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Introgression of the high grain protein gene *Gpc-B1* in an elite wheat variety of Indo-Gangetic Plains through marker assisted backcross breeding

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ABSTRACT

Grain protein content (GPC) in wheat has been a major trait of interest for breeders since it has enormous end use potential. In the present study, marker-assisted backcrossing (MABC) was successfully used to improve GPC in wheat cultivar HUW468. The genotype Glu269 was used as the donor parent for introgression of the gene *Gpc-B1* that confers high GPC. In a segregating population, SSR marker Xucw108, with its locus linked to *Gpc-B1* was used for foreground selection to select plants carrying *Gpc-B1*. Background selection, involving 86 polymorphic SSR markers dispersed throughout the genome, was exercised to recover the genome of HUW468. For eliminating linkage drag, markers spanning a 10 cM region around the gene *Gpc-B1* were employed to select lines with a donor segment of the minimum size carrying the gene of interest. Improved lines had significantly higher GPC and displayed 88.4–92.3 per cent of the recurrent parent genome (RPG). For grain yield, selected lines were at par with the recurrent parent HUW468, suggesting that there was no yield penalty. The whole exercise of transfer of *Gpc-B1* and reconstitution of the genome of HUW468 was completed within a period of two and half years (five crop cycles) demonstrating practical utility of MABC for developing high GPC lines in the background of any elite and popular wheat cultivar with relatively higher speed and precision.

1. Introduction

Wheat (*Triticum aestivum* L. em. Thell.) is one of the most important food crops in the world with global yield over 700 million tons annually, and providing 20% of the total calorie intake for the world population [1,2]. Wheat is grown in ~128 countries involving all the continents of the world, the top five leading producers being China, India, United States of America, France and Russia [3]. Among cereals involved in cross-border trading, wheat has the highest tonnage, with an estimate value of 135 Mt in 2012/13, with major importing countries being in Asia and Africa [3]. In India, wheat is a staple food for more than 65% of the population with annual production of around 94 Mt [4]. The demand of wheat is expected to keep growing due to steady population increase. The demand for better quality wheat grain will also increase due to increased urbanization [5]. Although, many Indian wheat varieties have been characterized for various end products, these varieties and their traits are spillovers of the routine breeding program for high yield and disease resistance, rather than the product of a systematic quality breeding program [6]. The current challenges for wheat breeding programs around the world are to maintain or improve agronomic performance along with improvement in wheat quality, thus maintaining competitiveness in the increasingly discriminating international market [7].

Wheat is a crop with several end-use products such as pasta, macaroni, biscuit, chapatti and bread. These end-use products differ for their requirements of GPC and the type of wheat. In general, GPC in Indian wheat cultivars is relatively lower than the standards of international market [7]. Under these conditions, either we need to accept wheat with a lower GPC or apply more nitrogen to achieve the desired level of GPC, since some increase in GPC through increased nitrogen application has been documented [8].

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GPC is also an important for bread-making quality, which is known to depend upon both, the content and composition of grain protein [9,10].

According to an estimate of World Health Organization [11], over 3 billion people were deficient in key micronutrients Zn and Fe, and about 160 million children below the age of 5 lack adequate protein, amounting to malnutrition [11–13]; this suggests that not only GPC, but also the content of micronutrients like Zinc (Zn) and Iron (Fe) need to be improved for improving the grain quality of wheat. Progress in breeding for high GPC wheat has been rather slow, because GPC is controlled by a complex genetic system and is also influenced by the environment, thus making it difficult to select effectively for this trait [8,14,15]. However, GPC and grain yield are reported to be negatively correlated [8,14], making it difficult to breed for high GPC without a yield penalty. Although the theoretical basis for this inverse correlation has been debated [16], high GPC cereals are unlikely to be commercially successful without a financial incentive to growers.

Yield is an essential trait for commercial success of a variety, hence developing wheat varieties combining improved grain quality with high grain yield is an important goal in wheat breeding. However specific quality parameters such as protein %, grain hardness, bread loaf volume and biscuit spread factor are getting increased attention due to growing demand for industrial end-products such as bread, biscuit, cake, pasta, etc. Wheat varieties with high GPC (>12%) and micronutrients (Zinc and Iron) are also important for providing nutritionally improved wheat based diet and for enhancing export potential of wheat. In addition, high yielding wheat with superior internal (protein %) and external (grain weight, luster) traits is easy to market and may provide extra cash to poor farmers. In India, although wheat is overwhelmingly consumed in the form of chapatti [7], the demand for other end-products like bread, biscuit, pasta and cakes is growing with expanding urbanization (estimated urban population in 2020 = 550 million) and growing industrialization [5]. Therefore, it is important to combine the high grain yield with better grain quality to meet the twin challenges of nutritionally superior and high quality wheat products [6].

In the recent past, the introgression and pyramiding of major genes/QTL for different traits through marker-assisted selection (MAS) has proved successful in wheat [17–24]. Several RFLP, SSR and CAPS markers were reported to be closely linked with high GPC locus (*Gpc-B1*) on the short arm of chromosome 6B [25–28]. Among these markers, a tightly linked marker at a narrow distance of 0.1 cM within a physical location of a 250 kb, was the SSR marker Xuhw89 for the locus *Gpc-B1* [29]. Since *Gpc B1* has been cloned and characterized, a "gene-specific" marker is also available for this locus [30]. The introgression of *Gpc-B1* has been achieved for improving GPC without yield penalty mostly in the developed countries [8,31], although a report of successful introgression of *Gpc-B1* in 10 elite varieties of India is also available [23].

Conventional breeding program, if supplemented with MAS, can become cost and time-effective [20]. For the last more than 20 years, MAS is being used on a large-scale in several countries including USA, Australia, Canada, and Mexico (CIMMYT). In majority of these MAS programs in wheat, MABC involving backcrossing has been deployed to ensure maximum recovery of the genome and particularly, the carrier chromosome [32]. According to a recent report, more than 60 genes/markers are being deployed for wheat improvement through MAS [33], of which more than 20 traits/genes belong to grain quality like gain hardness, dough strength and swelling volume [34]. Molecular markers for quality traits (protein content, pre-harvest sprouting tolerance, gluten strength and grain weight) are also being increasingly used in Indian wheat breeding program successfully [19,22–24].

The present study was planned to improve GPC through MABC coupled with stringent phenotypic selection into the genetic background of wheat cultivar HUW468, which is a very promising cultivar with good performance under conventional and zero-tillage conditions in the North Eastern Plains Zone (NEPZ) of India [6].

