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Effect of *Gpc-B1* gene on grain protein content and productivity traits in a set of high yielding wheat lines

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Abstract

The effect of *Gpc-B1* in the high yielding background of bread wheat DPW621-50 was studied in yield trials conducted during 2013-14 and 2014-15 at Punjab Agricultural University, Ludhiana by using a set of six gene positive and six gene negative lines derived from the cross DPW621-50//Glupro/3*PBW568/3/DPW621-50. The hard red US spring wheat "Glupro" served as primary donor, which was backcrossed twice to PBW568 to develop a large set of locally adapted lines. A high grain protein derivative with desirable agronomic features served as donor for the current set of lines in the background of DPW621-50. Presence of *Gpc-B1* resulted in significant increase of grain protein content with range of enhancement being 5.2 to 12.5% during 2013-14 and 3.8 to 11.0% during 2014-15. The enhancement in protein content did not show concomitant increase for grain zinc and iron concentration. Comparison of yield showed negative influence of *Gpc-B1* gene as yield was lowered to >25% in *Gpc-B1* lines. The yield reduction was primarily mediated through reduction in 1000-grain weight in the *Gpc-B1* positive lines.

Key words: DPW621-50, protein content, grain zinc & iron concentration, grain yield, yield components

Introduction

High carbohydrate, cereal based diets mostly lack required amount of dietary proteins. In Central Asian, Middle Eastern countries and the Indian Subcontinent wheat provides around 50 percent of the daily energy intake and this proportion can exceed 70 per cent in rural areas (Cakmak 2004). Providing ample food does not answer the hidden hunger, which is a devastating threat to health, education, economic growth and to human dignity in developing countries. According to the estimates made by FAO (www.fao.org), more than 36 percent of the populations in developing countries are undernourished (Mishra et al. 2015). A major ameliorative strategy involves genetic biofortification of staple crops like wheat. Grain protein content and micronutrient content constitutes the primary traits for this purpose. Grain protein content (GPC) is a regular breeding objective in wheat as protein is the basic determinant of wheat processing and nutritional quality. In recent years, widespread deficiencies of micronutrients likes iron and zinc in the diets of human population particularly in the third world has been recognized. Enhancing grain protein has some effect on enhanced levels of other micronutrients as well. This has led to adoption of enhanced grain density for protein (directly) and these micronutrients (indirectly) as breeding criteria. Conventional breeding for grain protein content as well as micronutrient density however, has not been very effective, because of complex genetic control and greater proneness to influence of environmental factors (Simmonds 1995; Loffler and Busch 1982; Lawlor 2002).

It is in this context that *Gpc-B1*, a major genetic factor for grain protein as well as micronutrient density can prove useful. *Gpc-B1* gene is derived from *Triticum turgidum* L. ssp. *dicoccoides*, the wild progenitor of durum wheat (Avivi 1978). The gene *Gpc-B1* has emerged as a gene of interest from a series of chromosomal (Joppa et al. 1997), molecular marker based (Khan et al. 2000; Olmos et al. 2003) and transformation studies (Uauy et al. 2006a). The locus encodes a NAC transcription factor (*NAM-B1*) that contributes to enhanced grain concentration of protein

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and micronutrients, by accelerating leaf senescence and thus remobilization of amino acids, Zn and Fe from flag leaves into seeds.

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 positionally cloned and extensively characterized (Uauy et al. 2006b; Fu et al. 2009) with functional molecular markers available to carry marker assisted selection. Introgressing a single chromosome segment would confer high grain protein, high iron and zinc and resistance to stripe rust, thereby making it valuable for wheat breeding programmes.

The present study explores the utility of *Gpc-B1* in the high yielding background of DPW621-50 (KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES) a bread wheat variety recommended for irrigated, timely sown cultivation practices in North Western Plains Zone of India. The hard red US spring wheat "Glupro" had served as primary donor, which was backcrossed twice to PBW568 resulting in generation of a large set of locally adapted lines. A high grain protein derivative with desirable agronomic features served as donor for the current set of lines in DPW621-50 background. Following intense selection for plant type and yield attributes in segregating phase, fixed lines representing *Gpc-B1* carriers as well as non carriers were evaluated for yield and quality. The study thus investigates the issue of commercial deployment potential of *Gpc-B1* by looking at the quality-yield tradeoffs.

