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Marker-assisted improvement of grain protein content and grain weight in Indian bread wheat

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Abstract The leading Indian wheat cultivar 'HUW234' produces grain with relatively low protein content (GPC) and thousand grain weight (TGW). Therefore, marker-assisted backcrossing was used to improve these two important traits by introducing favorable genes/alleles from cv. 'Glu133', which harbors both alleles for both GPC (*Gpc-B1*) and TGW. Foreground selection for GPC was achieved using microsatellite markers *Xucw108* linked with *Gpc-B1* and *Xgwm297* linked with TGW. Background selection applied to support recovery of the recurrent parent genotype was based on 86 genomically distributed microsatellites. A selected BC_2F_1 plant was the progenitor of 15 BC_2F_4 families, in which representation of the cv. HUW234 genome ranged from 89.5 to 93.0 %. The new derivatives of HUW234 were comparable in yield to the original cultivar, but with both significantly higher GPC and heavier kernels.

Keywords Background selection · Foreground selection · Marker assisted selection · *Triticum aestivum*

Introduction

Wheat provides over 20 % of the calories in the human diet and 25 % of the protein (FAO 2012). After many years of increasing productivity, wheat yields have shown evidence of plateauing since the turn of the century (Lobell et al. 2011) while the human population continues to grow. Although yield is the primary trait under selection in most breeding programs and grain destined for human consumption is processed before consumption, end-use quality is also an important breeding objective. Among quality traits, grain protein content (GPC) and thousand grain weight (TGW) are significant quality traits. GPC is a major determinant of nutritional and market value of wheat grain as well as processing quality (Brevis et al. 2010). High GPC varieties not only address the issue of end-use quality, but also provide a relatively cheap source of protein. This is of high significance for the population dense North

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Eastern Plains Zone (NEPZ) of India with a rice–wheat cropping system and with wheat having double the protein content of rice. TGW is correlated with flour yield as well as actual grain yield (GY) (Ketata et al. 1976; Campbell et al. 1999). Despite major advances in yield achieved by the Green Revolution, progress in increasing GPC has been slow (Gupta 2004). Although it is commonly reported that yield and GPC are negatively correlated (Simmonds 1995), there is little agreement on its biological basis (Cox et al. 1985; Brevis and Dubcovsky 2010).

A number of DNA-based markers for various quality-associated traits have been described in wheat (Gupta et al. 2011; Roder et al. 2008). Accession FA-15-3, which originated in Israel, accumulates high GPC (Avivi 1978) partly due to presence of the gene *Gpc-B1* (Joppa et al. 1997; Olmos et al. 2003). This gene has been tagged by a variety of DNA-based markers (Khan et al. 2000; Singh et al. 2001; Distelfeld et al. 2004, 2006; Olmos et al. 2003; Prasad et al. 2003; Kulwal et al. 2005; Wang et al. 2012) and was positionally cloned by Uauy et al. (2006). When *Gpc-B1* was introduced into low GPC cultivars, it showed little evidence of a yield penalty (Kade et al. 2005; Brevis and Dubcovsky 2010), and similar experiences were reported for Indian cultivar derivatives (Tyagi et al. 2014; Vishwakarma et al. 2014).

As an important component of GY, TGW is always a favored trait in wheat breeding programs (Gegas et al. 2010). QTLs on chromosomes 1A, 1B, 1D, 2B, 2D, 4B, 4D, 7A and 7D have been reported for this trait (Huang et al. 2003, 2004; McCartney et al. 2005; Wang et al. 2009; Mir et al. 2012). A major QTL (*QTgw.ipk-7D*) explaining 84.7 % of the phenotypic variation in TGW (Huang et al. 2003, 2004) was later fine-mapped by Roder et al. (2008). More recently, Mir et al. (2012) identified 11 new QTLs.

Marker assisted selection (MAS) has gained considerable attraction as a breeding tool in crop improvement (Gupta et al. 2010; Vishwakarma et al. 2014). Gupta et al. (2010) estimated that at least 60 genes/quantitative trait loci (QTL) have been used in wheat improvement programs, largely (but not exclusively) in more developed countries. Likewise, there has been successful implementation of MAS in India (Gupta et al. 2011). Here, we describe progress in a MAS program aimed at improving both GPC and grain weight in cultivar (cv.) HUW234.

