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Pyramiding of genes for grain protein content, grain quality, and rust resistance in eleven Indian bread wheat cultivars: a multi-institutional effort

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Abstract Improvement of grain protein content (GPC), loaf volume, and resistance to rusts was achieved in 11 Indian wheat cultivars that are widely grown in four different agro-climatic zones of India. This involved use of marker-assisted backcross breeding (MABB) for introgression and pyramiding of the following genes: (i) the high GPC gene *Gpc-B1*; (ii) HMW glutenin subunits $5+10$ at *Glu-D1* loci, and (iii) rust resistance genes, *Yr36*, *Yr15*, *Lr24*, and *Sr24*. GPC increased by 0.8 to 3.3%, although high GPC was generally associated with yield penalty. Further selection among high GPC lines allowed identification of progenies with higher GPC associated with improvement in 1000-grain weight and grain yield in the backgrounds of the following four cultivars: NI5439, UP2338, UP2382, and HUW468. The high GPC progenies (derived from NI5439) were also improved for grain quality using HMW glutenin subunits $5+10$ at *Glu-D1* loci. Similarly, progenies combining high GPC and rust resistance were obtained in the backgrounds of following five cultivars: Lok1,

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HD2967, PBW550, PBW621, and DBW1. The improved pre-bred lines developed following multi-institutional effort should prove a valuable source for the development of cultivars with improved nutritional quality and rust resistance in the ongoing wheat breeding programmes.

Keywords Wheat · Grain protein content · Marker-assisted backcrossing · Rust resistance

Introduction

Global food and nutritional security continue to be the subjects of world-wide discussions for several decades. Therefore, major global efforts are underway to increase food production and to improve nutritional value of the food products. Although, for the last few decades, we have been in a relatively comfortable situation with respect to meeting the demands of food production for growing world population, the problems of undernourishment and malnutrition have not been addressed adequately. As a result of recent efforts, a decline in undernourished population was witnessed, although a reverse trend started again after 2015, so that currently 9–11% of world population suffers from undernourishment/malnutrition, and this is likely to increase further (by ~0.8 to 1.0%) by 2030, when >850 million people are predicted to suffer with hunger and undernourishment/malnutrition (Purugganan and Jackson 2021). Particularly in India, malnutrition alone causes stunting (48% children), wasting (20% children), and low body weight (43% children) in children under the age of five, which is highest in the world (Bhutia 2014) and therefore, is a matter of concern.

While addressing the above problem of hunger, undernourishment, and malnutrition, wheat crop deserves major attention, since it is the second most important crop after maize in terms of staple food and second most important crop after rice in terms of

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food and nutritional security. While efforts are being made to increase global wheat production from the current level of ~764.39 Mt during 2019/2020 (http://www.worldagriculturalproduction.com/crops/wheat.aspx) to at least ~850 Mt in the year 2050, efforts are also underway to improve nutritional quality of wheat grain, both in terms of grain protein content (GPC) and health-promoting mineral nutrients like zinc (Zn) and iron (Fe) using the approach of biofortification (Gupta et al. 2020, 2021). Since ~20.4% of protein needs of growing global population is met by wheat grain (https://www.wheatinitiative.org/), the problem of protein deficiency can be partly addressed through increase in GPC in wheat to provide a healthy diet; other components of nutrition such as fibre and minor quantities of lipids, vitamins, minerals, and phytochemicals are also being addressed (Shewry and Hey 2015).

Considerable genetic variation for GPC is known to occur in wheat. However, the genetic architecture of GPC seems to be complex indeed (see Balyan et al. 2013), so that QTLs determining protein content in cultivated and wild wheats are now known to occur on all the 21 wheat chromosomes (for a summary of QTLs for grain protein content, consult wheatQTLdb at http://wheatqtldb.net/Quality.php; Singh et al. 2021). However, none of these QTLs is a major QTL and their expression is unstable and influenced by QTL×QTL interactions and QTL×environment interactions, rendering them relatively unsuitable for marker-assisted breeding (Prasad et al. 1999, 2003; Dholakia et al. 2001; Kulwal et al. 2005; Kumar et al. 2006; Krishnappa et al. 2017; Goel et al. 2019). In contrast to this gloomy picture, the gene *Gpc-B1* that was discovered originally from wild emmer wheat (*Triticum turgidum* var. *dicoccoides*) (Avivi 1978) has become the gene of choice and has been widely exploited during the last decade. The gene *Gpc-B1* encodes a NAC transcription factor that accelerates senescence resulting in increased mobilization and transfer of nitrogen (N) and minerals like Zn and Fe to the developing grains (Uauy et al. 2006). Therefore, lines expressing this allele contain relatively higher contents of GPC, Zn, and Fe in their grain (Distelfeld et al. 2006; Uauy et al. 2006). When transferred to hard red spring wheat, *Gpc-B1* led to an increase in GPC by up to 3% over the parental line (Khan et al. 2000). In the past, the derived lines carrying *Gpc-B1* were evaluated in 40 environments in seven different