2. Materials and methods

2.1. Plant materials

Plant materials used in the present work included recipient parent HUW468 and a donor parent Glu269 with high GPC procured from Punjab Agriculture University, Ludhiana, Punjab. HUW468 (CPAN-1962//TONI/LIRA'S'/PRL'S'). Glu269 is a high yielding, disease resistant and double dwarf wheat variety released in 1999 for timely sown high fertility irrigated conditions of NEPZ and since then has maintained resistance and popularity among farmers due to its superior agronomic performance. The donor parent Glu269 (DBW16/GluPro//2*DBW16) is a wheat breeding line that is resistant to yellow and brown rusts and is also amenable to late sowings. It also carries higher level of resistance to spot botch relative to all the existing varieties, and has been identified for cultivation in the North Western Plains Zone (NWPZ) of India. Since, it had GluPro as one the parents in its pedigree, it carries *Gpc-B1* providing higher level of GPC (>14%) relative to the recurrent parent HUW468 with only 10% GPC.

2.2. Molecular marker used

Seven GPC linked markers (Xuhw89, QGpc.ccsu-2D.1/2DL, CAPS/ASA/XNor-B2, Xwmc415, Xucw108 and Xucw109) were validated based on published results [26,29,30,35,36]. Out of seven, only one (Xucw108) [30] was selected for foreground selection. Primers were synthesized from Eurofins Genomics India Pvt Ltd., Bangaluru, India. To analyze the recovery of RPG of the segregating backcross progeny during background selection, a total of 744 SSR (simple sequence repeats) markers covering all the 21 chromosomes of wheat were selected to detect polymorphism between HUW468 and Glu269. The primer sequences were obtained from http://wheat.pw.usda.gov/GG2/index.shtml (GrainGenes 2.0: A database for Triticeae and Avena), Somers et al. [37] and Röder et al. [38]. Primers that were polymorphic between parents were used for background selection.

2.3. MABC breeding

2.3.1. DNA isolation, PCR conditions and electrophoresis

DNA isolation of parental genotypes and backcross progenies was carried out from one-month-old plants using a modified CTAB method [39]. The PCR amplification was carried out in a reaction mixture of 20 μL containing 200 μM dNTPs (MBI; Fermentas, Lithuania, USA), 0.75 U Taq DNA polymerase (MBI; Fermentas, Lithuania, USA), 5 pmole of each primer, 20–30 ng template DNA and 10 X PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$). PCR cycle consisted of an initial denaturation for 5 min at 94 °C, followed by 40 cycles each with 1 min at 94 °C, 1 min at annealing temperature (which differs for different primers), with a final extension of 7 min at 72 °C. The amplified products were resolved on 2.5% agarose gel for the foreground selection (involving use of gene specific marker Xucw108), and on 10% PAGE (followed by silver staining for visualization) for the background selection (used for RPG recovery).

2.3.2. Breeding scheme

MABC scheme [32] was followed to transfer the *Gpc-B1* gene from Glu269 into the genetic background of HUW468. Recurrent

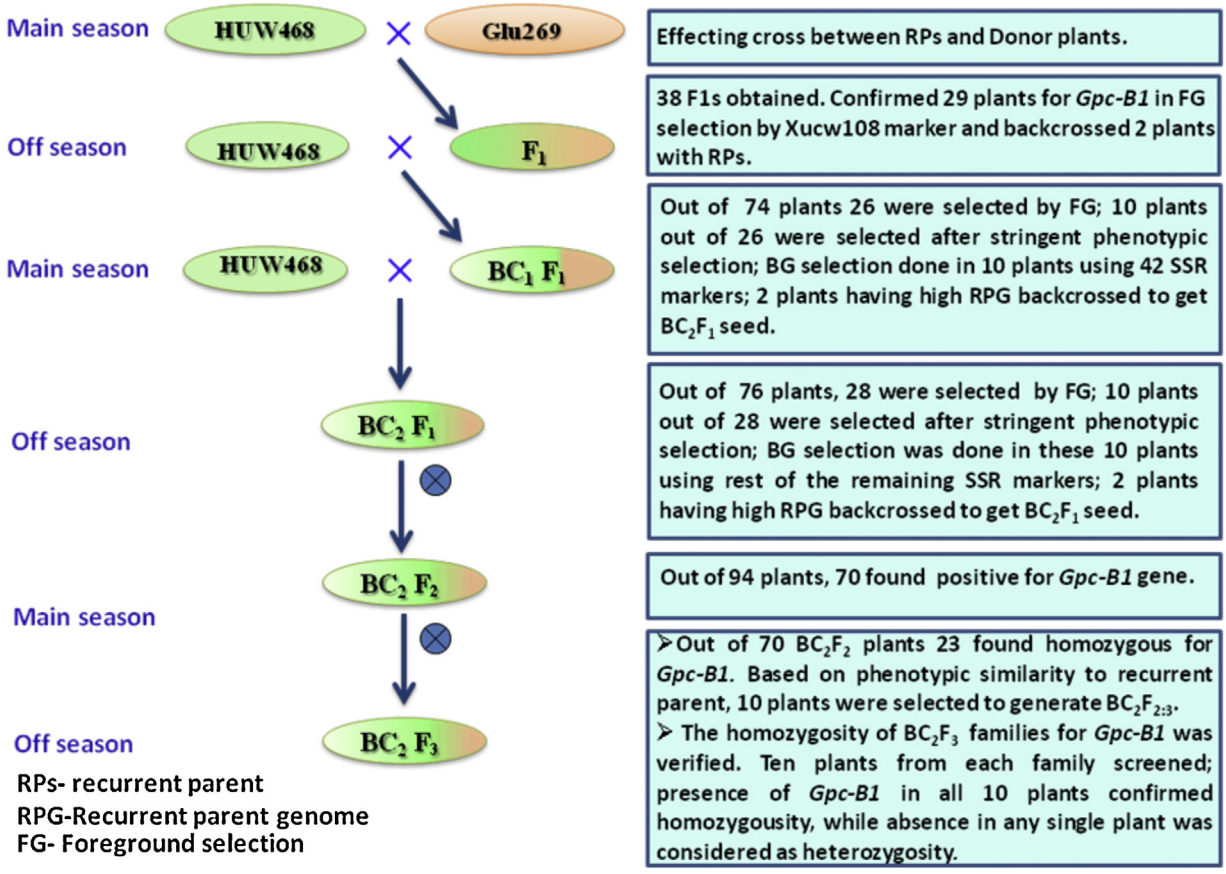

Fig. 1. Development of HUW468 for protein content with details of markers used for foreground and background selection and numbers of plants selected in each generation.

parent HUW468 was used as female and crossed with Glu269 as male to generate the F_1 seeds. The cross was designated HUW468-09. Detailed flowchart of MABC approach deployed to transfer *Gpc-B1* is given in Fig. 1. Following testing hybridity of F_1 plants using gene-linked marker, two true F_1 plants were crossed with the recurrent parent to obtain BC_1F_1 seeds. Both foreground and background selections were deployed. Foreground selection for a trait using molecular marker facilitates identification of positive plants for the gene of interest at early plant stage and thus enables a breeder to reduce the population size by around 50% in a backcross breeding program [40,41].