Material and methods

The material used in the present experiment comprised of twelve advanced breeding lines in the background of wheat variety DPW621-50 alongwith standard checks. Six lines carry *Gpc-B1* while others are negative for the gene. These lines were developed in a two-step process, the first being represented by cross of US hard red spring wheat 'Glupro' with locally adapted spring wheat line PBW568. This primary round of introgression involved two backcrosses to PBW568. A high grain protein derivative with desirable agronomic features was identified after quality and yield testing of fixed lines and was crossed with DPW621-50. The parentage of 12 test lines used in this study may thus be represented as "DPW621-50//Glupro/3*PBW568/3/DPW621-50". Initially, a large set of lines were generated in the Wheat Section (BWL and PBW series) and School of Agricultural Biotechnology (SABW series), PAU, Ludhiana. Preliminary yield data was used to shortlist best lines on which marker analysis was carried out using Xucw108 (Uauy et al. 2006). Six best *Gpc-B1* positive and six *Gpc-B1* negative lines were chosen for assessment of yield and quality. Trials were conducted under high fertility conditions having good nitrogen, phosphorus and potash, planted under randomised complete block design with three replications during 2013-14 and 2014-15 crop seasons at Ludhiana, Punjab. Besides 6 *Gpc-B1* positive and 6 negative lines, the trials included three checks represented by recipient parent DPW621-50, WH1105 and HD2967. All the three checks are recommended bread wheat varieties for irrigated, timely sown conditions of North Western Plains Zone of India. The experimental plot size was of 1.2m (6 rows) x 6 meter length each with row to row spacing of 20cm. All the recommended, standard cultural practices were followed to raise the crop.

Grain protein estimation and traits of economic importance

Protein content was estimated using "Infratec1241" grain analyser supplied by M/S Foss Analytical AB, Sweden. The instrument uses near infra-red light transmitted through the grains. The grain samples were scanned in the range of 850 to 1050nm with a bandwidth of 7nm and there were 100 data points per scan. The results were displayed as percent protein content and percent moisture in the grain. The final protein value was standardized for 14% moisture. Observations recorded in the field and laboratory were i.e., thousand grain weight (g), plot yield (kg/ha), grains per spike, plant height (cm), days to heading and maturity and tillers number per meter. Analysis of variance for randomized complete block design was applied by using SAS9.3.

Results

Agronomic and nutritional quality related observations on 6 entries positive for *Gpc-B1* and 6 non *Gpc-B1* entries alongwith recipient parent DPW621-50 and checks WH1105 and HD2967 were recorded for two crop seasons (Tables 1 & 2). Donor parent for *Gpc-B1*, Glupro being exotic, un-adapted line was not included in the experiment. Glupro was grown separately and data was recorded for parental reference only. The analysis of variance was carried out between *Gpc-B1* positive and negative lines (B), as well as comparisons were done over year (A) and between genotypes (C). The mean data for traits along with group means is given in Table 1 and 2 alongwith all

Table 1. Performance of the *Gpc-B1* and non *Gpc-B1* lines during the years 2013-14 and 2014-15

Genotypes	*Gpc-B1*	Heading days		Maturity days		Plant height (cm)		Tillers/meter		Grains/spike	
		2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
SABW226	+	102.00	93.00	148.33	138.00	95.00	93.67	100.67	86.33	55.00	54.67
SABW227	+	99.00	92.00	148.33	138.33	95.33	96.00	102.33	102.00	49.00	48.67
SABW228	+	103.00	93.67	150.33	136.67	93.33	92.67	91.67	94.33	54.00	51.00
BWL3280	+	105.33	97.00	149.33	138.33	91.33	94.33	99.67	87.33	59.67	54.33
BWL3281	+	103.00	96.00	148.67	137.67	91.67	92.33	89.00	101.67	56.00	49.33
BWL3560	+	97.00	91.00	147.67	137.00	93.67	87.33	102.00	103.67	48.00	48.33
Mean		101.56	93.78	148.78	137.67	93.39	92.72	97.56	95.89	53.61	51.06
SABW223	-	102.33	91.33	154.00	135.67	93.00	91.00	98.33	91.67	44.67	48.00
SABW231	-	101.33	94.00	152.00	135.33	89.67	89.67	90.67	96.33	52.00	42.33
SABW234	-	101.67	93.00	150.00	136.00	93.00	92.00	95.33	102.67	46.33	46.67
PBW725	-	102.00	91.00	151.33	137.00	104.00	104.33	93.00	91.00	57.33	49.67
BWL3586	-	96.67	91.33	150.67	137.67	90.67	89.00	84.33	88.67	54.00	49.33
PBW744	-	100.67	91.00	149.67	138.33	101.00	99.00	76.33	90.33	48.00	45.67
Mean		100.78	91.94	151.28	136.67	95.22	94.17	89.67	93.44	50.39	46.94
DPW621-50		104	93.00	150.70	139.30	102.30	104.00	95.00	88.70	56.70	48.70
WH1105		101	90.70	151.00	139.30	94.00	88.00	74.00	80.30	57.00	59.00
HD2967		104	93.00	150.70	137.70	101.3	100.30	91.00	96.30	59.00	52.00
cd Year (A)		0.666		0.791		N/A		N/A		1.769	
Gpc-B1+/- (B)		0.666		N/A		1.202		1.908		1.769	
A x B		N/A		1.118		N/A		2.698		N/A	
Genotype (C)		1.153		N/A		2.082		3.305		3.064	
A x C		1.631		N/A		N/A		4.674		N/A	
B x C		1.631		N/A		2.945		4.674		N/A	
A x B x C		N/A		2.738		N/A		6.61		N/A	