countries (for a review, see Tabbita et al. 2017). As a result, several cultivars of bread wheat (Lassik, Farnum, Lillian, Somerset, Burnsid) and durum wheat (Westmore and Desert King High Protein) with high GPC have been developed (for reviews, see Balyan et al. 2013; Gupta et al. 2020, 2021). Recently, in India also, many advanced lines containing *Gpc-B1* have been developed in different genetic backgrounds including cv. PBW550 at PAU, Ludhiana. We hope that some of these wheat lines will eventually be released as high grain protein wheat cultivars in foreseeable future. Overall, these studies established that *Gpc-B1* increases not only GPC, but also Zn and Fe contents, although it has a marginally negative impact on grain yield (Tabbita et al. 2017). However, high GPC lines containing *Gpc-B1* with no yield penalty have also been developed (Kumar et al. 2011).

Besides improved nutritional quality, functional and rheological properties of the wheat flour are also important for developing suitable end-products like bread and chapati (Indian flat bread). The seed storage proteins (~50% of the total protein) determine the processing quality of the wheat flour (Gupta et al. 1998). Among the storage proteins, the quantity and quality of glutenin proteins are the major determinants of the elasticity of wheat flour dough contributing to the quality of bread and chapati (D'Ovidio and Masci 2004; Figueroa et al. 2009; Payne et al. 1979). The composition of high molecular weight glutenin subunits (HMW-GS) determines the wheat baking quality (Dobraszczyk and Morgenstern 2003; Dhaka and Khatkar 2015). The HMW-GS 5 + 10 combination encoded by complex *Glu-D1* locus is associated with good baking quality and is therefore preferred over 2 + 10 subunit combination, which is associated with poor baking quality (see Nucia et al. 2019). However, the HMW-GS 5 + 10 combination is not common among Indian bread wheat cultivars (Ram 2003, Ram et al. 2015, Patil et al. 2015). Therefore, there is a need for the introgression and pyramiding of HMW-GS 5 + 10 along with high GPC into elite Indian bread wheat cultivars to improve the technological properties of the flour.

In the present multi-institutional study, five institutions worked together to introgress the high GPC gene *Gpc-B1* into 11 elite Indian bread wheat cultivars (including cultivars that are being grown in different wheat-growing zones of the country) using marker-assisted backcross breeding (MABB). In

one of these cultivars (NI5439), GPC was also pyramided with HMW-GS 5 + 10. The genes *Yr15* and *Yr36* for resistance against yellow rust (*Puccinia striiformis*) associated with *Gpc-B1*, the gene *Lr24* for resistance against leaf rust (*Puccinia triticina*), and the gene *Sr24* for resistance against stem rust (*Puccinia graminis*) were also pyramided over genes for enhanced grain quality in the derived lines. The results of this collaborative study are presented in this communication.

Materials and methods

Materials (recipients, donors, and markers)

Recipient parents The following 11 elite Indian bread wheat cultivars were used as recipient parents: (i) six cultivars (HD2967, PBW550 (*Yr15*), DBW17, PBW621, UP2338, and UP2382) from north western plain zone, (ii) two cultivars (HUW234 and HUW468) from north eastern plain zone, (iii) two cultivars (MACS2496 and NI5439) from peninsular zone, and (iv) a solitary cultivar (Lok1) from central zone (Figure S1). All recipient cultivars are cultivated under timely or late sown (HUW234) and irrigated conditions; only cv. NI5439 of peninsular zone is recommended for cultivation under rainfed and timely sown conditions.