Foreground selection for desirable BC_1F_1 plants with *Gpc-B1* gene was exercised using gene-linked marker. The plants possessing *Gpc-B1* were subjected to further phenotypic selection to identify top ten plant with desirable recurrent parent phenotype (RPP) for analyzing RPG recovery. One selected plant with high RPG recovery was then backcrossed to produce BC_2F_1 seed. Like BC_1F_1, foreground selection (for *Gpc-B1*), phenotypic selection (for plants having agronomic similarity to recurrent parent) and background selections (for RPG recovery) were again exercised to identify suitable plants for obtaining BC_2F_2 seeds. The selected BC_2F_2 plants with high RPG recovery were selfed and advanced up to BC_2F_3 using marker-assisted pedigree method of selection. The homozygosity of BC2F3 families for *Gpc-B1* gene was further confirmed by screening of randomly selected 10 plants from each family using the marker Xucw108 associated with *Gpc-B1*.

2.3.3. Estimation of linkage drag

Analysis of the size of introgressed donor segment on carrier chromosome was conducted to eliminate linkage drag [42]; this was possible through the use of six additional markers (Xgwm132, Xcfd190, Xgwm193, Xgwm361, Xgwm219 and Xcfd2110) from the 10 cM genomic region on either side of *Gpc-B1* marker Xucw108 given in http://wheat.pw.usda.gov/GG2/index.shtml (GrainGenes 2.0: A database for Triticeae and Avena).

2.3.4. Evaluation of MABC lines for agronomic performance

Field trials to evaluate MABC lines were conducted during main Rabi season (2008–2009 to 2011–2012) at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi, Uttar Pradesh, India and during off-season (2009–2011) at IARI, Regional Station, Wellington, Tamil Nadu during 2008–2009 to 2011–2012. In the different backcross generations, plants were hand sown in three rows of 2 meter with row to row spacing of 22.5 and plant to plant distance of 20 cm. After every third progeny row, recurrent parent HUW468 was planted as a check for facilitating morphological evaluation. Recommended agronomic and fertilization (120 kg N: 60 kg P_2O_5: 40 kg K_2O per ha) practices were followed at both locations and seasons. Zinc was not applied. Full doses of K_2O and P_2O_5 were applied at sowing; nitrogen was supplied in split applications, with 60 kg N per ha at sowing, 30 kg N per ha at the first irrigation (21 days after sowing), and 30 kg N per ha at the second irrigation (45 days after sowing) [56]. Data on phenotypic traits in BC_1F_1, BC_2F_1 and BC_2F_2 generations was recorded on single selected plants that were positive for *Gpc-B1* gene, as determined through associated marker. In BC_2F_3 generation, data were taken from ten randomly selected plants from each homozygous family for *Gpc-B1* identified in BC_2F_2. Following agronomic traits were used for recording data on phenotypes: days to maturity (DM), plant height (PH), number of effective tillers/plant (TP), spike length (SL), spikelet number (SN), thousand grain weight (TGW) and grain yield per plant (GY).

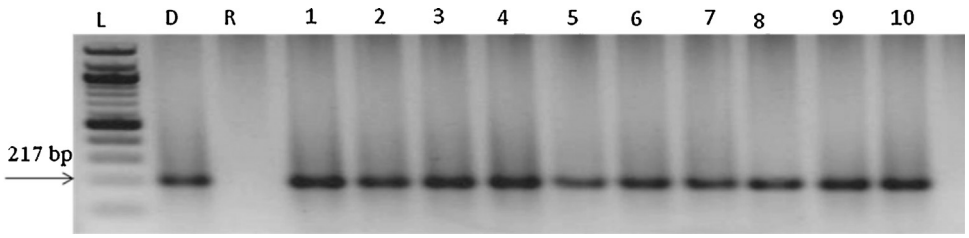

Fig. 2. Molecular profile of BC_2F_3 families of HUW468 × Glu269 cross for confirming homozygosity for *Gpc-B1* by marker Xucw108. L-100 bp ladder, D-Glu269, R-HUW468, lane 1–10 for each BC_2F_3 families.

2.3.5. Evaluation of MABC lines for protein, zinc and iron content

The seeds of BC_2F_3 lines of HUW468 having *Gpc-B1* gene were analyzed by Infratec™ 1241 Grain Analyser, Foss, Denmark. A total of 5–7 g sample of clean grain was used for Zn and Fe analysis, based on X-ray fluorescence (X-Supreme8000, Oxford Instruments, Oxford, UK). Grain protein content (GPC) was estimated using an Infratec 1241 Grain Analyser (Foss, Hilleroed, Denmark). Protein data was recorded at 12% of seed moisture level in (%) unit, while data on Zn and Fe content were taken as particle per molecule (ppm) [43].

2.3.6. Statistical analysis and determination of recurrent parent genome recovery

The data recorded as above was used for estimation of progeny means for each replication. The statistical analysis was performed on the basis of progeny means in each replication by the PAST software [44] and Microsoft excel. The extent of RPG recovery in backcross generations was calculated using the following formula.

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

where, R = number of marker loci homozygous for recurrent parent allele; H = number of marker loci still remaining heterozygous and N = total number of polymorphic markers used for background analysis. In BC_2F_3, the genetic similarity between the recurrent parent HUW468 and the high GPC introgressed lines was determined through data on morphological features. To check the robustness of the clustering, boot-strap analysis was carried out. Further graphical genotyping was done by using GGT software [45].

3. Results

3.1. MABC for GPC

Of seven markers for *Gpc-B1*, only two (Xucw108 and Xucw109) were found polymorphic between parents. The pipeline (crossing program) followed for MABC to incorporate *Gpc-B1* into HUW468 is presented in Fig. 1. Details of 10 phenotypically superior plants each among 26 BC_1F_1 and 28 BC_2F_1 plants carrying *Gpc-B1* are available in Supplementary Tables S1 and S2, respectively. The homozygosity of BC_2F_3 families for *Gpc-B1* by screening of randomly selected 10 plants from each family using the marker Xucw108 is given in Fig. 2.

