



RESEARCH ARTICLE

Assessment of *Gpc-B1/Yr36* introgressions in a popular Indian wheat (*Triticum aestivum* L.) cultivar PBW550 for grain yield and quality traits

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Abstract

The impact of the *GpcB1/Yr36* gene on agronomic attributes, grain quality parameters, and stripe rust resistance was studied in the background of the popular Indian wheat cultivar PBW550. Introgression lines SABW159 showed 21.01% enhancement in protein with no yield penalty and stripe rust-resistant reaction (10s). Similarly, SABW141 registered a 14.19% increase in protein content along with a 6.17 % yield advantage over PBW550 and the terminal disease reaction of 10S. The corresponding disease score for PBW550 was 80S. Similar trends for improvement across all three traits, viz. grain yield, GPC, and stripe rust resistance, were observed for several introgression lines, and no single yield component showed a reduction on account of enhanced protein.

Keywords: Grain protein content, yield, *Yr15*, stripe rust resistance, *Triticum aestivum*

Introduction

In bread wheat, grain protein content (GPC) is an important grain quality trait, which determines nutritional value, processing properties, quality of the end products (bread and pasta) and market value of the grain. Breeding efforts aimed at genetic improvement of grain protein content are generally perceived to result in lowering of grain yield (Simmonds, 1995). Efforts to increase GPC have also been hindered by high environmental influence and complex genetic control (Simmonds, 1995; Löffler and Busch 1982; Lawlor 2002). Identification of a single large-effect gene, *Gpc-B1*, in wheat, representing a relatively simple genetic control, is likely to help resolve the above issues (Uauy et al. 2005). It is a cloned, well-characterized gene that can be mobilized easily to understand its effect in isogenic backgrounds. In the present study its effect has been studied in the background of a popular Indian wheat cultivar PBW550. This variety serves a population largely dependent on cereals for calories as well as protein in their diet. Thus *Gpc-B1* can play an important nutritional role provided significant enhancement in protein content can be achieved without incurring serious yield reduction.

GpcB1 gene carrying chromosome segment also harbors high temperature induced adult plant resistance (HTAPR) gene, *Yr36* (Fu et al. 2009) against stripe rust of wheat. Stripe rust (*Puccinia striiformis* f. sp. *tritici*) is the most

damaging disease of wheat and is causing significant losses on susceptible wheat cultivars worldwide (Wellings 2011). The deployment zone of PBW550 in India overlaps with a vast stripe rust-prone tract along the Himalayan foothills spanning several states. The resistance to stripe rust in this variety has already succumbed to the widely prevalent

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pathotype possessing virulence to the *Yr27* gene. Thus, the present study was planned to assess the effect of the *GpcB1/Yr36* gene on agronomic attributes, grain quality traits, and stripe rust resistance in the genetic background of wheat cultivar PBW 550. A relatively smaller set of *Yr15* lines in PBW550 background was also included in the study to serve as benchmarks along with recipient variety PBW550 to help highlight the multi-trait impact of *Gpc-B1/Yr36*.

Materials and methods

Donor parents

A hard red US winter wheat Glupro was used as a source of the *Gpc-B1/Yr36* gene originally derived from *Triticum dicoccoides* accession FA15-3(Avivi 1978) for introgressing in PBW550. In a parallel effort Avocet isogenic line carrying *Yr15* gene derived from *Triticum dicoccoides* accession G-25 (Gerechter-Amitai et al. 1989) was introgressed in PBW550. This introgression programme was carried out at Punjab Agricultural University, Ludhiana, India