Donors with desirable genes The following two donor parents were used in the present study: (i) PBW343 (*Gpc-B1/Yr36 + Lr24*), which is an improved version of an erstwhile popular cultivar PBW343, which later became susceptible to yellow rust, and was also recently resurrected through pyramiding of leaf and stripe rust resistance genes (Kumar et al. 2011; Sharma et al. 2021). (ii) Glupro (*Gpc-B1/Yr36*), an exotic genotype earlier developed by Jorge Dubcovsky and his team at the University of California (Davis), USA (Khan et al. 2000; Uauy et al. 2006).

Markers for foreground and background selections. For foreground selection, the following markers were used: (i) *Xuhw89*, a marker closely linked (0.1 cM) to *Gpc-B1* (Distelfeld et al. 2006), and *Xucw108*, a gene-based co-dominant functional

marker for *Gpc-B1* (Uauy et al. 2006); (ii) SCAR marker *Xscs73* for *Lr24* (Prabhu et al. 2004); (iii) *Xgwm*413 marker for *Yr15* (Murphy et al. 2009); (iv) gene-based marker *Xucw130* for *Yr36* (linked to *Gpc-B1*) (Fu et el. 2009). For background selection, polymorphic SSRs ranging in number from 102 to 126 were used.

Scheme for MABB

The breeding scheme followed for MABB is presented in Fig. 1. Eleven crosses were made involving 11 recipients and the two donor parents [the donor genotype PBW343 (*Gpc-B1/Yr36 + Lr24*) was used for eight recipients whereas Glupro (*Gpc-B1/*
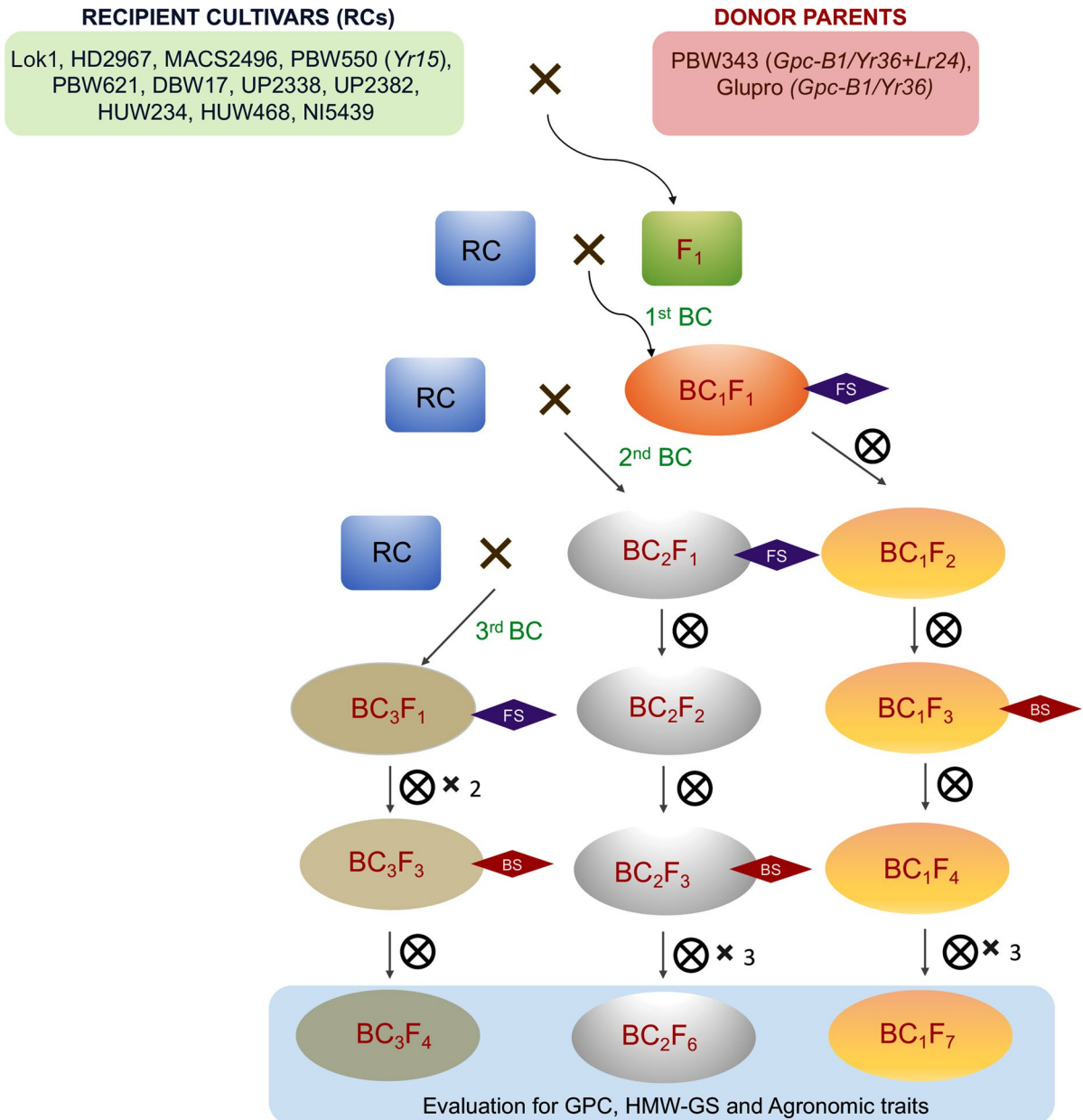