3.2. Grain quality in BC_2F_3

A summary of data on grain quality of 23 BC_2F_3 lines is presented in Table 1. The GPC of the BC_2F_3 lines ranged from 13 to 17.2% compared to 10% in HUW468 and 14.3% in donor parent Glu269. All these 23 lines had a significant increase in the mean value of GPC relative to the recurrent parent HUW468. Similarly, in these 23 BC_2F_3 lines, Fe content ranged from 39.3 to 53.8 ppm, while Zn content ranged from 35 to 54.2 ppm, the Zn and Fe in the recurrent parent HUW468 being 39 and 30 ppm respectively. For Zn and Fe content, all the 23 improved lines except HUW468-09-233 for Fe were superior to the recurrent parent HUW468, and only one line (HUW468-09-131) exceeded the level in the donor.

3.3. Evaluation of MABC lines for agronomic performances

The 23 BC_2F_3 MABC lines showed variable expression of agronomic traits. The plant height among the families ranged from 80-92 cm compared to 85.7 cm of HUW468 (Table 1). Six lines showed significantly higher tillers/plant relative to both parents HUW468 and Glu269. The number of spikelet per spike ranged from 41 to 51, while only one line HUW468-09-6 (12 cm) showed better spike length relative to HUW468 (11.2 cm). The TGW among selected families ranged from 37.8 to 42.4 g as against 35.10 g in HUW468. For TGW, all the 23 lines were better than the recurrent parent HUW468, but none was better than the donor Glu269. For yield per plant, 14 families showed significant improvement over HUW468 (7.85 g), while only one (HUW468-09-95) was better than the donor parent. On the basis of overall performance, three lines (HUW468-09-131, HUW468-09-132 and HUW468-09-59) were considered to be superior to the recurrent parent HUW468.

3.4. Recurrent parent genome recovery

3.4.1. Recovery for whole genome

Out of 744 SSR markers, 106 (14.0%) were polymorphic between both the parents. However, on the basis of the difference in product size (eliminated ≤10 bp difference), 86 SSR (11.5%) were selected for background analysis of 23 BC_2F_3 lines (Table 1); these 86 markers covered all the 21 chromosomes. The per cent RPG recovery among the 23 selected lines ranged from 90.7% (HUW468-09-3 and HUW468-09-6) to 95.4% (HUW468-09-244) (Table 1).

3.4.2. Carrier chromosome recovery with the minimum size of donor segment

As expected, donor segments were present in all the 23 improved lines, and a segment carrying the gene *Gpc-B1* was available in all the lines; this segment was not the minimum possible, so that some linkage drag was unavoidable. A screening for recombinants carrying the minimum size of donor fragment was undertaken using six markers, which were the only polymorphic markers among the 48 flanking markers that were tested for the *Gpc-B1* region. These six markers included Xgwm132, Xcfd190, Xgwm193, Xgwm361, Xgwm219 and Xcfd2110 (Fig. 3). Based on carrier chromosome analysis of 23 lines using these six markers, four (HUW468-09-96, HUW468-09-131, HUW468-09-132 and HUW468-09-244) were such, which did not carry any segment other than the small segment carrying *Gpc-B1*; all other lines carried additional segments, away from *Gpc-B1*.

4. Discussion

Breeding for agronomic and nutritional traits of wheat continues to be important for food security and human health especially in developing countries of south Asia, where demand of wheat is

Table 1
Quality characters and agronomic performance of BC_2F_3 families of the cross HUW 468 × Glu269 with high *GPC-B1* in the background of recurrent parent HUW468.

Grain quality				Agronomic traits							%RPG
Lines/traits	GPC (%)	Zn (ppm)	Fe (ppm)	DM	PH (cm)	SPN	SPL	TLN	TGW	YPP	
HUW468-09-3	14.5	45.8	47.5	107	86	47	10	13	38.80	7.56	90.70
HUW468-09-5	13.5	43.5	53.0	102	92	41	9	12	37.84	8.23	91.86
HUW468-09-6	13.0	43.0	52.3	109	89	45	12	16	41.44	8.45	90.70
HUW468-09-29	13.8	44.3	41.0	105	82	46	10	16	40.04	8.41	91.86
HUW468-09-30	13.8	44.5	43.0	104	80	45	9	16	41.94	8.50	91.86
HUW468-09-56	14.8	50.2	49.4	104	89	50	9	9	41.56	7.56	91.86
HUW468-09-57	13.6	48.4	47.4	102	89	45	9	10	42.43	8.23	93.02
HUW468-09-59	13.3	42.6	52.5	102	84	48	10	10	41.48	8.45	93.02
HUW468-09-88	13.1	48.0	52.0	107	87	51	11	12	38.96	7.90	91.86
HUW468-09-89	13.2	42.2	35.1	102	82	50	10	13	37.08	7.55	91.86
HUW468-09-95	13.2	47.3	44.8	108	85	47	11	15	41.20	9.24	93.02
HUW468-09-96	13.1	43.5	47.2	110	89	46	10	15	41.76	8.40	94.19
HUW468-09-128	13.8	47.2	46.9	104	84	46	10	9	40.40	8.60	93.02
HUW468-09-131	16.4	53.8	54.2	107	85	47	11	12	41.60	7.90	94.19
HUW468-09-132	14.1	44.6	47.0	107	87	48	10	13	40.84	7.85	94.19
HUW468-09-142	17.2	44.0	49.8	103	86	48	9	11	40.44	8.41	93.02
HUW468-09-144	13.3	46.5	43.1	104	87	50	10	12	39.04	8.50	93.02
HUW468-09-233	13.6	39.3	45.4	106	91	45	11	10	41.94	7.56	93.02
HUW468-09-235	13.6	43.7	46.8	104	91	47	10	12	41.56	8.23	91.86
HUW468-09-236	13.7	41.4	49.2	103	83	48	9	13	42.44	7.56	91.86
HUW468-09-241	13.8	43.3	51.1	104	88	45	10	13	41.44	8.23	91.86
HUW468-09-242	14.1	48.4	52.6	102	90	45	11	12	40.04	8.45	93.02
HUW468-09-244	14.2	47.2	43.3	111	89	47	10	14	41.94	7.90	95.35
HUW468	10.0	39.0	30.0	103	85.7	46.5	11.2	9	35.10	7.85	
Glu 269	14.3	45.2	49.0	120	82.6	53.6	11	11	42.24	8.54	
CD (0.05)	0.43	1.38	1.97	1.17	1.39	0.96	0.36	3.93	0.64	0.19	

