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Short Communication

Gpc-B1 mediated grain protein enhancement in wheat is compatible with high grain weight at moderated yield thresholds

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Abstract

A set of 94 BC_2F_7 lines (BC RILs), selected for presence of *Gpc-B1* gene from the cross Glupro/3*PBW568 were evaluated for morpho-physiological characters including yield and its components, grain protein content, grain zinc and iron concentration for two seasons. *Gpc-B1* mediated grain protein enhancement as influenced by combination of other agronomic traits was studied through inter-trait correlations. Grain protein content was positively correlated with plant height (r=0.552, 0.676**) and 1000-grain weight (r=0.257**, 0.287**) in both years. Lines with high yield conferring combinations of yield components showed lower grain protein content. The results showed that there were no constraints for combining grain weight with *Gpc-B1* mediated proteins enhancement provided yield moderation can be exercised. Grain protein contents showed significant and positive correlation with grain zinc (0.557**, 0.764**) and iron concentration (0.542**, 0.595**) indicating that *Gpc-B1* mediated enhancement in protein content and not just presence of *Gpc-B1* was necessary. Zinc and iron concentration were also positively associated with each other (0.567**, 0.648**).**

Key words: *Triticum aestivum*, back cross RILs, inter-trait correlations, nutritional quality, grain zinc and iron

Grain protein content (GPC) is among the most important factors determining the processing and nutritional quality of wheat. The selection for high GPC in conventional breeding programmes, however, is not very effective because of the greater influence of environment on this trait and an often encountered negative correlation with yield (Barneix 2007). *Gpc-B1* is tightly linked (0.3 cM) to *Yr36*, a gene conferring high-temperature adult-plant (HTAP) resistance against stripe rust (Fu et al. 2009). These genes have been cloned and extensively characterized (Uauy et al. 2006) leading to development of a suite of perfect molecular markers for marker assisted selection. The locus encodes a NAC transcription factor (*NAM-B1*) that contributes to enhanced grain concentration of protein and micronutrients, by accelerating leaf senescence and thus hiking up mobilization of amino acids, zinc, iron, etc., from leaves into seeds. A major trend in these studies is however, represented by decrease in grain weight. Mesfin et al. (1999) showed a significant decrease in test weight associated with the presence of the *Triticum dicoccoides* 6B introgression in one population. The presence of the *Gpc-B1 T. dicoccoides* allele was associated with 6-10% increases in GPC and 3-4% decreases in TGW. The present study is based on diverse, recombinant set of lines, all of them carrying *Gpc-B1* gene. The objective was to show how the expression of the gene measured in terms of grain protein content is modulated in response to combination of morpho-physiological attributes, thus bringing out the opportunities and constraints related to use of *Gpc-B1* in wheat breeding.

Backcross recombinant inbred lines for *Gpc-B1* were developed from the cross of *Gpc-B1* carrying line Glupro (Columbus/*T. turgidum* var*. dicoccoides*//

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Len) with PBW568. One backcross was performed with PBW568. About 250 randomly chosen BC_1F_1 plants were monitored for presence of *Gpc-B1* and positive plants advanced to BC_1F_2. Five plants from each BC_1F_2 were screened with Xucw 108 (Distelfeld et al. 2006). Most of the progenies yielded at least one plant homozygous for *Gpc-B1*. About 100 such plants emanating from different BC_1F_2 progenies were advanced by single seed descent to BC_1F_7. These were confirmed to be positive for *Gpc-B1* and ninety four bulked lines from this generation were taken up for evaluation in the present study. DNA extraction using CTAB method and molecular marker analysis followed standard procedures (Allen et al. 2006). The field experiments were laid out in a simple lattice design with three replications in 2013-14 and 2014-15 in the Department of Plant Breeding and Genetics at Punjab Agricultural University, Ludhiana. Experimental units consisted of 4 rows of 1.75m length spaced 20 cm apart. Total entries used in this experiment were one hundred including 94 BC-RILs from cross Glupro/ 2* PBW568, recipient line PBW568 and five high yielding varieties as checks. Observations were recorded on days to flowering, plant height, days to maturity, tillers per metre row length, grains per spike, thousand grain weight, grain yield per plot (converted to Kg/ha), grain protein content (%), grain zinc concentration (ppm) and grain iron concentration (ppm). Grain protein content was determined using Infratec1241 grain analyzer supplied by M/S Foss Analytical AB, Sweden.