Scheme for development of introgression lines

The elite variety PBW550 was crossed with Avocet isogenic line carrying *Yr15* (Avocet+*Yr15*) and backcrossed twice with PBW550 to sufficiently recover the genetic background of PBW550. BC_2F_4 introgression lines were derived under intense selection for plant type and stripe rust resistance. In a parallel effort, PBW550 was crossed with the winter wheat variety Glupro (carrying *T. dicoccoides* segment harboring *Yr36* and *Gpc-B1*). BC_2F_4 introgression lines were developed in the same manner as indicated above. Two generations were taken per year with the help of summer planting in high altitude zone (PAU Offseason Research Station at Keylong, Himachal Pradesh). In both introgression streams, stripe rust epiphytotic based on pathotype 78S84 was employed at the main season. Selection for *Yr15* in the segregating phase was largely based on resistance to stripe rust. Markers were used for introgressing *Yr36/Gpc-B1*, as the conferred resistance is temperature dependent and misclassification is likely. Several hundred progenies were generated and screened at each backcross and selfing step to ensure a good probability of desirable segregants. Bulking of desirable lines was carried out in BC_2F_4 . A small set was finally chosen for the replicated trial after grain selection and marker-based gene assay. The markers used for screening the target genes *Yr15* and *Gpc-B1/Yr36* were *Xgwm498* (Yavin et al. 2015) and *Xucw130* (Uauy et al. 2005). Gene-positive and agronomically desirable lines were chosen for further evaluation in replicated trials.

Evaluation of introgression lines and data analysis

The evaluation set generated comprised 24 $BC_2F_{4;5}$ introgressions for genes *Gpc-B1/Yr36* and other five lines carrying *Yr15* in PBW 550 background. These were evaluated

in a randomized block design with three replications at Punjab Agricultural University, Ludhiana. The introgression lines and recipient cultivar PBW 550 were grown in 3.6 m² plots. Standard agronomic practices were followed for raising the crop. The trial was protected from stripe rust infection with a foliar spray of propiconazole (0.1%) at maximum tillering and anthesis stages to obtain an unbiased comparison of introgression lines with the susceptible recipient parent for agronomic traits. The productivity trial was conducted over two years.

Data were recorded on days to heading, plant height (cm), number of tillers per meter, number of grains per spike, thousand-grain weight (g), and grain yield per plot (g). The data on grain yield per plot(g) was converted to kg/ha. For the trial conducted in the 2014-15 crop season, the following quality parameters, including protein content (%), test weight (kg/hl), grain hardness (kg), Grain appearance (score out of 10), and sedimentation value(cc) were recorded. Protein content was determined using "Infratec1241" grain analyser (M/S Foss Analytical AB, Sweden). The sodium dodecyl sulfate (SDS) method was used for calculating the sedimentation value of the whole meal. The grain hardness was measured by using the grain hardness tester (M/S Ogawa Seiki Co. Ltd., Japan) by crushing randomly taken ten grains one by one, considering the weight, diameter and moisture of the grain. The mean force (kg) required to crush the grain was recorded. The grain appearance score was evaluated subjectively based on the size, shape, color, and luster of the grain scored out of 10. Test weight was measured using standard container of 100 ml capacity (Mishra et al. 1998). The grains were weighed, and the test weight was expressed in Kg/hl. The analysis of variance, means, least significant differences, and correlation analysis were computed using SAS ver 9.3.

Screening for stripe rust resistance

The entire set of lines evaluated in the second season was planted in a disease-screening nursery. The stripe rust disease scoring based on percent infection on leaf was recorded as traces Ts, 5S, 10S, 20S, 40S, 60S, and 80S as per modified Cobb's scale as given by Peterson et al. (1948). Disease scoring was done at three stages: maximum tillering, booting, and initiation of grain filling.

Results and discussion

Evaluation for agronomic traits

The analysis of variance revealed that genotypic differences were significant for yield, days to heading, plant height, number of grains per spike, and thousand-grain weight. Genotypes and years of interaction were found to be significant for yield, grains per spike, number of tillers, and days to heading. Out of 24 lines carrying *Gpc-B1/Yr36* introgression, 23 lines yielded at par with the parent PBW550