GPC (%), grain protein content (per cent); Fe (ppm), iron content; Zn (ppm), zinc content, DM, days to maturity; PH, plant height; SPN, spikelet number; SPL, spikelet length; TLN, number of tillers; TGW, 1000-grain weight; % RPG, per cent recurrent parent genome recovery.

growing due to increasing population and income levels. For a long time, conventional breeding methods have given desired success in wheat improvement in the absence of alternative approaches. However conventional methods are time consuming, needing up to 12 years for the development leading to release of a new variety [46]. It has been suggested that conventional breeding methods, if supplemented with MAS, could help reduce time for development of a new cultivar and offer an approach for reliable improvement of elite breeding material [20]. During the last few years, use of molecular tools has grown substantially due to development of high-throughput markers that can be used in a cost-effective manner [33]. There are several examples of successful use of MAS for introgression or pyramiding of major genes/QTL for different traits in wheat [18,21,22,33]. There are also examples of introgression of *GpcB1* through MAS for improving GPC without yield penalty [8,31], but most of these examples are from developed countries. There are at least two reports of its introgression in elite wheat lines of India, one of them involving pyramiding of eight genes including *Gpc-B1* [23,24]. In the present study, *Gpc-B1* was incorporated into elite variety HUW468 using MABC combined with stringent phenotypic selection within a short period. HUW468 is a popular cultivar of NEPZ of India but has low protein content. The derived lines HUW468 (*Gpc-B1*) showed significant improvement in GPC, Fe and Zn as compared to the recipient parent. In addition, the improved lines were superior in TGW, TLN and YPP, while at par for other agronomic traits. Distelfeld et al. [29] reported that GPC was significantly ($P < 0.001$) correlated with grain Zn and Fe content, since the locus *Gpc-B1* (imparting high GPC) on the short arm of chromosome 6B was also effective in increasing Zn and Fe in the grain. In our study, some MABC lines were found to show higher Zn content compared to Fe. Additive main effects and multiplicative interactions (AMMI) analysis of genotype × environment interactions for grain Fe and Zn content also revealed that Fe content in a large measure is genetically controlled, whereas Zn was almost totally dependent on location effects [47].

4.1. Marker assisted background selection with stringent phenotypic selection

The expected average RPG recovery in the BC_2F_3 generation was 93.75%. However, RPG in the final product (HUW468-09-244) in BC_2F_3 was 95.35% as revealed by background analysis with polymorphic SSR markers. Eighty six (86) genome-wide polymorphic SSR markers distributed at an average interval of 5 Mb (~20 cM) were employed to analyze the recovery of RPG in 23 improved lines in the background of HUW468. The higher recovery of RPG was achieved mainly through stringent phenotypic selection followed by background selection using molecular markers. Background analysis exercised through phenotypic evaluation is reported to be useful in efficient recovery of the RPG [48]. It has been suggested that stringent phenotypic selection for the recovery of recurrent parent phenotype is a good substitute for background selection through molecular markers in terms of judicious use of resources [42,49–51]. Recently, the problem of linkage drag has also been reported in rice when stringent phenotypic selection with MAS was used [51]. In the present study, the progenies selected through foreground markers were subjected to phenotypic selection for background traits followed by background analysis using molecular markers. This approach reduces the cost of MAS and also helps to retain useful interactive loci of both the parents [42]. Cost of molecular genotyping in routine marker-assisted breeding programs is an important factor especially in developing countries.

4.2. Marker assisted step-wise background selection (MASBS)

Most of the MABC studies conducted earlier utilized all the background polymorphic markers in early generation for the recovery of RPG [23]. The use of a large number of markers in a single generation is a tedious job and keeps molecular breeders engaged for a longer period of time. In the present study, we utilized stepwise background selection for RPG recovery. We judicially used two markers per chromosomes and six markers on carrier chromosome