Materials and methods

Plant materials

The recurrent parent was cv. HUW234 (HUW12/Sparrow//HUW12), and the donor of both *Gpc-B1* and the TGW QTL was line Glu133 (DBW16/GluPro//2*DBW16). The introgression scheme is illustrated in Fig. 1. The hybrid status of two F_1 plants from the cross HUW234 × Glu133 (designated HUW234-09) was confirmed by genotyping with the *Xucw108* marker linked to *Gpc-B1* (Uauy et al. 2006). These plants were backcrossed to HUW234. Foreground selection on the BC_1F_1 plants was applied for *Gpc-B1* using the *Xucw108* marker (Uauy et al. 2006) and background selection in conjunction with phenotypic selection for HUW234 morphology was applied using 86 genome-wide (four to six per chromosome) polymorphic microsatellite markers, selected following an initial screen of the parents using 592 microsatellite primer pairs. The chromosomal locations and primer sequences are given in http://wheat.pw.usda.gov/GG2/ (Roder et al. 1998; Somers et al. 2004). The procedure was repeated on the BC_2F_1 materials. BC_2F_2 homozygotes at *Gpc-B1* were selected by progeny tests on 10 plant samples because marker *Xucw108* is dominant. Homozygous *Gpc-B1* BC_2F_3 families were screened with *Xgwm297* to identify those carrying the TGW QTL (Mir et al. 2012). BC_2F_4 populations were generated from selected homozygous plants.

DNA isolation, PCR conditions and electrophoresis

DNA was isolated from one-month-old plants using a CTAB method (Saghai-Maroof et al. 1984). Each PCR in a 20 μL volume was formulated as described by Vishwakarma et al. (2014). *Xucw108* and *Xgwm297* amplicons were resolved in 2.5 % agarose gels. PCR products for background selection were separated in 10 % polyacrylamide gels and visualized by silver staining.

Agronomic performance and assessment of end-use quality

Each BC_2F_4 selection, and both parents, were grown in three–row, 3 × 3 m plots with 22.5 cm between rows

