ash concentration and semolina ash concentration with increases of 0.9 g kg^{-1} and 0.4 g kg^{-1} "respectively" for both analyzed cultivars.

The lines used in this study showed large differences in maturity between environments, which may be correlated with differences in quality (Brevis et al., 2010). In the study performed in El Centro, smaller differences in senescence were observed between genotypes than the study performed in Davis. However, a number of quality traits exhibited significantly larger differences in El Centro than in Davis (including GPC; +7.5 and +5.7 g kg^{-1} respectively) suggesting that the effects of *GPC-B1* on quality are not directly associated with changes in senescence.

Several undesirable effects in some genetic backgrounds were associated with the wild-type *GPC-B1* allele. Because of the reduction on TKW and TW some lines presented significant decreases in FYLD and increases in flour and semolina ash concentration. However, decreased grain weight is not always correlated with lower extraction rates or increased ash content (Breseghello and Sorrells, 2007). Significant decreases in TW associated with the wild-type *GPC-B1* allele have been reported which did not affect flour extraction (Mesfin et al., 2000). Another population in this study showed no differences in TW but did show significant increases in flour extraction in lines with the wild-type *GPC-B1* allele.

6. Considerations for the application of *GPC-B1* in wheat breeding

Several breeding programs have begun introgressing the functional *GPC-B1* allele into elite material and 18 commercial varieties

carrying this allele have already been released in different wheat markets (Table S5). Protocols and recommendations for *GPC-B1* introgression in durum and bread wheat cultivars using MAS are provided on MASWheat web (http://maswheat.ucdavis.edu/protocols/HGPC/index.htm).

The data summarized in this review show that the impact of the wild-type *GPC-B1* allele on several of the measured traits is proportionally greater in tetraploid durum wheat than in hexaploid bread wheat. This effect can likely be explained by gene dosage (Brevis and Dubcovsky, 2010). Tetraploid varieties carrying the non-functional *GPC-B1* allele carry only one functional *GPC1* gene (*GPC-A1*), whereas hexaploid varieties carry two (*GPC-A1* and *GPC-D1*), meaning that the introgression of a functional *GPC-B1* allele into tetraploid wheat would be expected to have a proportionally greater impact in tetraploid than in hexaploid wheat.

This data also highlights the negative pleiotropic effects that the functional *GPC-B1* allele can have in some genetic backgrounds and environments. GPC is a quantitative trait (Kulwal et al., 2005; Kuspira and Unrau, 1957; Law et al., 1978; Snape et al., 1996; Stein et al., 1992) and the wildtype *GPC-B1* allele exhibits significant interactions with genotype and environment. Consequently, adequate genotype and environment combinations should be identified during breeding and MAS programs for a positive cost-benefit balance (Brevis and Dubcovsky, 2010). When introgressing the functional *GPC-B1* allele into elite varieties, breeders should consider the complex pleiotropic effects on grain size, FYLD and ash concentration described in this review to reduce the negative impact of the *GPC-B1* allele on these traits.

Different strategies can be applied to ameliorate some of the negative effects of the wild-type *GPC-B1* allele in wheat breeding. One example is the use of MAS to select for the *GPC-B1* allele while simultaneously applying phenotypic selection for other yield and quality traits. This approach was applied in a series of multi-location field trials and generated several derived progenies exhibiting significantly higher GPC without a yield penalty (Kumar et al., 2011). Another possible strategy is to deploy the wild-type *GPC-B1* allele in combination with other maturity alleles or QTLs related to yield components. For example, one study describes the use of the wild-type *GPC-B1* allele in combination with a QTL conferring increased TKW. All fifteen of the lines with this allelic combination exhibited significantly increased levels of GPC, Fe and Zn and 12 lines also showed a significant increase in TKW compared to control lines with the non-favorable alleles (Vishwakarma et al., 2016).

An alternative strategy to improve wheat grain quality will be to target the functional pathways acting downstream of *GPC1*. Progress has been made in other crop species towards understanding nutrient translocation by manipulating genes involved in micronutrient transport using genetic engineering. Transgenic rice lines overexpressing the phytosiderophore biosynthesis genes encoding nicotianamine synthase and nicotianamine aminotransferase accumulate higher concentrations of Fe and Zn in their grains (Johnson et al., 2011; Lee et al., 2011, 2009a; Masuda et al., 2013, 2009). Phytosiderophores are chelators of Fe and Zn ions and appear to be a limiting factor for their transport since they are required to maintain ion solubility in the alkaline environment of the phloem (Takagi et al., 2008; Takahashi, 2003). Therefore, phytosiderophore biosynthesis genes are attractive targets for biofortification strategies in wheat (White and Broadley, 2011). The overexpression of membrane-bound transporters (*OsYSL15, OsYSL2* and *HvZIP7*, Ishimaru et al., 2010; Lee et al., 2009b; Tiong et al., 2014) and genes encoding the Fe storage protein FERRITIN (*SoyferH1, SoyferH2+, OsFer2 and OsFerC* genes) also result in increased Fe and Zn content in cereal grains (Goto et al., 1999; Oliva et al., 2014; Paul et al., 2014, 2012; Vasconcelos et al., 2003). Conversely, knockout mutants of *OsVIT1* and *OsVIT2* genes resulted in significant increases in Fe and Zn in rice (Zhang et al., 2012). The wheat orthologs of many of these transporters, including *TaFRO*, *TaHMA*, *TaNRAMP* and *TaZIFL* have been identified and represent promising targets to improve wheat grain quality, either through transgenic manipulation or screening for natural allelic variation among wild relatives of wheat (Borrill et al., 2014; Pearce et al., 2014).

7. Conclusions

In summary, ten years of studies show that the wild-type *GPC-B1* allele is associated with significant positive increases in GPC, Fe and Zn content in the grain in a wide variety of genetic backgrounds and environments, as well as benefits in bread- and pasta-making quality traits. Despite minor negative effects on individual yield components, the impact on yield *per se* are, in many environments and genetic backgrounds, negligible. Overall reductions in yield were found in only four lines with the functional *GPC-B1* allele (of ninety-eight in total). Because the functional wild-type allele derived from emmer wheat is rare among modern cultivars (Table 1), its introgression into breeding programs has great potential to confer significant improvements in wheat grain quality in a wide range of germplasm. Notably, the effects of the functional *GPC-B1* allele have not yet been described in varieties adapted to either European or African environments. The high prevalence of malnutrition in Africa means the introgression of the functional *GPC-B1* allele could have a significant impact on public health in this region.

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Competing interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jcs.2017.01.003.

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