(Table 1). In terms of percent increase/decrease over the recipient parent a range of 12.47 to -14.11% was observed. Highest-yielding cluster of lines in the trial consisted of *Yr15* introgression. Unlike *GpcB1/Yr36* introgressions where no lines exceeded the recipient, four of the *Yr15* introgressions out yielded PBW550. The yield trial in both years was protected with systemic fungicide propoacanazole(0.1%), and yield differences seem to accrue from inherent genetic differences. However, a low threshold of disease occurrence (10S) could not be prevented. It is possible that the *Gpc-B1/Yr36* introgression lines, on account of their lowered protection in the early growth stages, may have been at a disadvantage. This difference is likely to affect their comparison with *Yr15* introgression, possessing all-stage protection, but not with PBW550, which is susceptible. Though only one line shows significant decreases in yield over PBW550, there are several other lines showing varying levels of percentage reduction in yield among the *Gpc-B1/Yr36* subset. A yield ceiling imposed by *Gpc-B1/Yr36* is indicated. Multilocation evaluation of these lines with larger plot sizes is likely to clarify further the influence of introgressed genes on yield. In the present study, however, the relative contribution of yield component traits can indicate the basis of yield difference in the two introgression groups. The relatively higher yield of the *Yr15* carrying subset is partly explained by the data above having more average grains per spike, slightly higher tillers per meter, and thousand-grain weight at par with PBW550.

The donor(s) from which the genes were introgressed were greatly divergent from the recipient in terms of their adaptation, growth habits, and other characteristics. In spite of intensive selection towards recipient plant type, considerable variation was observed for morphological traits. In case of days to heading and plant height, derivatives close to the recipient are generally preferable. With respect to other yield components, favorable variations may be capitalized on the evaluated set, thus representing the yield equivalence or superiority over PBW550. It merits further evaluation as a replacement candidate for PBW550, providing significant improvement in nutritional quality and better rust resistance. These two aspects are taken up in the sections that follow.

Evaluation of quality traits

Genotypic differences for all the quality traits studied, viz., grain protein content (%), test weight, grain hardness, grain appearance, and sedimentation value, were significant. A clear impact of the *Gpc-B1* gene on grain protein content was observed. Fifty percent of the *Gpc-B1/Yr36* lines (12 of 24 evaluated) showed significant superiority for protein content (Table 2). In the case of *Yr36/Gpc-B1*, an improvement in grain protein content of about twenty percent was observed in SABW143 and SABW159. In the case of *Yr15*, none of the introgression lines deviated from the recipient

parent with regard to grain protein content. Tabbita et al. (2013) reported that when the *Gpc-B1* gene was introgressed into germplasm, it consistently increased GPC across environments, ranging from 3.6 to 9.9 g/kg.

Brevis and Dubcovsky (2010) compared and reported near isogenic lines (NILs) with contrasting *Gpc-B1* alleles in tetraploid and hexaploid wheat and showed that the functional *Gpc-B1* is associated with increases in both protein content and total protein yield. The recipient parent PBW 550 is known for its superior test weight and thereby good flour recovery. A striking trend of reduced test weight in *Gpc-B1/Yr36* lines is observed.

On the other hand, all the lines carrying *Yr15* showed parity with PBW550. PBW 550 possesses very hard grain, and several derivative lines from both sets are able to match it for hardness. However, a few lines with *Yr36/Gpc-B1* (SABW161, SABW148) tend to be significantly softer. There was a wider range for hardness in *Gpc-B1/Yr36* lines. Grain hardness is a primary attribute for superior chapatti quality, for which PBW 550 is known to excel. There is an indication that high grain protein and grain hardness (along with chapatti quality) can be combined. Traditionally, the superior chapatti quality landraces typically possess moderate protein content (Mishra et al. 1998). However, nutritional considerations demand that the protein content of wheat varieties meant for chapatti making should receive priority.

Grain appearance score is based on luster besides shape, size, and color and, therefore, tends to be associated with grain hardness. Sedimentation is by and large improved in the *Gpc-B1/Yr36* lines and augers well for processing quality. Unexpectedly, however, similar improvement is also evident for some of the *Yr15* introgressions. Besides the *Gpc-B1* locus which can influence sedimentation value through enhanced grain protein content, the donor lines are expected to contribute other minor genes for sedimentation trait. Having discussed productivity and quality parameters separately, it is important to understand how grain yield relates to the quality parameters and the possibility of generating desirable germplasm combining both these aspects. The correlations (Table 3) between quality traits and grain yield offer important insights in this regard. The grain protein content is negatively associated with test weight, grain appearance score, and grain yield. This relationship is further reiterated by the positive correlation of grain yield with test weight and grain appearance. Thousand-grain weight, grain hardness, and sedimentation value remain neutral in this matrix of correlation. In spite of a negative correlation between protein content and yield, a large number of *Gpc-B1/Yr36* derivatives have higher protein and yield as compared to recipient PBW550.