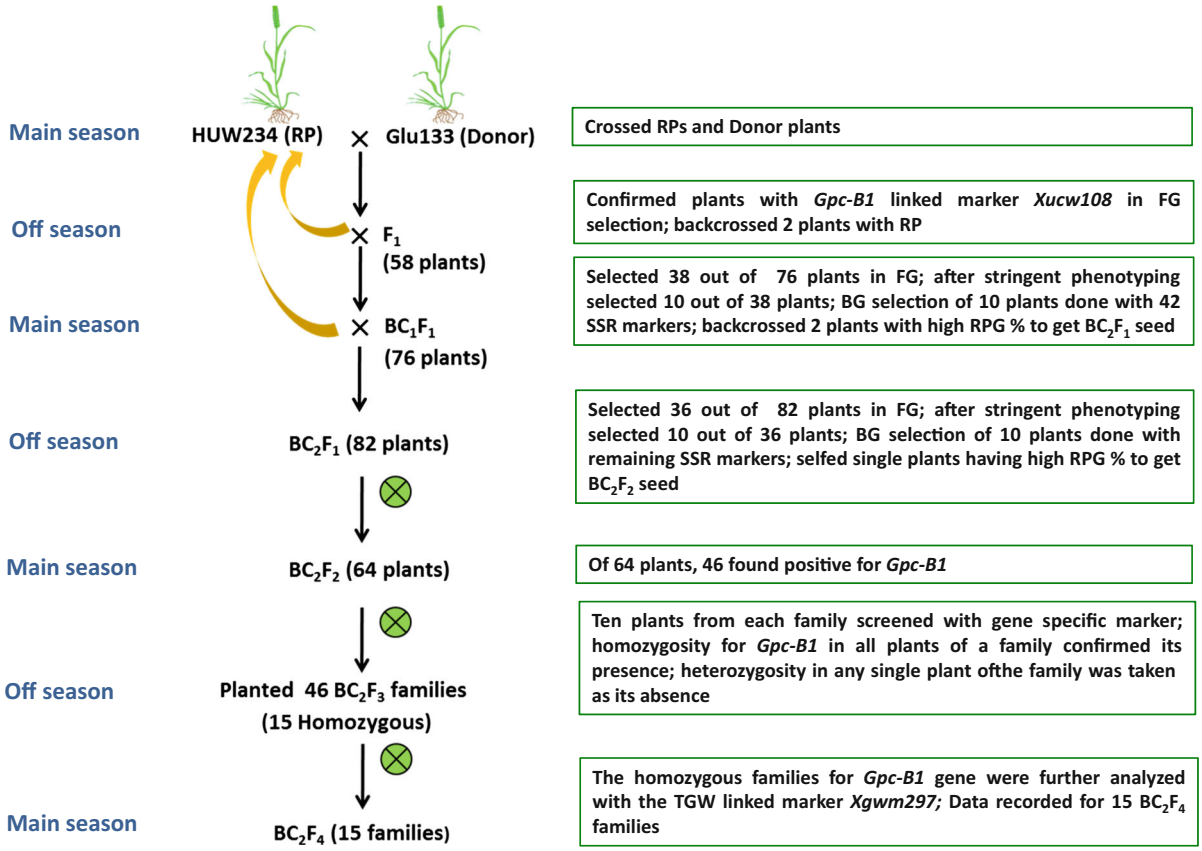

Fig. 1 Crossing and MAS selection scheme to improve GPC and TGW in cv. HUW234. The numbers of plants selected at each generation are indicated in *parentheses*. *RP* Recurrent parent, *FG* foreground selection, *BG* background selection, *RPG* recurrent parent genome

during the 2012–2013 main season at Varanasi. Recommended fertilizer treatments (120 kg N, 60 kg $P2O_5$ and 40 kg K_2O per ha) were applied and the trial managed as a commercial crop (Vishwakarma et al. 2014). P and K dressings were given at sowing, and the N application was split between 50 % at sowing, and 25 % each at the time of first (21 days after sowing) and second (45 days after sowing) irrigations. Data from ten plants per family were recorded for plant height (cm), number of tillers per plant, DM (number of days to maturity), spike length (cm), number of spikelets per spike, TGW, and yield per plant (YPP) in BC_2F_1. BC_2F_4 grain yields were obtained from 3 × 3 m row plots. BC_2F_3 grain was dried to a moisture content of 12 %, then used to measure GPC (%) and zinc (ppm) and iron (ppm) contents. GPC was obtained using an Infratec 1241 Grain Analyser (Foss, Hilleroed, Denmark), and Zn and Fe contents were measured using an X-ray fluorescence device (EDXRF X-Supreme 8000 spectrometer) following the procedure of Paltridge et al. (2012).

Recovery of recurrent parental genotypes

The proportion of the recurrent parent genome (RPG) in each backcross derivative was estimated from:

$$\text{RPG } \% = \frac{2(\text{R}) + (\text{H})}{2\text{N}} \times 100$$

where R represents the number of homozygous recurrent parent marker alleles, H, the number of heterozygous marker loci, and N, the number of background selection marker loci. The level of similarity between the recurrent parent and the BC_2F_4 derivatives was a measure of the effect of

marker selection on the recurrent parent genome. Graphical genotypes were generated with GGT software (Van Berloo 1999).

For determining significant differences in performance between the introgressed families and the HUW234 recurrent parent, multiple comparisons were made of least square means using a significance level of $\alpha = 0.05$ by a two-sided Dunnett test (Dunnett 1955) in SPSS 16.0 (SPSS Inc. 2007) software.

Results

The *Xucw108* amplicon derived from the cv. Glu133 template was 217 bp, whereas there was no amplification from HUW234. Two F_1 HUW234-09 plants backcrossed to HUW234 produced 76 progeny, of which 38 carried the Glu133 *Xucw108* allele. The use of 86 polymorphic background selection markers, along with phenotypic selection for the recurrent parent phenotype, identified families HUW234-09-69 and -36, for which the RPG recoveries were 81.8 and 79.5 %, respectively (Table S1). These plants were used to produce 82 BC_2F_1 progeny, 36 of which had the *Xucw108* allele. The latter plants were subjected to background genotypic selection (44 microsatellite loci) and phenotyping (Table S2). The BC_2F_1 selection with the highest RPG (HUW234-09-69-8, 91.9 %) generated 64 BC_2F_2 progeny, of which 46 retained the *Xucw108* allele in the hetero- or homozygous state. Progeny testing identified 15 BC_2F_3 families that were homozygous for the *Xucw108* allele. These 15 BC_2F_3 families were used to generate BC_2F_4 progeny that were evaluated for agronomic performance (Table 1). Six of a further 48 microsatellite loci located near *Gpc-B1* on chromosome 6B were polymorphic between the recipient and donor cultivars (Fig. 2). Based on genotyping with these six markers, nine lines with the shortest introgressed segments were selected for further investigation.