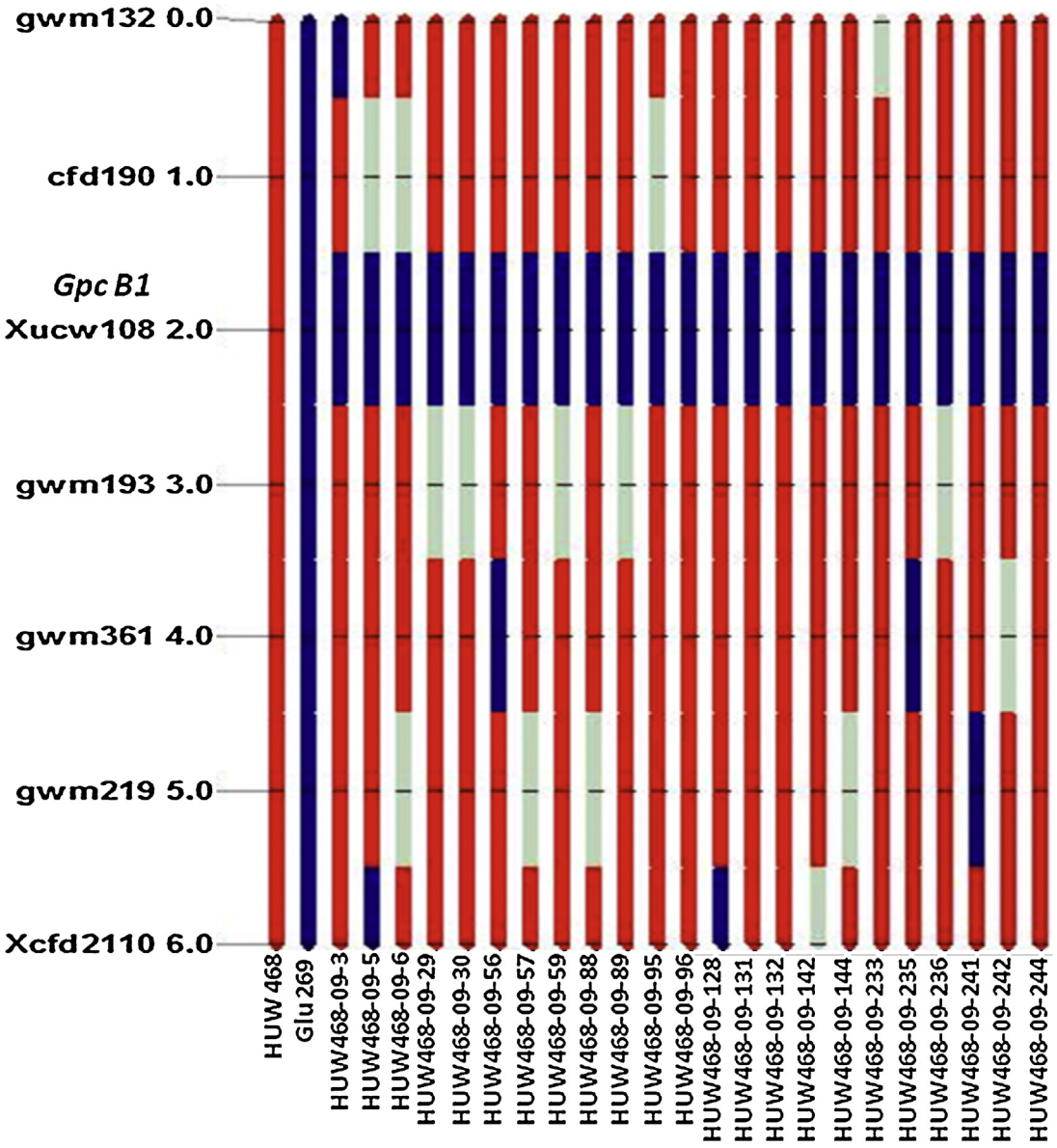

Fig. 3. Analysis of improved lines of HUW468 for introgressed genomic regions of chromosome 6B, including segments carrying *Gpc-B1* (blue segments from donor; red segments from recurrent parent, and gray segments showing heterozygosity). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

in the first backcross generation after stringent phenotypic selection. While the remaining markers out of a total of 86 were used in the second backcross. The markers that were heterozygous in BC_2F_1 were again used in BC_2F_2. In this manner, RPG recovery could be fastened in a cost-effective manner. The application of this step-wise background selection has also been demonstrated in rice [52].

4.3. Linkage drag and its elimination

During introgression of desirable/target gene(s) in the backcross-breeding program the probability of other closely linked genes getting introgressed is quite high, which affects the performance of the end product [53]. The probability of undesirable end product due to linkage drag is high, if the donor genotype is of unadapted/wild type. This can be significantly reduced by selecting the genomic region of recurrent parent flanking the desirable gene(s) [42,54].

In the present study, the donor parent Glu269 (DBW16/GluPro//2*DBW16) carrying the gene *Gpc-B1* was a non-adaptive genotype for NEPZ and had poor grain texture and chapatti making quality. This could be because the introgressed gene *Gpc-B1* for high GPC was originally identified in wild emmer wheat *Triticum turgidum* ssp. dicoccoides accession FA15-3 (referred hereafter as DIC; [55]). Hexaploid cultivar Glu-pro was developed from a three-way cross between two bread wheat cultivars and the *dicoccoides* accession earlier used to develop the substitution line LDN(DIC6B) [26]. Therefore, the only option to get rid of undesirable effects, particularly poor grain texture and chapatti quality, was to minimize linkage drag of the donor parent Glu269 used for introgressing the gene *Gpc B1*. For this, we used flanking markers (*Xcfd190* and *Xgwm193*) on the carrier chromosome 6B. These markers were approximately 12 cM apart from each other covering the gene *Gpc-B1*. In a previous study by Kumar et al. [23] flanking markers *XNor-B2* (*CAPS*) and Xgwm193 were used for selection of *Gpc-B1* gene to eliminate the risk of losing the gene segment due to lack of closely linked markers. Later, more precise (Xucw108 *and* Xucw109) markers (within 500 bp region in gene) became available for the transfer of *Gpc-B1*

segment [30]. This facilitates the flanking PCR markers XNor-B2, Xucw66, Xgwm508 and Xgwm193 to minimize the linkage drag during MAS [27]. One of the BC_2F_3 line HUW468-09-244 had a single small donor segment (no other segment in the vicinity of *Gpc-B1*) in the carrier chromosome 6B in the region flanking the *Gpc-B1* gene. In this study we utilized carrier chromosome genome recovery in the plants selected through foreground selected in each of the backcross generations. After foreground selection in BC_2F_1, we covered genome of all 21 chromosomes by utilizing single marker for each chromosome along with 6 markers for carrier chromosomes, covering 12 cM region of recurrent parent flanking the donor gene segment. Previously, two backcrosses along with maximum recovery of carrier chromosomes were reported in wheat [32].

4.4. Agronomic performance in the backcross derived lines

Marker-assisted backcrossing has been used for introgression of the gene *Gpc-B1* in a few landmark wheat cultivars in India [23,24]. However, only seven desirable selected progenies showed high GPC without yield penalty. The RPG recovery varied from 72.0 to 95.71%. However in the present study, the improved HUW468 derivatives showed high GPC as well as agronomic superiority. Due to directional phenotypic selection for yield and yield related traits, we obtained superiority in four HUW468 derived lines (HUW468-09-95, HUW468-09-131, HUW468-09-132 and HUW468-09-59) in the background of HUW468. Thus, the present work is a successful example of an integrated approach of combining phenotypic selection with marker assisted backcross breeding in wheat for introgression of *Gpc-B1*, high grain weight and leaf rust resistance in the background of HUW468. The results indicate that the marker-assisted selection with stringent phenotypic selection is effective in rapid recovery of the RPG in a time and cost-effective manner.

Author's contribution

All authors contributed significantly in different ways. In the planning, coordination, conduct of the field experiments, data collection, tabulation, field and molecular analysis, interpretation and writing of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical standards

All experiments complied with the current laws of the India, the country in which they were performed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cpb.2014.09.003.