Introgression lines like SABW159 showed 21.01% enhanced protein with no yield penalty, and SABW 141 registered a 14.19% increase in protein content along with a 6.17% yield advantage over PBW550. A similar trend

Table 1. Performance of introgression lines and check evaluated for agronomic traits over two years

Genotype	Yield (kg/ha)	Percent increase over check	Days to heading	Plant height (cm)	Tillers/m	Grains/spike	Thousand-grain weight (g)
PBW550+Gpc-B1/Yr36 lines							
SABW96	5376	4.23	97	82.3	99.3	38.3	40.2
SABW105	5408	4.85	97	82.7	100.2	33.9	39.7
SABW125	5412	4.92	96	82.6	94.3	36.4	44.1
SABW133	5176	0.35	100	85.0	91.0	34.1	42.8
SABW134	4866	-5.66	99	80.8	96.7	38.8	43.4
SABW135	5344	3.61	100	83.4	94.7	35.0	41.7
SABW136	5769	11.85	102	82.7	99.5	41.9	39.7
SABW140	5168	0.19	97	78.2	96.2	36.0	40.3
SABW141	5476	6.17	98	77.9	103	39.9	41.6
SABW142	4932	-4.38	98	75.1	97.5	39.5	40.1
SABW143	5063	-1.84	98	82.1	102.5	40.7	40.5
SABW146	4449	-13.75	98	75.6	101.8	40.7	40.3
SABW147	4430	-14.11	98	73.3	101.3	44.8	40.1
SABW148	4564	-11.52	99	74.9	98.5	41.6	36.3
SABW150	5065	-1.80	96	76.0	101	34.7	37.3
SABW154	5469	6.03	97	75.7	92.7	39.0	39.1
SABW157	5801	12.47	95	77.9	89.7	41.0	40.0
SABW158	5162	0.08	95	80.5	97.5	36.0	40.3
SABW159	5225	1.30	96	79.3	101.5	38.1	40.6
SABW160	4711	-8.67	96	76.2	94.3	37.4	42.9
SABW161	4567	-11.46	96	74.7	94.2	43.2	39.8
SABW163	5323	3.20	99	74.5	95.5	49.9	38.9
SABW165	4924	-4.54	99	75.8	97.2	37.2	40.0
SABW166	5643	9.40	98	74.8	94.2	39.4	37.9
PBW550+Yr15 lines							
BWL2748	6424	24.54	94	89.5	102	44.9	42.9
BWL2760	6471	25.46	94	86.5	98.3	44.8	39.9
BWL2761	6159	19.41	96	80.9	97.8	43.3	40.9
BWL2763	6048	17.25	93	86.1	99.3	47.1	39.9
BWL2764	5231	1.42	98	86.0	94.3	41.9	39.3
PBW550 (Check)	5158	0.0	98	76.2	94.5	35.9	40.3
LSD (0.05)	726.01		2.96	6.32	NS	6.13	2.59

was observed for introgression lines SABW96, SABW 105, SABW 125, SABW 154, SABW 157, and SABW 166. Brevis & Dubcovsky (2010) elaborate that *Gpc-B1* introgression on grain yield and grain protein yield will depend on the genotype where it is introgressed and the environment where it is used. They further suggested that the negative

impact of the *Gpc-B1* allele on grain weight might be reduced by breeding.