All of the selected BC_2F_4 families had at least 13 % protein, compared to 11.1 % for the recurrent parent. The range in GPC was 13–14.8 % (Table S3). Fe contents of the 15 families ranged from 40.3 to 49.3 ppm, compared to 38 ppm for HUW234; and Zn contents ranged from 41.4 to 55.7 ppm, well above the recurrent parent level of 28 ppm (Table S3). All characteristics of the selected families were similar to HUW234 (Table S4; Fig. 3); DM ranged from 110 to 117 days (mean 112 days), close to that of cv. HUW234; and mean spike length ranged from 8.0 to 11.0 cm, with a mean across families somewhat below

Table 1 Comparison of the parents and 15 HUW234*4 × Glu133 introgression lines for agronomic traits using Dunnett tests

Parent/line	Trait						
	DM	PH	SPL	SPN	TLN	TGW	GY
HUW234	112	99	43.6	11	7	37.3	44.7
Glu 133	120	82.7	49.4	11	11	41.2	46.3
HUW234-09-21	2.00	−21.9*	1.60*	−1.00	3.00*	4.10*	2.00
HUW234-09-47	−2.00	−9.9*	0.70	−2.00*	3.00*	2.70*	1.00
HUW234-09-48	−2.00	−0.80	7.50*	−2.00*	2.00*	4.60*	4.00*
HUW234-09-49	−1.00	−9.7*	1.70*	0.00	4.00*	4.30*	0.70
HUW234-09-65	−1.00	−5.8*	5.80*	−1.00	5.00*	5.10*	1.00
HUW234-09-112	3.0*	−1.60	3.70*	−2.00*	5.00*	1.50*	0.20
HUW234-09-114	5.0*	−3.6*	4.50*	−2.00*	4.00*	−0.50	0.40
HUW234-09-116	3.0*	−9.7*	4.00*	0.00	4.00*	4.20*	0.50
HUW234-09-117	−1.00	−20.1*	5.90*	−1.00	5.00*	4.70*	1.50
HUW234-09-128	−2.00	−11.4*	4.60*	−3.00*	2.00*	−0.20	0.70
HUW234-09-213	−1.00	−2.8*	2.10*	−3.00*	1.00	3.90*	0.00
HUW234-09-214	0.00	−8.2*	5.50*	−3.00*	1.00	4.50*	−1.50
HUW234-09-239	2.00	−6.5*	2.30*	−2.00*	2.00*	1.10	0.10
HUW234-09-255	−2.00	−1.70	−0.10	−1.00	4.00*	4.30*	−0.80
HUW234-09-256	0.00	−7.3*	8.50*	−2.00*	4.00*	4.50*	0.50
SE±	0.821	0.810	0.480	0.400	0.394	0.410	0.990

Positive and negative values are releative to HUW234

DM Days to maturity, *PH* plant height, *SPN* spikelet number, *SPL* spikelet length, *TLN* number of tillers, *TGW* 1000-grain weight, *GY* grain yield qt/ht, *SE* standard error