the interactions. Differences for Year, genotypes and affect of *Gpc-B1* were found to be significant for economic traits. The results are being discussed based on their means.

Yield data revealed that non-*Gpc-B1* entries significantly outperformed for grain yield against the *Gpc-B1* lines in both the crop seasons *viz*., 2013-14 and 2014-15 (Table 2). *Gpc-B1* lines showed 23 to 34% yield decrease during 2013-14 and 27 to 34% decline in yield during 2014-15 over parental line DPW621-50. Among the non *Gpc-B1* lines, three of the six lines out-yielded the parent. Non-*Gpc-B1* line, PBW725, gave highest yield in the trials (5925.93 kg/ha during 2013-14 & 5995.37 kg/ha during 2014-15). In *Gpc-B1* positive set, highest yielding lines were SABW227 (3877.32 kg/ha) in 2013-14 and SABW226 (3564.82 kg/ha) during 2014-15. The observations on yield components are likely to explain the yield differences between the two groups. The component which contributed to high yield of non *Gpc-B1* lines was thousand grain weight. *Gpc-B1* lines displayed significantly low grain weight (36.67g & 36.61g) during both the years. This was in contrast to thousand grain weight of 42.72g (during 2013-14) and 40.72g (during 2014-15) registered in the *Gpc-B1* negative set.

Surprisingly, the other two yield components i.e. number of tillers per meter and grain number per spike were superior in *Gpc-B1* lines. The average number of tillers per meter was significantly higher for *Gpc-B1* lines (97.56 and 95.89 during 2013-14 and 2014-15, respectively) as compared to the non *Gpc-B1* lines (89.67and 93.44 tillers per metre). In the *Gpc-B1* group,

Table 2. Performance of the *Gpc-B1* and non *Gpc-B1* lines during the years 2013-14 and 2014-15 and improvement over recipient parent

Genotypes	*Gpc-B1*	1000-grain weight (g)		Yield (kg/ha) and per cent over parent				Protein (%) and per cent increase over parent			
		2013-14	2014-15	2013-14	% inc	2014-15	% inc	2013-14	% inc	2014-15	% inc
SABW226	+	34.00	37.00	3,587.96	-29%	3,564.82	-27%	11.48	8.3%	12.87	3.8%
SABW227	+	38.67	37.00	3,877.32	-23%	3,368.06	-31%	11.85	11.8%	13.39	8.0%
SABW228	+	39.67	39.50	3,692.13	-27%	3,217.59	-34%	11.62	9.6%	13.39	8.0%
BWL3280	+	33.00	33.33	3,321.76	-34%	3,402.78	-30%	11.38	7.4%	13.09	5.5%
BWL3281	+	36.67	32.00	3,518.52	-30%	3,298.61	-32%	11.15	5.2%	13.72	10.6%
BWL3560	+	38.00	39.00	3,842.59	-24%	3,483.80	-28%	11.92	12.5%	13.76	11.0%
Mean		**36.67**	**36.31**	**3640.05**	-28%	**3389.28**	-30%	**11.57**	9.1%	**13.37**	7.8%
SABW223	-	36.33	37.33	4,814.82	-5%	3,657.41	-25%	11.11	4.8%	12.27	-1.0%
SABW231	-	42.67	38.67	3,958.33	-22%	3,483.80	-28%	10.92	3.0%	12.14	-2.1%
SABW234	-	38.00	38.67	3,969.91	-21%	3,842.59	-21%	10.59	-0.1%	12.15	-2.0%
PBW725	-	46.67	42.00	5,925.93	17%	5,995.37	23%	10.54	-0.5%	12.76	2.9%
BWL3586	-	45.33	42.67	5,324.07	6%	5,055.56	4%	10.34	-2.5%	12.06	-2.8%
PBW744	-	47.33	45.00	5,578.70	11%	5,750.00	18%	10.29	-3.0%	12.68	2.2%
Mean		**42.72**	**40.72**	**4928.63**	-2%	**4630.79**	-5%	**10.63**	0.3%	**12.34**	-0.5%
DPW621-50		56.7	48.70	5046.30		4861.10		10.60		12.40	
WH1105		57	59.00	5185.20	3%	5324.10	10%	10.00	-5.7%	11.40	-8.1%
HD2967		59	52.00	5180.60	3%	4449.10	-8%	10.30	-2.8%	11.90	-4.0%
cd Year (A)		1.146		115.502				0.198			
Gpc-B1+/- (B)		1.146		115.502				0.198			
A x B		N/A		N/A				N/A			
Genotype (C)		1.984		200.055				N/A			
A x C		N/A		282.921				0.484			
B x C		2.806		282.921				N/A			
A x B x C		N/A		400.11				N/A			