Reaction to stripe rust

The productivity and quality enhancements discussed above are realizable only if adequate resistance against stripe rust

Table 2. Performance of introgression lines evaluated for quality traits

Genotype	Protein (%)	Percent Increase over PBW550	Test weight(kg/hl)	Grain hardness(kg)	Grain appearance (score out of 10)	Sedimentation value (cc)
PBW550+<i>Gpc-B1/Yr36</i> lines						
SABW96	14.06	14.03	78.2	10.9	5.5	43.3
SABW105	13.75	11.52	77.0	11.0	5.7	49.0
SABW125	13.80	11.92	76.3	11.0	5.4	46.3
SABW133	13.57	10.06	74.8	10.2	5.2	44.0
SABW134	13.88	12.57	76.0	11.6	5.4	49.7
SABW135	14.39	16.71	77.3	10.1	5.4	48.7
SABW136	12.93	4.87	76.3	12.4	5.8	42.3
SABW140	13.48	9.33	77.7	12.3	5.9	47.3
SABW141	14.08	14.19	76.8	10.5	6.1	47.0
SABW142	13.25	7.46	77.0	10.5	5.8	46.0
SABW143	14.81	20.11	76.8	10.9	5.6	43.7
SABW146	13.29	7.79	75.3	9.3	5.7	48.3
SABW147	13.99	13.46	75.8	9.8	5.6	43.0
SABW148	12.97	5.19	74.3	8.7	5.7	44.3
SABW150	13.40	8.68	77.0	9.8	5.7	44.0
SABW154	13.19	6.97	76.7	10.4	6.0	47.0
SABW157	12.82	3.97	77.0	12.0	5.7	43.3
SABW158	13.72	11.27	77.7	11.7	5.6	48.3
SABW159	14.92	21.01	77.0	11.9	5.5	42.0
SABW160	13.47	9.25	74.3	9.1	5.5	44.3
SABW161	13.27	7.62	75.0	8.2	5.1	47.0
SABW163	12.85	4.22	77.2	9.8	5.2	46.0
SABW165	13.44	9.00	75.8	11.2	5.4	52.7
SABW166	13.55	9.89	77.3	10.5	5.6	47.3
PBW550+<i>Yr15</i> lines						
BWL2748	12.39	0.49	78.8	9.5	5.7	42.0
BWL2760	11.99	-2.76	78.3	10.4	5.9	50.7
BWL2761	12.79	3.73	78.2	9.8	5.8	45.7
BWL2763	11.17	-9.41	80.0	10.6	6.2	50.7
BWL2764	11.60	-5.92	79.0	9.4	5.8	55.7
PBW550	12.33	0.0	78.8	11.9	5.9	41.3
LSD(0.05)	1.32		1.72	2.25	0.27	5.69

is available. The resistance behavior of *Gpc-B1/Yr36* and *Yr15* was observed in a screening nursery employing intensive epiphytotics of relevant stripe rust pathotype. A distinct resistance pattern emerged for the two sets of derivative lines. Rust score was recorded at three stages, namely maximum tillering, booting, and initiation of anthesis (Table 4).

The three growth stages also represent successive rises in temperature, generally demarcated by a difference of about 8-10 degrees in average daily temperature in spring wheat cultivation areas. The percent infection on parental line PBW550 is seen to increase in evenly spaced steps from 40S to 60S and eventually 80s over the three recordings. Lines

Table 3. Correlations among quality traits and grain yield

	Test weight (kg/hl)	Grain hardness (kg)	Grain appearance#	Sedimentation value (cc)	Thousand-grain weight	Grain Yield
Protein%	-0.467*	0.196	-0.445*	-0.0317	0.257	-0.434*
Test weight		0.306	0.527*	0.194	-0.113	0.683*
Grain hardness			0.224	-0.123	0.193	0.252
Grain appearance				0.104	-0.269	0.412*
Sedimentation value					-0.046	0.0271
Thousand-grain wt						0.0157

* Significant at 5% level ** Significant at 1% level; ns=non significant # (score out of 10)