Fig. 1 MABB scheme followed for introgression of genes for grain quality and pyramiding genes for rust resistance into the backgrounds of 11 Indian bread wheat cultivars. FS, Foreground selection; BS, Background selection

Yr36) was used for the remaining three recipients]. In BC$_1$F$_1$ to BC$_3$F$_2$ populations, foreground selection was exercised in order to recover plants heterozygous/ homozygous for the desired alleles from the donor parent. The selected plants in BC$_1$F$_2$/BC$_2$F$_2$/BC$_3$F$_2$ were selfed to obtain BC$_1$F$_3$/BC$_2$F$_3$/BC$_3$F$_3$ progenies, which were advanced up to BC$_{1-3}$F$_{4-7}$ before conducting field trial of MABB-derived progenies. Background selection accompanied the foreground selection up to BC$_1$F$_3$/BC$_2$F$_3$/BC$_3$F$_3$ generations; in some cases, background selection was performed after the recovery of the homozygous and stable progenies in order to estimate the percent recurrent parent genome recovery. Phenotypic selection was carried out simultaneously in order to derive agronomically superior progenies.

Laboratory experiments

DNA isolation, PCR, and marker analysis DNA isolation was carried out using leaf samples of 30–40-day-old plants using a modified CTAB method (Weising et al. 1995). DNA quality was checked on 1% agarose gels and quantification was carried out using Pico2000. Polymerase chain reaction (PCR) was carried out in 12–20-μL reaction volume containing 25–50 ng of genomic DNA, 1-Unit Taq polymerase (Sigma-Aldrich, USA/Bangalore Genie, India), 0.02 mM of each of four dNTPs (Sigma-Aldrich, USA/Bangalore Genie, India), 0.2 μM of forward and reverse primers (synthesized by IDT, USA), and 1X PCR buffer (10 mM Tris–HCl pH 8.4). PCR was performed using Applied Biosystem's Thermal Cycler using the following PCR profile: 94 °C for 4–5 min, followed by 35 cycles of 94 °C for 30–60 s, 57–60 °C (depending on the primer sequences) for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 7–8 min. The amplified products were resolved either on 2–2.5% agarose gel, visualized following ethidium bromide staining, or on 6% PAGE, visualized following silver staining.

Grain quality tests Percent grain protein content (GPC) was determined using Near-Infrared Transmittance in 1241 Grain Analyzer (FOSS, Sweden). The concentration of Fe (ppm) and Zn (ppm) in the grains was estimated using Energy-Dispersive X-ray Fluorescence (EDXRF Spectrometer X-Supreme8000) (Paltridge et al. 2012). Sedimentation volume was

worked out using micro-SDS sedimentation volume (MSDS-SV) test following Dick and Quick (1983).

