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Introgression of a gene for high grain protein content (*Gpc-B1*) into leading cultivars of wheat

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A wheat genotype named 'PBW343+*Gpc-B1+LR24'* containing *Gpc-B1* gene linked with the DNA-based marker *Xucw108*, developed at Choudhary Charan Singh University (CCSU), Meerut, through marker-assisted selection, was used as the donor parent to transfer the gene (*Gpc-B1*) for high grain protein content (GPC) into two popular cultivars (HUW234 and HUW468) of Eastern Gangetic Plains (EGP) of India. In both the cultivars, *Gpc-B1* gene was introgressed through marker-assisted backcross breeding (MABB) which involved the following three steps: (i) Foreground selection; (ii) Screening for the carrier chromosome, and (iii) Recovery of recipient parent genome (RPG). Data on GPC (percent grain weight) was recorded for all selected individual plants from $BC_2F_{2:3}$ generation. The dominant marker *Xucw108* was used for foreground selection, and heterozygous plants were identified through progeny testing. For RPG recovery, both genotypic and phenotypic selections were used. Introgression of high GPC gene into recipient background without yield loss was completed in 5 years, starting from F_1 (2009-10) and completing it in BC_2F_5 (2013-2014). Ten selected single plants from $BC_2F_{3:4}$ had comparable yield with 26% higher GPC than the recurrent parent HUW 234. For the other parent HUW 468, eight selected plants had comparable yield with 34% higher GPC. Multi-row progenies (BC_2F_4 and BC_2F_5) of each selected plant were evaluated for yield traits with donor and recipient parents during 2012-2013 and 2013-2014. Two lines with significantly higher GPC were identified in each of the crosses with no yield penalty. The study reinforced the belief that marker assisted selection (MAS) in combination with phenotypic selection could be a useful strategy for the development of wheat genotypes with high GPC without sacrificing grain yield.

Key words: *Triticum aestivum*, grain protein content (GPC), marker assisted selection (MAS), carrier chromosome, recipient parent genome.

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INTRODUCTION

Among cereals, wheat has highest GPC ranging from 8 to 15% (Johnson et al., 1985) and is one of the most to 15% (Johnson et al., 1985) and is one of the most important components of nutritional value that directly affects bread making and pasta quality (Avni et al., 2014). India has successfully maintained its 2nd position among wheat producing countries of the world after China by producing 95.9 mt wheat from an area of 30.61 m ha in 2013-14 (Anonymous, 2014). Assuming an average of 10% GPC in all wheat varieties grown in India, 95.9 m tons of annual produce will amount to 9.59 million mega gram of protein for human and livestock nutrition. Although wheat production in India has been able to keep pace with India's population growth, which is of paramount importance for food security, malnourishment continues to be a serious issue in most developing countries, particularly those in south Asia. According to the estimate made at FAO (www.fao.org), it is predicted that by the year 2015, more than 36% of the people in developing countries can be affected from undernourishment. Since wheat is the most widely consumed food grain in the world, and provides staple food to 35% of the world population, occupying 17% of total arable land and 21.8% of the total area under food grain crops (Ortiz et al., 2008), breeding for nutritious wheat grain may be the most effective way of providing nutrition to human beings. The importance of this breeding objective is particularly relevant to the thickly populated EGP of India, where 90% of the farmers are small land holders, who consume most of their farm produce as food. More than two decades ago, Joppa and Cantrell (1990), developed substitution lines of the *Triticum turgidum* ssp. *Dicoccoides* accession (DIC) and mapped high GPC gene on chromosome 6BS. Olmos et al. (2003) first mapped the QTLs for GPC within a 2.7 cM region. Distelfeld et al. (2004), using wheat-rice collinearity developed a more specific map, which narrowed down the *Gpc-B1* region to a 0.3 cM interval. Using MABB, efforts were later made to introgress *Gpc-B1* gene in some popular cultivars in India (Tyagi et al., 2014). In USA also, two hexaploid wheat cultivars (Lassik at University of California, Davis and Farnum at Washington State University, Pullman, USA) were developed using *Gpc-B1* gene. Recently, a donor Glu-79 was used to successfully introgress *Gpc-B1* gene in a wheat cultivar HUW468 (Vishwakarma et al., 2014) at Banaras Hindu University where the present investigation was conducted.

This study was undertaken to improve GPC using MABB in a popular cultivar (HUW234) of EGP of India using a high GPC line 'PBW343+*Gpc-B1+LR24'* that contains *Gpc-B1* gene linked to a known marker *Xucw108.* The methodology of MABB involved foreground selection, screening for the carrier chromosome and recovery of recipient parent genome. The cultivar HUW 468 was also improved to see whether GPC can be improved through MABB using a donor different from that reported by Vishwakarma et al. (2014).