References

[1] F.J.P.H. Brouns, V.J. van Buul, P.R. Shewry, Does wheat make us fat and sick, J. Cereal Sci. 58 (2013) 209–215.
[2] A. Rasheed, X. Xia, Y. Yan, R. Appels, T. Mahmood, Z. He, Wheat seed storage proteins: advances in molecular genetics, diversity and breeding applications, J. Cereal Sci. (2014), http://dx.doi.org/10.1016/j.jcs.2014.01.020.
[3] FAOSTAT (2012) faostat.fao.org/site/339/default.aspx and www.fao.org/worldfoodsituation/wfs-home/csdben
[4] M.J. Hawkesford, J. Araus, R. Park, D. Calderini, D. Miralles, T. Shen, J. Zhang, M.A.J. Parry, Prospects of doubling global wheat yields, Food Energy Secur. 2 (1) (2013) 34–48.
[5] R.J. Pena, Current and future trends of wheat quality needs, in: H.T. Buck, J.E. Nisi, N. Salomon (Eds.), Wheat Production in Stressed Environments, Springer, Berlin, 2007, pp. 411–424.
[6] A.K. Joshi, B. Mishra, R. Chatrath, G.O. Ferrara, R.P. Singh, Wheat improvement in India: present status, emerging challenges and future prospects, Euphytica 157 (2007) 431–446.
[7] P.K. Gupta, Quality of Indian wheat and infrastructure for analysis, in: A.K. Joshi, R. Chand, B. Arun, G. Singh (Eds.), A Compendium of the Training Program on Wheat Improvement in Eastern and Warmer Regions of India: Conventional and Non-Conventional Approaches, NATP Project, (ICAR), BHU, Varanasi, India, 2004.
[8] J.C. Brevis, J. Dubcovsky, Effect of chromosome region including the Gpc-B1 locus on wheat protein and protein yield, Crop Sci. 50 (2010) 93–104.
[9] P.R. Shewry, The HEALTHGRAIN programme opens new opportunities for improving wheat for nutrition and health, Nutr. Bull. 34 (2009) 225–231.
[10] R. Avni, R. Zhao, S. Pearce, Y. Jun, C. Uauy, F. Tabbita, T. Fahima, A. Slade, J. Dubcovsky, A. Distelfeld, Functional characterization of GPC-1 genes in hexaploid wheat, Planta 239 (2014) 313–324.
[11] P.J. White, M.R. Broadley, Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine, New Phytol. 182 (2009) 49–84.
[12] R.M. Welch, R.D. Graham, Breeding for micronutrients in staple food crops from a human nutrition perspective, J. Exp. Bot. 55 (2004) 353–364.
[13] T.D. Thacher, P.R. Fischer, M.A. Strand, J.M. Pettifor, Nutritional rickets around the world: causes and future directions, Annu. Trop. Paediatr. 26 (2006) 1–16.
[14] N.W. Simmonds, The relation between yield and protein in cereal grain, J. Sci. Food Agric. 67 (1995) 309–315.
[15] D.W. Lawlor, Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems, J. Exp. Bot. 53 (2002) 773–787.
[16] B.J. Miflin, Nitrogen metabolism and amino acid biosynthesis in crop plants, in: PS Carlson (Ed.), The Biology of Crop Productivity, Academic Press, London, 1980, pp. 255–296.
[17] A.L. Gao, H.G. He, Q.Z. Chen, Pyramiding wheat powdery mildew resistance genes Pm2, Pm4a and Pm21 by molecular marker-assisted selection, Acta Agron. Sin. 31 (2005) 1400–1405.
[18] T. Miedaner, F. Wilde, V. Korzun, E. Ebmeyer, M. Schmolke, L. Hart, C.C. Schon, Marker selection for Fusarium head blight resistance based on quantitative trait loci (QTL) from two European sources compared to phenotypic selection in winter wheat, Euphytica 166 (2009) 219–227.
[19] P.K. Gupta, H.S. Balyan, J. Kumar, P.K. Kulwal, N. Kumar, R.R. Mir, A. Kumar, K.V. Prabhu, QTL analysis and marker assisted selection for improvement in grain protein content and pre-harvest sprouting tolerance in bread wheat, in: 11th Wheat Genetics Symposium (IWGS), Brisbane, Australia, 2008, pp. 1–3.
[20] P.K. Gupta, J. Kumar, R.R. Mir, A. Kumar, Marker-assisted selection as a component of conventional plant breeding, Plant Breed. Rev. 33 (2010) 145–217.
[21] D. Barloy, J. Lemoine, P. Abelard, A.M. Tanguy, R. Rivoal, J. Jahier, Marker assisted pyramiding of two cereal cyst nematode resistance genes from *Aegilops variabilis* in wheat, Mol. Breed. 20 (2007) 31–40.
[22] J. Kumar, R.R. Mir, N. Kumar, A. Kumar, A. Mohan, K.V. Prabhu, H.S. Balyan, P.K. Gupta, Marker-assisted selection for pre-harvest sprouting tolerance and leaf rust resistance in bread wheat, Plant Breed. 129 (2010) 617–621.
[23] J. Kumar, V. Jaiswal, A. Kumar, N. Kumar, R.R. Mir, S. Kumar, R. Dhariwal, S. Tyagi, M. Khandelwal, K.V. Prabhu, R. Prasad, H.S. Balyan, P.K. Gupta, Introgression of a major gene for high grain protein content in some Indian bread wheat cultivars, Field Crop Res. 123 (2011) 226–233.
[24] S. Tyagi, R.R. Mi, P. Chhuneja, B. Ramesh, H.S. Balyan, P.K. Gupta, Marker-assisted pyramiding of eight QTLs/genes for seven different traits in common wheat (*Triticum aestivum* L.), Mol. Breed. 34 (2014) 167–175.
[25] A. Mesfin, R.C. Frohberg, J.A. Anderson, RFLP markers associated with high grain protein from *Triticum turgidum* L. var. dicoccoides introgressed into hard red spring wheat, Crop Sci. 39 (1999) 508–513.
[26] I.A. Khan, J.D. Procunier, D.G. Humphreys, G. Tranquilli, A.R. Schlatter, S. Marcucci-Poltri, R. Frohberg, J. Dubcovsky, Development of PCR-based markers for a high grain protein content gene from *Triticum turgidum* ssp. dicoccoides transferred to bread wheat, Crop Sci. 40 (2000) 518–524.
[27] S. Olmos, A. Distelfeld, O. Chicaiza, A.R. Schlatter, T. Fahima, V. Echenique, J. Dubcovsky, Precise mapping of a locus affecting grain protein content in durum wheat, Theor. Appl. Genet. 107 (2003) 1243–1251.
[28] A. Distelfeld, C. Uauy, S. Olmos, A.R. Schlatter, J. Dubcovsky, T. Fahima, Microcolinearity between a 2-cM region encompassing the grain protein content locus GPC-6B1 on wheat chromosome 6B and a 350-kb region on rice chromosome 2, Funct. Integr. Genomics 4 (2004) 59–66.
[29] A. Distelfeld, C. Uauy, T. Fahima, J. Dubcovsky, Physical map of the wheat high-grain protein content gene Gpc-B1 and development of a high-throughput molecular marker, New Phytol. 169 (2006) 753–763.