% inc= Per cent increase

grain numbers per spike was also significantly higher in both the years (53.61 and 51.06). This superiority of *Gpc-B1* lines was however; completely negated by their consistently low thousand grain weight. The lines on the average were almost a week earlier in flowering during 2014-15 (93.78 & 91.94 days) as compared to 2013-14. *Gpc-B1* positive lines were late by a day or two in flowering as compared to non *Gpc-B1* lines. The two sets were however at par in days to maturity. Plant height in *Gpc-B1* group was found to be few centimetres short than the non-*Gpc-B1* set. PBW725 having 104cm average plant height during both the years was tallest genotype and similar to parent DPW621-50 for the trait. None of these traits seemed to affect yield in a particular direction.

The effect of the *Gpc-B1* gene on the protein content was quite evident as all *Gpc-B1* positive lines showed significantly higher grain protein. The average grain protein content in *Gpc-B1* set was 11.57 % (2013-14) and 13.37% (2014-15) as compared to the non-*Gpc-B1* set (10.6 % & 12.4%). The grain protein content of non *Gpc-B1* set was at par with the parent DPW621-50. Maximum improvement for grain protein was observed in *Gpc-B1* positive line BWL3560 which showed enhancement to the tune of 12.5% and 11.0% over DPW621-50 during 2013-14 and 2014-15, respectively. Donor parent, Glupro, gave 16.71% grain protein content with 21.74g thousand grain weight and 3725kg/ha yields.

Discussion

In this set of derivatives, protein levels tended to be modest (11-13%) but percent increase over the recipient was impressive. The effect of *Gpc-B1* depends on both environment and genetic background, and has been reported to give an average increase in grain protein concentration of approximately 10% in tetraploid wheat's and 6% in hexaploid wheat's (Brevis and Dubcovsky 2010). In the present study the extent of protein enhancement obtained was higher than expected (e.g., 12.5% and 11% during 2013-14 and 2014-15, respectively, for eg. in BWL3560).

Over both the seasons, the best yielding lines were invariably *Gpc-B1* non carriers. This negative association of yield and protein content has been earlier established by many authors viz. Austin et al. 1980, Masclaux et al. 2001, Lawlor 2002, Triboi and Triboi-Blondel 2002, Groos et al. 2003, Gonzalez-Hernandez et al. 2004 and Barneix 2007, Tabbita et al. 2017. Use of *Gpc-B1* for protein enhancement has been shown to be non-detrimental to yield by several researchers. (Oury et al. 2006; Morgounov et al. 2007; Vishwakarma et al. 2016). Most of these studies however, reported a reduction in thousand grain weight. This reduction was seen to be compensated by other yield components. The present study also shows improved grains per ear and tiller number, but a point of complete compensation was not reached. The highest yielding derivative, PBW725, though negative for *Gpc-B1* and its linked stripe rust resistance gene *Yr 36*, showed complete resistance to stripe rust and is a now a released cultivar having 14.15% area under its cultivation in Punjab (personal communication). BWL3560 is available as *Gpc-B1* carrying, high grain protein and medium bold grained stock for breeding purpose.

In conclusion, results indicated that *Gpc-B1* gene in DPW621-50 background moderately enhanced grain protein. Thus we observed that *Gpc-B1* gene must be allowed sufficient flexibility in productivity trait combinations for manifestation of protein and grain enhancement effects. Balancing yield and quality together in a wheat cultivar is tedious task and can be achieved through some inter trait tradeoffs.

Authors' contribution

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

Declaration

The authors declare no conflict of interest.

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