The range and mean of BC-RILs, parental lines and checks for no. of grains/spike, 1000 grain weight, yield (t/ha) and quality characters such as grain protein concentration, grain zinc and iron concentrations pooled over two years is presented in Fig. 1. Days to heading and maturity in the BC RIL set remain below the Glupro levels though transgression beyond PBW 568 while plant height almost the entire span between the two parents was reflected in the BC RILs. In case of yield and yield components, transgressive segregation seems to be the norm. In case of protein content, the recombinant lines span the wide parental difference (11.97-16.70%), while transgressive segregation is evident for zinc and iron concentrations in the grain. Analysis of variance for genotypic differences was highly significant for all traits and values representing least significant differences are given. The frequency distribution graphs based on 94 BC-RILs (Fig. 1) further substantiate the high level of variation observed for six traits. Additionally, it was revealed a clear bimodal tendency in frequency distribution of plant height and tillers per metre row length. Alleles for *Rht, Vrn* and *Ppd* genes known to govern height, vernalisation response and photoperiod response, respectively in wheat are likely to be divergent in the two parents, and multiple peaks in the frequency distribution may be a manifestation of segregation of major genes or large effect QTL. The distribution of grain protein content also shows the high protein lines forming a small second peak. The mode of BC RIL derivation, wherein each RIL traces back to an independent BC_2F_1 plant ensured a wide spectrum of variation for almost all the traits investigated. The effect of *Gpc-B1*on quality and other characters has largely been studied through NILs and that too in a small number of genetic backgrounds in any one study (Brevis and Dubcovsky 2010; Tabbita et al. 2017). Interplay of *Gpc-B1* with a wide range of trait combinations as explored in this study can address this gap to some extent. Use of an exotic donor Glupro as source of *Gpc-B1* thus proved to be beneficial as it was highly divergent from the locally adapted parent PBW568. The parents, however, had to be kept outside the ambit of character correlations for which 94 BC RILs were used. Glupro, despite being spring wheat, has a duration which is long enough to make it almost un-testable in our conditions and an unbiased assessment of particularly the grain characters is not possible.

All the BC-RILs carry the *Gpc-B1* gene. The protein content across this set however, varies greatly showing a range of 11.86 to 15.87. To what extent is this variation influenced by other traits varying in this set is brought out by the matrix of correlation coefficients obtained separately for years 2013-14 and 2014-15 and given in Table 1. The inter-trait correlation trends for the two years match well in terms of magnitude, direction and statistical significance. The greatest positive influence on protein content is seen to be plant height (r=0.552 and 0.676 in 2013-14 and 2014-15, respectively). Plant height has not been implicated as a factor in higher protein content in case of *Gpc-B1*. The other major positive influence, consistent for both years, on protein content is 1000-grain weight. This is again in contrary to earlier reports on influence of *Gpc-B1* gene on grain weight. Majority of the reports show a reduction in grain weight. Among the more plausible trends observed in this study is the significant and consistent negative correlation of grain protein content with number of grains per spike (–0.230 and –0.640 for year 1 and 2) and grain yield (–0.359 and –0.252).