MATERIALS AND METHODS

The experiment was conducted at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi (25.15° N latitude; 82.59° E longitudes, 75.5 m above mean sea level, soil pH 7.4), which is located in the EGP of India. Off-season facility for growing an additional crop in the summer was also utilized at Wellington, Tamil Nadu (11.33° N latitude; 76.80° E longitudes, 1854 m above mean sea level). The crop at Wellington was protected with the fungicide Propiconazole.

Plant material

Two well adapted bread wheat genotypes (HUW234 and HUW468) of EGP of India were used as recipient parents. The donor parent PBW343+*Gpc-B1+Lr24* was earlier developed at the Department of Genetics and Plant Breeding, CCSU Meerut, under the Department of Biotechnology (DBT) funded Quality Networking Project. This donor is a hexaploid wheat genotype having high GPC (16%) due to *Gpc-B1* and carries leaf rust resistance gene *Lr24*.

DNA isolation and the markers

DNA isolation for all experiments was carried out using leaves of 30 days old seedlings following the procedure of Saghai-Maroof et al. (1984). The extraction buffer contained 1 M Tris pH 8.0, 0.5 M EDTA, 5 M NaCl, 1.7 g/L PVP 40 (0.3 ml per mg lyophilized leaf), and the resulting DNA was dissolved in 0.2 ml HPLC water. *Xucw108* (*Gpc-B1*) was used as the marker for foreground selection.

PCR amplification and amplicon separation

Each 15 µl of PCR reaction mixture comprised 40 ng template DNA, 0.2 µM of each primer, 150 µM dNTP, 1× PCR buffer (10 mMTris pH 8.4, 50 mMKCl, 1.8 mM $MgCl_2$, 0.01 mg/ml gelatin) and 0.25 U Taq DNA polymerase. The cycling regime was initiated by a denaturation (94°C / 4 min), followed by 40 cycles of 94°C / 45 s, 60°C / 60 s and 72°C / 60 s and completed with a final extension (72°C / 10 min). Amplicons were separated via agarose gel (2.5%) electrophoresis and visualized by EtBr staining.

For polymorphic screening of many markers simultaneously between parents a touch-down PCR protocol was also used for amplification. The cycling regime for this PCR consisted of an initial denaturation (94°C / 4 min), followed by 20 cycles of 94°C / 30 s, Tm / 45 s from 65 to 55°C, reducing by -0.5°C per cycle, 72°C / 120 s, then 20 cycles of 94°C / 30 s, 55°C / 45 s, 72°C / 120 s, and completed with a final extension of 72°C / 10 min. Amplicons were separated on agarose gel (2.5%) electrophoresis and visualized by EtBr staining.