[30] C. Uauy, A. Distelfeld, T. Fahima, A. Blechl, J. Dubcovsky, A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat, Science 314 (2006) 1298–1301.
[31] M. Kade, A.J. Barneix, S. Olmos, J. Dubcovsky, Nitrogen uptake and remobilization in tetraploid 'Langdon' durum wheat and a recombinant substitution line with the high grain protein gene Gpc-B1, Plant Breed. 124 (2005) 343–349.
[32] H.S. Randhawa, J.S. Mutti, K. Kidwell, C.F. Morris, X. Chen, K.S. Gill, Rapid and targeted introgression of genes into popular wheat cultivars using marker-assisted background selection, PLoS ONE 4 (6) (2009) e5752, http://dx.doi.org/10.1371/journal.pone.0005752.
[33] P.K. Gupta, P. Langridge, R.R. Mir, Marker-assisted wheat breeding: present status and future possibilities, Mol. Breed. 26 (2010) 145–161.
[34] H.M. William, R.M. Trethowan, E.M. Crosby-Galvan, Wheat breeding assisted by markers: CIMMYT's experience, Euphytica 157 (2007) 307–319.
[35] M. Prasad, R.K. Varshney, A. Kumar, H.S. Balyan, P.C. Sharma, K.J. Edwards, H. Singh, H.S. Dhaliwal, J.K. Roy, P.K. Gupta, A microsatellite marker associated with a QTL for grain protein content on chromosome arm 2DL of bread wheat, Theor. Appl. Genet. 99 (1999) 341–345.
[36] H. Singh, M. Prasad, R.K. Varshney, J.K. Roy, H.S. Balyan, H.S. Dhaliwal, P.K. Gupta, STMS markers for grain protein content and their validation using near-isogenic lines in bread wheat, Plant Breed. 120 (2001) 273–278.
[37] D.J. Somers, P. Isaac, K. Edwards, A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.), Theor. Appl. Genet. 109 (2004) 1105–1114.
[38] M.S. Röder, V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy, M.W. Ganal, A microsatellite map of wheat, Genetics 149 (1998) 2007–2023.
[39] M.A. Saghai-Maroof, K.M. Soliman, R.A. Jorgensen, R.W. Allard, Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics, Proc. Natl. Acad. Sci. U.S.A. 81 (1984) 8014–8018.
[40] B.C.Y. Collard, D.J. Mackill, Marker-assisted selection: an approach for precision plant breeding in the twenty-first century, Philos. Trans. R. Soc. B 363 (2008) 557–572.
[41] F. Hospital, A. Charcosset, Marker-assisted introgression of quantitative trait loci, Genetics 147 (1997) 1469–1485.
[42] S. Gopalakrishnan, R.K. Sharma, Rajkumar, A. Kumar, M. Joseph, V.P. Singh, A.K. Singh, K.V. Bhat, N.K. Singh, T. Mohapatra, Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice, Plant Breed. 127 (2008) 131–139.
[43] N.G. Paltridge, L.J. Palmer, P.J. Milham, G.E. Guild, J.C.R. Stangoulis, Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grains, Plant Soil 361 (2012) 251–260.
[44] Ø. Hammer, D.A.T. Harper, P.D. Ryan, PAST: paleontological statistics software package for education and data analysis, Palaeontol. Electron. 4 (2001) 9.
[45] R. Van Berloo, GGT: software for display of graphical genotypes, J. Hered. 90 (1999) 328–329.
[46] R.P. Singh, J. Huerta-Espino, R. Sharma, A.K. Joshi, R. Trethowan, High yielding spring bread wheat germplasm for global irrigated and rainfed production systems, Euphytica 157 (2007) 351–363.
[47] A. Morgounov, H.F. Go'mez-Becerra, A. Abugalieva, M. Dzhunusova, M. Yessimbekova, H. Muminjanov, Y. Zelenskiy, L. Ozturk, I. Cakmak, Iron and zinc grain density in common wheat grown in Central Asia, Euphytica 155 (2007) 193–203.
[48] V.K. Singh, A. Singh, S.P. Singh, R.K. Ellur, D. Singh, S. Gopalakrishnan, P.K. Bhowmick, M. Nagarajan, K.K. Vinod, U.D. Singh, T. Mohapatra, K.V. Prabhu, A.K. Singh, Marker-assisted simultaneous but stepwise backcross breeding for pyramiding blast resistance genes Piz5 and Pi54 into an elite Basmati rice restorer line 'PRR78', Plant Breed. 132 (2013) 486–495.
[49] M. Joseph, S. Gopalakrishnan, R.K. Sharma, Combining bacterial blight resistance and basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice, Mol. Breed. 13 (2004) 377–387.
[50] S.H. Basavaraj, V.K. Singh, A. Singh, A. Singh, A. Singh, S. Yadav, R.K. Ellur, D. Singh, S. Gopalakrishnan, M. Nagarajan, T. Mohapatra, K.V. Prabhu, A.K. Singh, Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid, Mol. Breed. 26 (2010) 293–305.
[51] V.K. Singh, A. Singh, S.P. Singh, R.K. Ellur, D. Singh, S. Gopalakrishnan, M. Nagarajan, K.K. Vinod, U.D. Singh, R. Rathore, S.K. Prasanthi, P.K. Agrawal, J.C. Bhatt, T. Mohapatra, K.V. Prabhu, A.K. Singh, Incorporation of blast resistance gene in elite Basmati rice restorer line PRR78, using marker assisted selection, Field Crop Res. 128 (2012) 8–16.
[52] S.H. Basavaraj, V.K. Singh, A. Singh, D. Singh, M. Nagarajan, T. Mohapatra, K.V. Prabhu, A.K. Singh, Marker aided improvement of Pusa6B, the maintainer parent of hybrid Pusa RH10, for resistance to bacterial blight, Indian J. Genet. Plant Breed. 69 (2009) 10–16.
[53] P. Stam, A.C. Zeven, The theoretical proportion of the donor genome in near isogenic lines of self-fertilizers bred by backcrossing, Euphytica 30 (1981) 227–237.
[54] C.N. Neeraja, R.R. Maghirang, A. Pamplona, S. Heuer, B.C.Y. Collard, E.M. Eptinigsih, G. Vergara, D. Sanchez, K. Xu, A.M. Ismail, D.J. Mackill, A marker assisted backcross approach for developing submergence tolerant rice cultivars, Theor. Appl. Genet. 115 (2007) 767–776.
[55] L. Avivi, High grain protein content in wild tetraploid wheat Korn, in: S. Ramanujam (Ed.), 5th Wheat Genetics Symposium, Indian Society of Genetics and Plant Breeding, New Delhi, 1978, pp. 372–380.
[56] A.K. Joshi, J. Crossab, B. Arun, R. Chand, R. Trethowan, M. Vargas, I. Ortiz-Monasterio, Genotype × environment interaction for zinc and iron concentration of wheat grain in eastern Gangetic plains of India, Field Crop Res. 116 (2010) 268–277.