* Significantly different at P = 0.05 level

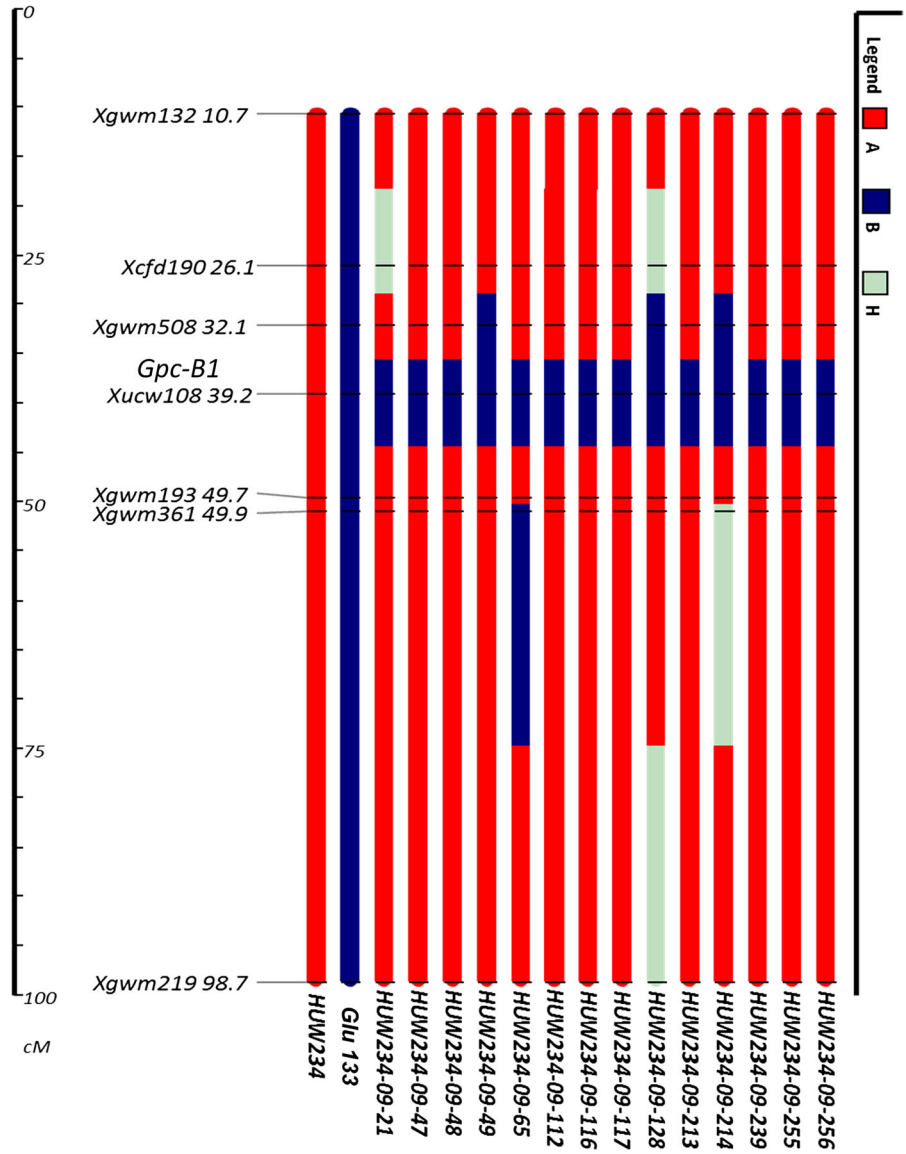

Fig. 2 Graphical representations of the selected BC_2F_4 lines with respect to the *Gpc-B1* region on chromosome 6B. *Colored segments* represent segments from the donor, RP and heterozygotes. (Color figure online)

that of the recurrent parent (9.3 vs 11.0 cm); maximum effective tiller number was superior to HUW234 and TGW ranged between 38.4 and 42.4 g, 2.9 and 13.7 % higher than HUW234. Thirteen selected lines out-yielded HUW234; the best by up to 8.9 %, while two were lower yielding.

The performances of BC_2F_4 families were evaluated by Dunnett test. This test treats one family as a control, and compares all others to it. Dunnett tests were significant ($P < 0.05$) for 72 % of 150 comparisons between introgressed families and recurrent parent HUW234 (Table 2). We found significantly higher GPC, Fe and Zn contents in almost all BC_2F_4 families compared to HUW234. Dunnett tests were significant ($P < 0.05$) for days to maturity, plant height, spikelet number, spikelet length, number of tillers, TGW and GY for 3, 12, 13, 9, 13, 12 and 1 BC_2F_4 families, respectively.

Discussion

The addition of genotype to conventional phenotypic selection succeeded in recovering over 90 % of the

Fig. 3 BC_2F_4 families selected for the presence of *Gpc-B1* and TGW compared to the recurrent parent

Table 2 Comparison of the parents and 15 HUW234*4 × Glu133 introgression lines for GPC and Fe and Zn contents using Dunnett tests

Parents/lines	Traits		
	GPC (%)	Fe (ppm)	Zn (ppm)
HUW234	11.15	38	28
Glu 133	14	45.2	49
HUW234-09-21	2.55*	4.00*	14.10*
HUW234-09-47	2.75*	7.20*	23.60*
HUW234-09-48	3.65*	8.00*	20.30*
HUW234-09-49	2.65*	8.30*	18.00*
HUW234-09-65	2.25*	5.60*	16.40*
HUW234-09-112	1.85*	2.50*	13.40*
HUW234-09-114	2.55*	9.30*	21.90*
HUW234-09-116	1.85*	3.30*	19.00*
HUW234-09-117	2.65*	3.20*	20.20*
HUW234-09-128	2.05*	2.30*	27.70*
HUW234-09-213	2.35*	11.30*	21.80*
HUW234-09-214	1.95*	10.40*	20.70*
HUW234-09-239	2.75*	7.40*	17.80*
HUW234-09-255	2.05*	3.30*	15.90*
HUW234-09-256	2.25*	7.40*	21.80*
SE±	0.50	0.23	0.41