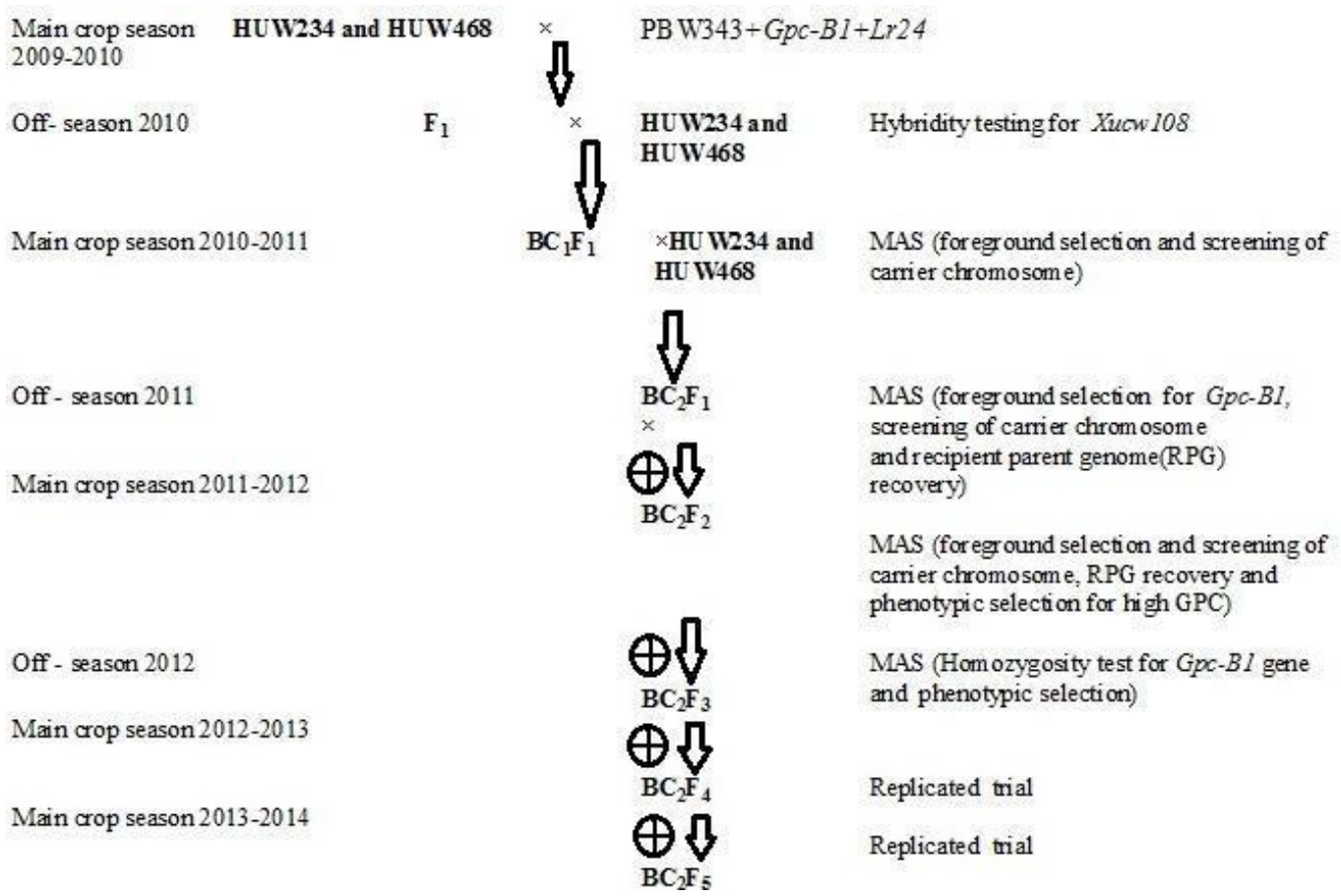


Figure 1. Flow diagram showing steps involved in marker-assisted beckcross breeding programme used for introgressing high grain protein content gene (Gpc-BI) in wheat cultivars HUW234 and HUW468 from PB W343+GpcBI+Lr24.

Background selection

As many as 498 SSR markers were screened to select the polymorphic markers between donor and the two recipient parents for the purpose of background genome recovery. For each chromosome, 20-55SSR markers were tested; 2 to 7 markers per chromosome were found to be polymorphic. For the carrier chromosome 6B (carrying the gene *GpcB1*), as many as 55 markers were tested, of which 6 were polymorphic between the donor and the recipient cultivar HUW234, while 7 were polymorphic between the donor and the recipient cultivar HUW468. For the whole genome, 55 and 53 markers were polymorphic in the crosses of HUW234 and HUW468, respectively.

Recipient parent genome

The proportion of the genome of the recipient parent present in the backcross generations (G) was estimated using the following formula of Sundaram et al. (2008):

RPG = 100(X +1/2Y)/N

Where, N represented the number of parental polymorphic markers screened, X the number of markers homozygous for recurrent parent allele and Y the number of markers in the heterozygous state. The molecular data were scored manually.

Marker-assisted breeding

The schematic diagram of the experimental program is summarized in Figure 1. The heterozygosity of F_1 plants was confirmed by *Gpc-B1*-linked marker (*Xucw108*); the selected individual plants were backcrossed with each corresponding recipient genotype. In each backcross, foreground selection was exercised using the marker *Xucw108*, and plants showing 217 bp amplicon were selected. These selected plants were also screened for carrier chromosome and RPG recovery. Plants that carried maximum proportion of RPG and highest phenotypic similarity with the recipient parent were selected.

In BC_2F_1, after foreground selection and screening of carrier chromosome, individual plants heterozygous for *Xucw108* were selected and selfed to obtain BC_2F_2. The marker *Xucw108* being dominant, both heterozygous and homozygous plants were identified in BC_2F_2. Both types of plants were screened for carrier chromosome as well as RPG. Plants with maximum proportion of RPG were selfed to obtain BC_2F_3 seed. Seeds of individual selected plants (BC_2F_3 seed) were also analyzed for GPC using FOSS 1241 Grain protein analyzer and high GPC plants were selected/confirmed.