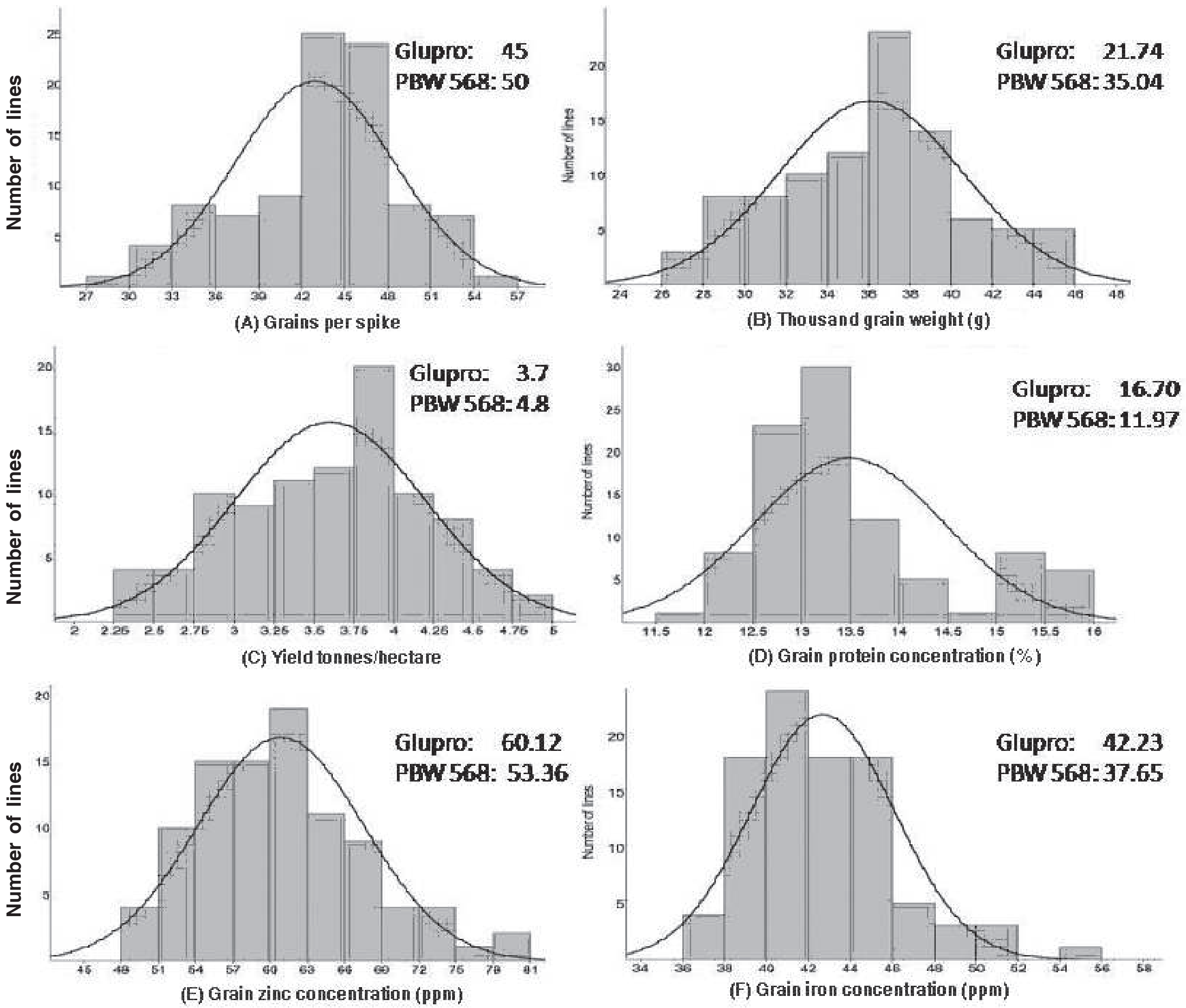

Fig. 1. Frequency distribution of different traits (A to F) along with its expected normal distribution. Parental and check values are also given in each graph

The positive correlation of grain protein and height is largely explained by plant height having a significant, consistent and negative correlation with yield (–0.361 and –0.407) and grains per spike (–0.308 and –0.530) in both years. Plant height is also positively associated with higher grain weight (0.272 in year 2013-14). Correlation coefficients between different agronomical traits with *GPC-B1* positive progenies has been reported earlier (Kaur et al. 2019) where positive influence of agronomic traits on protein content has been demostrated. The 94 RILs are thus seen to split into distinct plant types, possibly on account of major gene/large QTL segregation. Character associations clearly show that one major plant type is represented by tall lines with small ears (fewer number of grains per ear) and bold grains, but low yield. The second major set has dwarf plants with higher grain number per spike and high yield. High grain protein content is associated with former group while the latter category has low protein content. The inference thus is very clear, that negative correlation of yield and grain protein content is the fundamental constraint, while the yield component affected depends upon its relationship with yield in a particular set. In this set, grains per spike are a significant contributor to yield as indicated by significant positive correlation, while 1000-grain weight does not contribute significantly to yield.