GPC (%) Grain protein content, *Fe* iron content, *Zn* zinc content, *SE* standard error

* Significantly different at P = 0.05

genome of the recipient parent after two backcrosses (the average recovery using only foreground selection is 87.5 %) and a significant improvement to the GPC was attributed to the introduction of *Gpc-B1*. In addition to significantly increased GPC, both Fe and Zn contents of the grain were higher than for HUW234, and progress was also made in improved TGW and some other agronomic traits. Grain Fe and Zn contents were shown to be positively correlated with GPC (Distelfeld et al. 2006; Uauy et al. 2006), and ascribed to a pleiotropic effect of *Gpc-B1* rather than linkage. The significant progress in TGW appeared to be novel as the usual negative correlation between yield and GPC was broken without affecting days to maturity. The beneficial effects on TGW and other traits could be due to pleiotropic effects of *Gpc-B1* as experienced in previous studies (Kumar et al. 2011; Tyagi et al. 2014; Vishwakarma et al. 2014). Pyramiding of eight QTLs including those of GPC and TGW using MAS was demonstrated by Tyagi et al. (2014) in the then most popular Indian cultivar PBW343. Likewise Kumar et al. (2011) introgressed *Gpc-B1* into ten elite Indian varieties, and Vishwakarma et al. (2014) successfully introgressed *Gpc-B1* into HUW468 by backcrossing. In these studies only HUW468 was a NEPZ cultivar; the others were from the north western plains region.

MAS has made a significant impact on crop improvement programs (Gao et al. 2005; Barloy et al. 2007; Gupta et al. 2010; Kumar et al. 2011; Tyagi et al. 2014). Backcrossing strategies are particularly suited to MAS approaches, with markers providing opportunities for both foreground and background selection. MAS allows an accelerated recovery of the recipient parent genotype, the actual purpose of backcrossing. Foreground selection requires a tightly linked single marker (such as *Xucw108*), or less closely linked flanking markers (such as *Xgwm508* and *Xgwm193* for *Gpc-B1* (see Davies et al. 2006) in order to ensure selection of the target gene and its associated trait. To select superior recombinant lines with minimal donor parent contributions we used six flanking markers (*Xgwm132, Xcfd190, Xgwm508*, *Xgwm193, Xgwm361 and Xgwm219*) covering the gene *Gpc-B1* (Olmos et al. 2003; Vishwakarma et al. 2014).

The goal of the present work was to obtain an improved version of a well-established cultivar. The *Xucw108* marker used to track the presence of *Gpc-B1* is dominant and could not discriminate between the homozygous and heterozygous conditions. While this is not a problem during backcrossing, it necessitates progeny testing of the first selfing generation (BC_2F_2). Phenotypic selection for agronomic features and genotypic selection based on microsatellites to restore the HUW234 genetic background were also undertaken. To select both GPC and TGW in the same lines, we followed a stepwise screening method, by which we screened for *Gpc-B1* with linked marker *Xucw108* up to BC_2F_3, along with phenotypic selection for agronomic traits with special emphasis on TGW since the donor Glu 133 had 15 % higher TGW than the recurrent parent. Following phenotypic selection, screening for TGW was conducted using the linked marker *Xgwm297* in BC_2F_3 (Mir et al. 2012). A benefit of this strategy was to reduce the number of progenies requiring screening during the selfing generations (Kumar et al. 2011).

Although HUW234 is still grown over a large area in the NEP zone it is disadvantaged by modest GPC and low TGW, traits that were targeted in this work. Background selection was considered to be desirable in order to reconstitute the overall genetic background HUW234 as rapidly and thoroughly as possible. Phenotypic selection as recommended by previous workers (Kumar et al. 2011; Tyagi et al. 2014; Vishwakarma et al. 2014) was also used. We believe this approach provides a model for breeding for wheat quality in areas such as the NEP zone of India and neighboring countries with limited investments in wheat improvement programs.

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Compliance with ethical standards

Conflicts of Interest The authors declare that they have no conflicts of interest.

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