In BC_2F_3 generation, individual plant progenies (family) were grown. Ten random plants in a family were tested for foreground selection. If any one plant showed absence of the amplicon, entire family was rejected. A family where all the plants showed presence of amplicon for *Xucw108*, was considered a homozygous family.

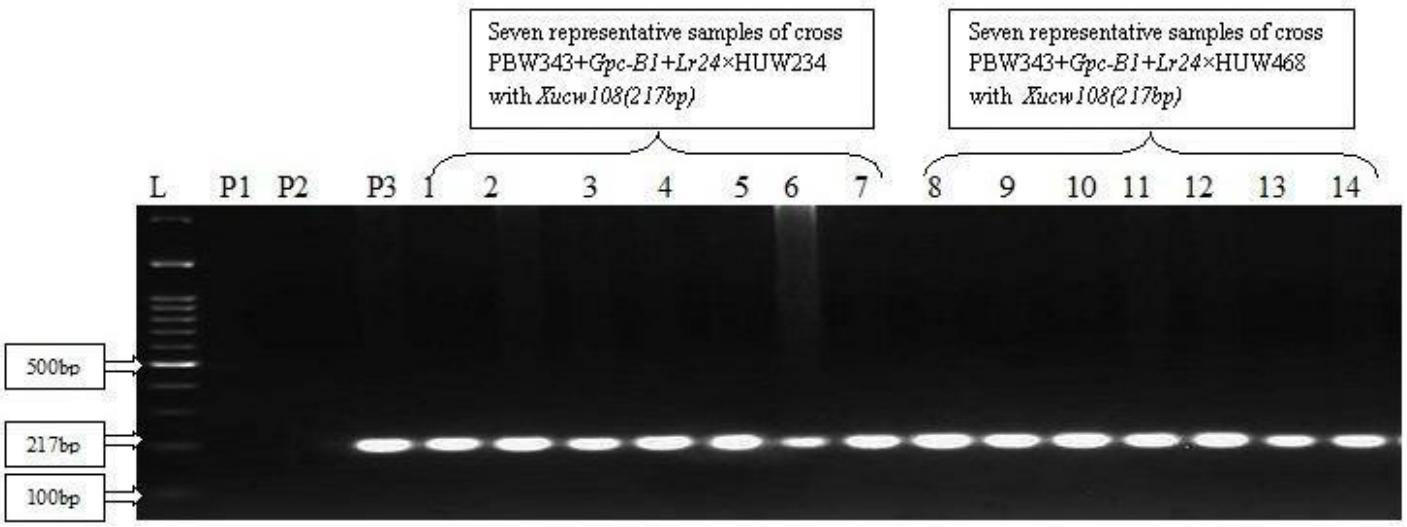


Figure 2. SSR amplification profiles. L: 100 bp ladder. PI: HUW234, P2: HUW468; PB W343+ GpcB1+Lr24. (a) Xucw108, Lanes 1-7; MAS-derived BC_2F_3 selections from the cross cv. PB W343+ GpcB1+Lr24×HUW234 and Lane 8-14: MAS-derived BC_2F_3 selections from the other cross PB W343+GpcB1+Lr24× HUW468. #1: HUW234-184, #2:-286, #3: -145, #4: -387, #5: -166, #6: -388, #7: -143, #8: huw 468-375, #9: -376, #10: -358, #11: -181, #12: -379, #13: -55, #14: -234.

After threshing, BC_2F_4 seed of individual homozygous family was bulked and analyzed for GPC. Families with more than 14% GPC (having an additional 3% more than the recipient's 11% GPC) were selected.

Protein estimation

The seed of $BC_2F_{2:3}$ and all the selected plants in the following generations of both crosses were evaluated for GPC using FOSS Infratec 1241 grain protein analyzer and compared with recipient parents.

Evaluation of BC_2F_4/F_5 progenies for GPC (%) and grain yield

The BC_2F_4 and BC_2F_5 generations obtained by selfing of each of the BC_2F_3 and BC_2F_4 progenies derived through MAS were used for conducting replicated yield trials at BHU, Varanasi during crop season 2012–2013 and 2013-2014. The trial was laid in RBD with three replications. Each genotype was planted with 4 rows each 3 m long, and with a row-to row distance of 25 cm. Full dose of K_2O and P_2O_5 were applied at sowing; nitrogen was applied in split application, with 60 kg N per ha at sowing, 30 kg N per ha at first irrigation (21 days after sowing) and 30 kg N per ha at second irrigation (45 days after sowing) (Joshi et al., 2010). All other recommended agronomic practices were followed. Data on days to heading (DH), plant height (PH), thousand-grain weight (TGW), grain yield (GY) and grain protein content (GPC) were recorded and used for statistical analysis. The data on GY was converted into grain yield (q/ha) for further statistical analyses. The GPC (%) at 12% moisture content was estimated for each genotype using Infratech 1241 Grain Analyzer, Foss, Denmark at BHU, Varanasi.