Grain protein content shows significant and highly positive correlation with grain zinc (0.557 in 2013-14 and 0.764 in 2014-15) and iron concentration (0.542 in 2013-14 and 0.595 in 2014-15). Other traits such as

Table 1. Phenotypic correlation coefficients among traits recorded on *Gpc-B1* positive BC RILs of Glupro/3* PBW568 cross during 2013-14 and 2014-15 trial

	Days to heading	Plant height	Days to maturity	Tillers no./m length	No. of grains/spike	1000-grain weight	Grain yield	Grain protein content	Grain zinc concentration	Grain iron concentration
Days to heading		−0.070 0.102	0.583** 0.269**	−0.005 0.162	0.073 0.309**	−0.523** −0.337**	−0.138 −0.199*	−0.121 −0.236*	−0.048 −0.238*	−0.135 −0.166
Plant height (cm)			0.128 0.265**	0.070 0.355**	−0.398** −0.530**	0.272** 0.123	−0.361** −0.407**	0.552** 0.676**	0.466** 0.529**	0.531** 0.452**
Days to maturity				0.090 0.263**	0.020 −0.161	−0.125 −0.163	−0.271** −0.096	0.146 0.178	0.203* 0.172	0.126 0.121
Tiller no. per m length					0.004 −0.197*	−0.176 −0.350**	0.086 −0.071	0.114 0.131	0.049 −0.042	0.022 −0.036
No. of grains/spike						−0.203* −0.331**	0.338** 0.185	−0.234* −0.640**	−0.195 −0.528**	−0.285** −0.377**
1000-grain weight (g)							0.053 0.072	0.257** 0.287**	0.237* 0.357**	0.432** 0.273**
Grain yield (t/ha)								−0.359** −0.252*	−0.340** −0.302**	−0.180 −0.128
Grain protein content (%)									0.557** 0.764**	0.542** 0.595**
Grain zinc concentration (ppm)										0.567** 0.648**
Grain iron concentration (ppm)										

*Significant at 5% level of significance, **Significant at 1% level of significance, 2013-14 values in normal font, 2014-15 values in italics font

plant height or grains per spike which are positively or negatively associated with protein content (for whatever reasons) also showed the same trend with micronutrient concentration. Further zinc and iron concentrations were positively associated with each other (0.567 in 2013-14 and 0.648 in 2014-15).

The consistency of correlations over the two seasons clearly indicates that these correlations were driven by fairly large morpho-physiological distinctions that remained stable over the two years. The corroboration of trends across the two seasons allow for more reliable generalizations. A very strong trend was represented by correlation of grain zinc and iron concentration with each other and protein content. This is largely in consonance with several reports on association of grain protein and micronutrient content in wheat (Peleg et al. 2008; Zhao et al. 2009; Velu et al. 2011a, b; Michel et al. 2019). Grain micronutrient content may thus be said to be enhanced essentially by *Gpc-B1* mediated enhancement in protein content and not just by presence of *Gpc-B1* or its action through other means.

As part of practical implications of the study it can be argued that it is possible to combine bold grains and protein content. This is a desirable combination with respect to milling and processing needs of several wheat products. Flat Indian breads have bold grains as a major quality indicator and if this can combine with high protein content, it can serve both processing and nutritional needs. This balance will however require manipulation of number of grains per ear, so as not to encounter the more fundamental constraints posed by high yield. The present material represents a random recombinant set. The empirical results obtained in situations presented by use of *Gpc-B1* in wheat improvement at our centre provide an alternate perspective to the issues discussed here. In our efforts to transfer *Gpc-B1* to released high yielding varieties, ear size or grains per ear tend to be conserved through deliberate or unconscious selection. Grain size is often reduced in these situations. Yield parity, if achieved, is primarily through improvement in tillering ability, provided there is scope to improve this component in a particular material. Sharp increase in grain protein content is generally incompatible with maintaining yield ability of an adapted, productive line. Considering both conditions (this study as well as breeding situations) it is evident that yield moderation may be important for a strong expression of high grain protein conferring ability of *Gpc-B1* gene in any wheat population.

Authors' contribution

Conceptualization of research (NS, VS, CS); Designing of the experiments (NS, VS, CS); Contribution of experimental materials (CS, PS, NS, AS); Execution of field/lab experiments and data collection (CS, PS, KK, VS); Analysis of data and interpretation (CS, PS, HK, AS); Preparation of manuscript (CS, NS, PS, VS).

Declaration

The authors declare no conflict of interest.

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