Table 4. Stripe rust disease score data for parental lines in two years

Genotype	1st score (maximum tillering)		2nd score (booting)		3rd score (anthesis)	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
PBW550+Gpc-B1/Yr36 lines						
SABW96	40S	20S	20S	20S	20S	10S
SABW105	20S	10S	20S	20S	10S	10S
SABW125	20S	20S	20S	20S	10S	10S
SABW133	40S	40S	20S	20S	10S	10S
SABW134	40S	20S	20S	20S	20S	20S
SABW135	40S	20S	40S	20S	20S	20S
SABW136	20S	20S	20S	20S	10S	10S
SABW140	40S	20S	20S	20S	20S	10S
SABW141	40S	20S	20S	20S	10S	10S
SABW142	20S	20S	20S	20S	10S	10S
SABW143	20S	20S	10S	10S	10S	5S
SABW146	40S	20S	20S	20S	20S	10S
SABW147	20S	20S	20S	20S	20S	10S
SABW148	40S	20S	20S	20S	20S	20S
SABW150	40S	20S	20S	20S	20S	10S
SABW154	40S	40S	20S	20S	20S	10S
SABW157	40S	40S	20S	20S	20S	10S
SABW158	20S	10S	20S	10S	10S	5S
SABW159	40S	20S	20S	20S	20S	10S
SABW160	20S	20S	20S	20S	10S	10S
SABW161	40S	20S	20S	20S	20S	10S
SABW163	40S	40S	20S	20S	20S	10S
SABW165	20S	20S	20S	20S	10S	10S
SABW166	20S	20S	20S	10S	10S	10S
PBW550+Yr15 lines						
BWL2748	10S	10S	10S	10S	5S	5S
BWL2760	10S	5S	10S	10S	5S	5S
BWL2761	10S	10S	10S	10S	10S	5S
BWL2763	5S	5S	10S	10S	5S	5S
BWL2764	10S	10S	10S	10S	10S	5S
PBW550	40S	20S	60S	40S	80S	60S

possessing *Yr15* maintain their resistance level at all three stages- a typical feature of all-stage major rust resistance genes. The entire set of lines with *Gpc-B1/Yr36* shows a clear pattern of gradual enhancement of resistance in consonance with the high-temperature inducible nature of the gene. The resistance kicks in prior to the critical grain filling phase and is expected to provide adequate yield protection.

Uauy et al. (2005) showed that lines carrying this gene show enhanced resistance when maintained at 25°C or above. Controlled environment and field studies in California (U.S.) corroborated the partial resistance provided by *Yr36* at temperatures $\geq 25^\circ\text{C}$ against a wide range of U.S. *P. striiformis* isolates. Another study in the UK shows that *Yr36* partial resistance is affected by the plant developmental stage and inoculum pressure (Segovia et al., 2014). The wheat-growing environment in North Western Plains of India is known for sharp rise in temperature during grain filling stage. The terminal temperature encountered in this environment may rank among the highest for wheat anywhere in the world. The grain filling stage is now also seen to overlap with the incidence of current widely adapted stripe rust pathotypes. This seems to be an appropriate context for the deployment of this potentially durable resistance.

Overall worth of *GpcB1/Yr36* in PBW550 background

Introgression lines SABW159 showed 21.01% enhancement in protein with no yield penalty and stripe rust-resistant reaction (10S). Similarly, SABW 141 registered a 14.19% increase in protein content along with a 6.17% yield advantage over PBW550 and terminal disease reaction of 10S. The corresponding disease score for PBW550 was 80S. A similar trend for improvement across all three traits, viz. yield, GPC, and stripe rust resistance, was observed for introgression lines SABW96, SABW 105, SABW 125, SABW 154, SABW 157, and SABW 166. No single yield component showed a reduction on account of enhanced protein. Most of the studies on *Gpc-B1* had indicated a reduction in thousand-grain weight with yield parity being restored via increased grain number and tiller per unit area (Tabbitta et al. 2013). The *Gpc-b1* gene has been successfully deployed commercially for e. g., 'Lassik' (UC Davis, CA) which shows a highly significant increase in GPC with no yield penalty relative to the recurrent parent Anza. Brevis and Dubcovsky (2010) emphasized that the effect of *Gpc-B1* introgression on grain yield and grain protein content depends on genetic background as well as growth environment. The present study strongly indicates that these two considerations are favorable in the case of *Gpc-B1/Yr36* introgression in PBW550, and wider testing in northwestern plains of India is warranted. In a further initiative, the *GpcB1/Yr36* gene has been combined with *Yr15* in PBW550 background to further strengthen stripe rust resistance.

Authors' contribution

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

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