Statistical analysis

The amplicon patterns produced by gene-linked SSR marker was classified in the foreground selection as + (pattern of donor parent), and - (pattern of recurrent parent) to construct a data matrix. In the background selection, the patterns of ampliconproduced by SSR markers were classified into - (pattern of donor parent), + (pattern of recurrent parent), H (heterozygous pattern), and U (missing data) to construct the data sheet.

The analysis of variance (ANOVA) was conducted for six different traits using two years data for all the three replications. Significance of differences between means of recipient parents and selected plants in BC_2F_4 andBC_2F_5 was tested by software SAS 9.3 (SAS Institute Inc., Cary, NC, USA) in PROC GLM.

RESULTS

Informativeness of the foreground and background selection markers

The 217 bp long expected amplicon due to *Xucw108* marker was absent in both the recipient parents (HUW234 and HUW468), but was present in the donor (PBW343+*Gpc-B1+LR24)* as resolved by electrophoresis through a 2.5% agarose gel (Figure 2).

Introgression of *Gpc-B1* from PBW343+*Gpc-B1+LR24* to cv.HUW234

The details of selection of desirable plants after foreground selection, screening of carrier chromosome, RPG recovery and GPC% analysis in PBW343+*Gpc-B1+Lr24* ×HUW234 cross are given in Table 1. The first step started with selection of 50 hybrid plants among 53 F_1s for being hybrids by testing with *Xucw108* (Table 1). Their two backcrosses with HUW234 followed by selfing and subsequent selections led to recovery of 116 BC_2F_2

Table 1. Selection of desirable plants after foreground selection, screening of carrier chromosome, recipient parent genome recovery and GPC% analysis in PBW343+*Gpc-B1*+*Lr24* ×HUW234 and PBW343+*Gpc-B1*+*Lr24*×HUW468 crosses.

Generation	Number of plants Scored	Number of plants heterozygous/ homozygous for *Gpc-B1*	Selected plant after carrier chromosome screening	Number of selected plants after background selection and their RPGR in range	Selected plants after phenotypic selection for GPC %	Criteria of selection for desirable plants
PBW343+ *Gpc-B1*+*Lr24* ×HUW234						
F_1	53	50				Marker *(Xucw108)*
BC_1F_1	850	403	10			FGS and CCS
BC_2F_1	413	208	32	18 (90.12 to 93.45)		FGS, CCS and RPGR
BC_2F_2	403	290	187	116 (93.05 to 95.11)	55	FGS, CCS, RPGR and PS
BC_2F_3	55 (Family)	15 (Family)			10	Testing for homozygosity of *Gpc-B1* locus and PS
BC_2F_4	10	10				Replicated yield trial
BC_2F_5	10	10				Replicated yield trial
PBW343+*Gpc-B1*+*Lr24* ×HUW468						
F_1	67	61				Marker *(Xucw108)* FGS
BC_1F_1	753	367	13			and CCS FGS,CCS and
BC_2F_1	455	221	43	6 (90.05 to 92.13)		RPGR FGS, CCS, RPGR
BC_2F_2	764	503	117	63 (93.44 to 95.12)	42	and PS
BC_2F_3	42 (Family)	11 (Family)			8	Testing for homozygosity*of Gpc-B1*locusand PS
BC_2F_4	8	8				Replicated yield trial
BC_2F_5	8	8				Replicated yield trial

FGS = Foreground selection, CCS = carrier chromosome selection, RPGR = recurrent parent genome selection and PS = phenotypic selection for GPC%.

plants that showed >93% RPG with a range of 93.05-95.11%. Self-seeds of all 116 BC_2F_2 plants showed higher GPC% than the recipient parent with 55 showing >14% GPC. In BC_2F_3, seeds of 55 plants were grown in multi row considered as 55 families and 15 were identified to be homozygous. Ten BC_2F_3 families that showed >14% GPC were selected (Table 1).

A highly significant variation was observed among BC_2F_4 and BC_2F_5 lines for all the traits except plant height (Table 2). Both the years were significantly different for GPC (%) and grain yield (q/ha) (Table 2). The replicated agronomic performance data of the ten families (PBW343+*Gpc-B1*+*Lr24* × HUW234) in BC_2F_4 and BC_2F_5 is shown in Table 3. Range of increase in GPC over recipient parent was 20.9 to 26.1% (Figure 3). Grain yield was observed as a mixed performance as it ranged from -3.99 to 0.46% over the recipient parent (Table 3), but the difference was statistically non-significant.

Introgression from PBW343+*Gpc-B1*+Lr24 in cv. HUW468

Like the previous cross, the details of selection of desirable plants after foreground selection, screening of carrier chromosome, RPG recovery and GPC% analysis in PBW343+*Gpc-B1*+*Lr24* ×HUW468 cross are given in Table 2. The 61 F_{1s} found as true hybrid were crossed with recipient parent HUW468 and using *Xucw108,* 764 plants were obtained in BC_2F_2. Since *Xucw108* did not distinguish between homozygous and heterozygous, foreground selection resulted into 503 plants. These were screened for carrier chromosome with 7 SSR markers and 117 plants were obtained which had all the 7 markers in homozygous condition on carrier chromosome with target gene *Xucw108*. These 117 plants were screened with polymorphic markers and 63 plants showed >93% RPG recovery. Of 63 BC_2F_2 plants, 42 showed >14% GPC. In BC_2F_3, out of 42 families, 11 were identified as homozygous. Of these, 8families showed >14% GPC and were selected.

The ANOVA of 8 families in BC_2F_4 and BC_2F_5 with donor (PBW343+*Gpc-B1+Lr24)* and recipient (HUW468) parents showed that entries significantly differed to each other for the six traits (Table 2). There was significant difference for GPC (percent grain weight) and grain yield across the two years (Table 2). The selected 8 MAS derived lines were at par to recurrent parent for DH, PH and GY but GPC was significantly higher. The superiority in GPC was to the range of 24.8 to 34.3% (Figure 3). Grain yield improvement though non-significant, ranged -1.20 to 2.95% over recipient parent (Table 3). Thousand

Table 2. Analysis of variances for days to flowering (DF), plant height (PH) , grains per spike (GPS), thousand kernel weight (TKW), grain protein content (GPC) and grain yield (GY) of BC_2F_4 and BC_2F_5 MAS-derived progenies in two crosses PBW343+*Gpc-B1*+*Lr24*×HUW 234 and PBW343+*Gpc-B1*+*Lr24*×HUW 468.

Cross/lines	df	Mean sum of square					
		DF (days)	PH (cm)	GPS (No.)	TKW (g)	GPC (%)	GY (q/ha)
		PBW343+*Gpc-B1*+*Lr24*×HUW 234					
Year	1	0.22	0.01	18.48	0.04	21.68**	6.67*
Entry	11	99.91**	114.25	14.98**	5.90**	4.07**	8.23**
Rep.	2	0.65	3.63	11.22	0.85	0.03	0.44
Year & Entry	11	0.55	8.17	2.99	0.65*	0.29	1.98
Error	46	0.66	1.28	5.5	0.28	0.21	1.72
CV		1.12	1.15	5.25	1.33	3.28	3.00
		PBW343+*Gpc-B1*+*Lr24*×HUW 468					
Year	1	0.14	107.12**	62.81**	0.14	7.49**	5.48*
Entry	9	67.14**	121.71**	32.18**	6.92**	7.02**	22.98**
Rep.	2	1.82	2.91	1.39	2.08	0.08	0.18
Year & Entry	9	1.32	3.81	5.67	0.83	0.26	1.79
Error	38	0.94	3.95	4.46	0.86	0.72	1.38
CV		1.27	2.01	4.35	2.35	5.99	2.61

*Significant at P = 0.05, **Significant at P = 0.01.

grain weight in MAS derived lines was variable with differences from recipient parent ranging from -8.75 to 5.93% (Table 3).

DISCUSSION

MAS is becoming increasingly common in crop improvement programs (Collard and Mackill, 2008; Gupta et al., 2010), even in developing countries like India (Kumar et al., 2011; Vishwakarma et al., 2014) with greater integration of MABB approach in crop breeding programs around the world. Although back cross breeding was advocated and used for a long time in conventional wheat breeding programs (Joshi et al., 1997; Singh and Huerta-Espino 2004, Singh et al., 2007), an increased adoption of MABB approach in recent years is due to manifold advantages, the major one being accelerated introgression of the target gene(s) by reducing the number of backcrosses and avoiding the linkage drag (Hospital, 2005; Herzog and Frisch, 2011). In this study, a gene enabling high GPC in wheat was successfully introgressed separately into two popular cultivars cv. HUW234 and HUW468 of the EGP of India.

For genome recovery, we mainly focused on carrier chromosome for initial screening, which was essential for avoiding linkage drag as well as rapid recovery of recipient genome. Transferring *Gpc-B1* gene through marker was validated by GPC analysis. Brevis and Dubcovsky (2010) introgressed *Gpc-B1* gene and successfully increased GPC in tetraploid and hexaploid wheat lines. Vishwakarma et al. (2014) transferred *Gpc-B1* gene from Glu269 (donor parent) into CV. HUW468 and got 14% GPC in the improved HUW468 compared to 10% of the recipient parent. In this study, same recipient parent (HUW468) was shown to achieve similar level (>14%) of GPC in the improved version using another donor 'PBW343+*Gpc-B1+LR24'*. The improved version of the other recipient parent (HUW234) also showed GPC enhancement from 11% to >14%. The results proved the robustness of *Gpc-B1* gene and confirmed the findings of earlier reports that the *Gpc-B1* gene can be introgressed using molecular breeding in various cultivars with significant benefit. Compared to recipient parents, the GPC in the back cross derived lines was enhanced 20.9 to 26.1% in HUW234 and 24.8 to 34.3% in HUW468. Of these 18 improved GPC BC_3F_5 lines from the two parents, none showed any yield penalty. Other agronomic traits (days to heading and plant height) also remained unaffected. This indicated that high GPC lines may be developed without any penalty of yield and other agronomic traits. A large variation in GPC was observed among lines with *Gpc-B1* gene in early generations. This variation could be due background effect as Brevis and Dubcovsky (2010), reported its complex genetic nature.

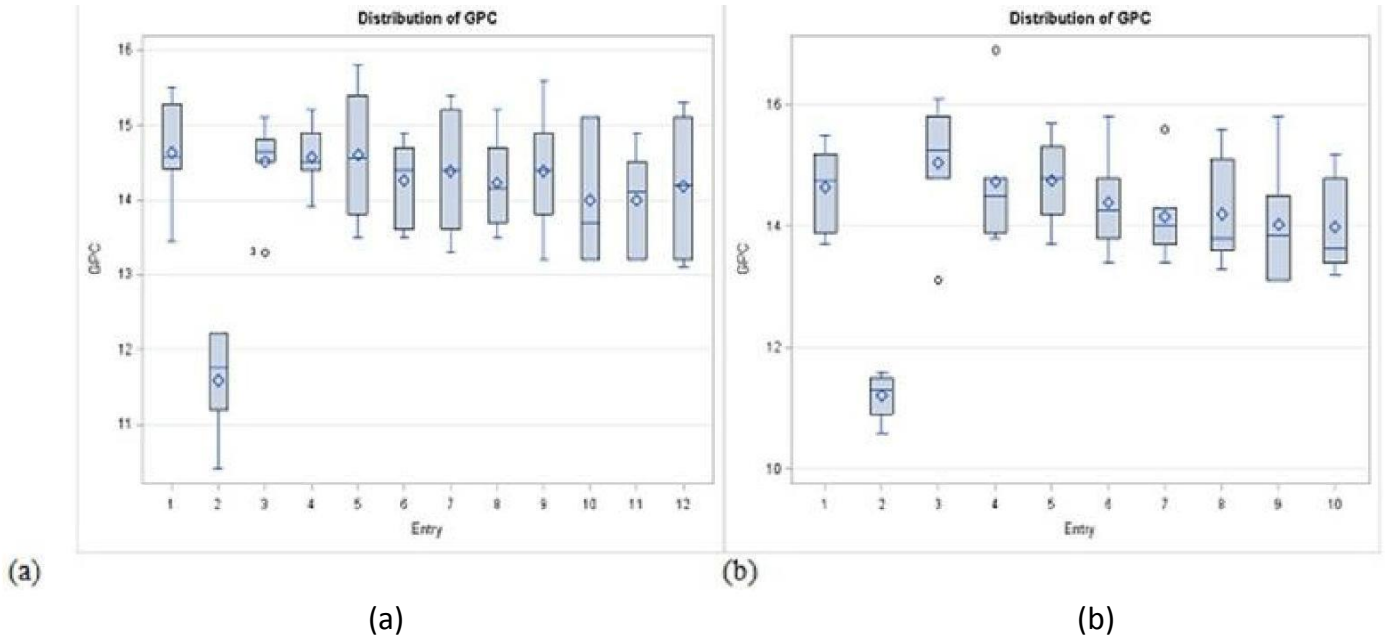
The GPC is not only the result of genotype but environmental factors such as weather conditions, nitrogen application, water access and temperature during growth and grain filling period also plays a significant role (Daniel and Triboi, 2000; López-Bellido and López-Bellido, 2001; Rharrabti et al., 2001; Tea et al., 2004; Abedi et al., 2011). In the present study, the lines improved for GPC were little bit taller than recipient variety (the difference being statistically non-significant);

Table 3. Mean performance of the improved lines for days to flowering (DF), plant height (PH), grains/spike (GPS), thousand grain weig ht (TGW), grain protein content (GPC), grain yield / ha (GY), their increase/decrease over recipient parents (%) and per cent recovery of the recipient parent genome in BC2F4 and BC2F5 selection from the two crosses PBW343+ *Gpc-B1+Lr24* ×HUW 234 and *PBW343*+ *Gpc-B1+Lr24* ×HUW 468.

Cross/lines	Days to flowering (days)	Plant height (cm)	Grains / Spike (No.)	Thousand Kernel weight (gm)	Grain protein content (%)	Grain yield/ha (q/ha)	Increase GPC over recipient parents (%)	Increase/decrease TGW over recipient parents (%)	Increase/ decrease GY over recipient parents (%)	Percent recovery of the recipient parent genome
PBW343+*Gpc-B1+Lr24* × HUW 234										
PBW343+*Gpc-B1+Lr24*	85.24	86.45	43.60	37.15	14.63	40.76				
HUW 234	72.45	99.36	42.70	38.27	11.58	44.72				
184	70.15	100.85	44.95	34.92	14.20	43.42	22.63	-8.75	-2.91	95.44
286*	72.45	101.53	46.50	39.73	14.57	44.93	25.82	3.81	0.46	93.33
145*	71.41	101.52	44.25	39.07	14.60	44.41	26.08	2.09	-0.68	93.22
387	72.45	100.22	44.75	36.24	14.25	42.93	23.06	-5.30	-3.99	94.33
166	70.15	99.66	45.01	35.68	14.38	42.97	24.18	-6.77	-3.92	94.11
388	71.41	99.35	45.05	36.12	14.23	43.06	22.88	-5.62	-3.72	93.33
143	71.41	101.57	42.12	37.56	14.38	43.48	24.18	-1.86	-2.77	95.00
233	72.45	98.65	45.88	39.96	14.00	44.53	20.90	4.42	-0.41	94.44
242	71.41	98.57	47.73	39.93	14.00	44.50	20.90	4.34	-0.48	94.44
123	70.15	99.29	43.58	40.54	14.18	44.38	22.45	5.93	-0.75	93.11
LSD <5%	2.67	1.48	2.72	0.53	0.55	1.43				
PBW343+*Gpc-B1+Lr24* × HUW 468										
PBW343+*Gpc-B1+Lr24*	85.00	88.62	47.19	40.70	14.63	39.81				
HUW 468	75.67	95.22	42.7	38.27	11.20	45.27				
375*	77.64	98.910	42.79	38.52	15.05	45.95	34.38	0.65	1.52	93.75
376	74.23	100.67	48.45	37.57	14.33	45.48	27.95	-1.83	0.47	95.00
358*	76.52	98.87	42.47	38.61	14.75	45.75	31.70	0.89	1.07	91.25
181	74.23	99.21	48.38	38.75	14.38	44.72	28.39	1.25	-1.20	92.50
379	75.67	100.12	48.39	38.82	14.17	45.52	26.52	1.44	0.56	93.75
53	74.23	97.09	48.58	40.51	14.20	46.60	26.79	5.85	2.95	92.50
234	75.67	100.44	51.15	39.75	14.03	46.54	25.27	3.87	2.81	92.50
258	75.67	97.36	50.31	40.29	13.98	45.92	24.82	5.28	1.45	91.25
LSD <5%	3.43	2.17	2.31	1.14	1.03	2.22				

*Improved lines would go to multilocation trial under All India Coordinated Wheat Improvement Project.

we speculate that high GPC lines could have efficient nitrogen uptake and/or nitrogen re-mobilization from leaf and stem.



(a) (b)

Figure 3. The distribution of grain protein content (GPC) within (a) PBW343+Gpc-BI+Lr24× HUW234 and (b) PBW343+ Gpc-B1+Lr24× HUW468 derived lines compared to recipient parents; Entry 1: Donor, Entry 2: Recipient parent and entries 3 onwards: Improved line.

Conflict of Interest

The authors have not declared any conflict of interest.

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