Micro-baking for loaf volume For estimation of loaf volume, the formulation included flour (100 parts), water (65%), salt (2 parts), and fresh yeast (2.5 parts), which were mixed in the 10-g mixograph. The resulting dough, which was moulded, rested for 20 min at 28 °C, remoulded, proofed for 45 min at 28 °C and 90% relative humidity (RH), and baked at 200 °C for 17 min (Gras and Bekes 1996). Loaf volume was measured by the mustard seed displacement method. Baking tests were done in duplicate.

SDS-PAGE (polyacrylamide gel electrophoresis) The proteins of flour samples were extracted and fractionated in 10% polyacrylamide gels using the method of Laemmli (1970) as modified by Payne et al. (1980).

Field experiments

The details of field experiments conducted for the evaluation of MABB-derived progenies in the background of 11 recipient cultivars are summarized in Table S1. The number of progenies in the different recipient genetic backgrounds varied from 1 to 24. The experiments were conducted in randomized block designs each with 2–3 replications over 1–3 years and 1–5 locations. The plot size differed in different experiments with the largest plot size comprising 12 rows of 6 m each and smallest plot size comprising 4 rows of 3 m each (Table S1). Standard cultural practices were followed to raise the crop at different locations and in different years (https://www.krishisewa. com/production-technology/132-wheat-growing. html). In each plot, data were recorded on 1000-grain weight (g), grain yield (tons/hectare (t/ha) or quintals per hectare (q/ha)), and GPC (%).

Screening for rusts

Glass-house screening against three rusts Altogether, 16 pathotypes of the three rust pathogens occurring in different wheat-growing zones of India were used for screening for resistance of the MAS-derived progenies in the backgrounds of cvs. Lok1 and HD2967; the avirulence/virulence formulae of pathotypes are listed in Table 1. Screening of the

Table 1 Avirulence/virulence formula of the 16 pathotypes of the three pathogens responsible for the three rusts

Pathotypes	Avirulence/virulence formula
(a) Puccinia triticana	
12–5	*Lr1, 2a, 9, 10, 13, 15, 19, 24, 25, 28, 29, 32, 36, 39, 42, 43, 45, 47/Lr 2b, 2c, 3, 11, 12, 14a, 14b, 14ab, 16, 17a, 17b, 18, 20, 21, 22a, 22b, 23, 26, 27+31, 30, 33, 34, 35, 37, 38, 40, 44, 46, 48,49*
77–5	*Lr9, 19, 24, 25, 28, 29, 32, 39, 42, 43, 45, 47/Lr1,2a, 2b, 2c, 3,10, 11, 12,13, 14a, 14b, 14ab, 15, 16, 17a, 17b, 18, 20, 21, 22a, 22b, 23, 26, 27, 30, 33, 34, 35, 36, 37, 38, 40, 44,48, 49*
77–8	*Lr9, 23, 24, 25, 26, 27+31, 28, 29, 32, 36, 39, 45, 47/ Lr1 2a, 2b, 2c, 3a, 10, 11, 13, 14a, 14b, 14ab, 15, 16, 17, 18, 19, 20, 21, 22a, 22b, 30, 33, 35, 37, 38, 44, 48, 49*
77–9	*Lr2a, 2b, 2c, 9, 19, 24, 25, 28, 32, 39, 42, 45, 47/ Lr1, 3, 10, 11, 12, 13, 14a, 14b, 14ab, 15, 16, 17a, 17b, 18, 20, 21, 22a, 22b, 23, 26, 27+31, 30, 33, 34, 35, 36, 37, 38, 44, 46, 48, 49*
104–2	*Lr9, 10, 13, 15, 19, 20, 24, 25, 28, 29, 32, 36, 39, 42, 43, 45, 47 /Lr1,2a, 2b, 2c, 3, 11,12, 14a, 14b,14ab, 16, 17a, 17b, 18, 21, 22a, 22b, 23, 26, 27+31, 30, 33, 34, 35, 37, 38, 40, 44, 48,49*
(b) Puccinia striiformis f. sp. tritici	
238S119	*Yr1* (Chinese 166*), 5* (TSA*), 10* (Moro*), 15,* Cappelle-Desprez, *24, 26, Sp,* PBW752, PBW757/*2, 3, 4, 6, 7, 8, 9, 11, 17, 18, 19, 21, 22, 23, 25, 27, A, AvS, SD, SU,* Riebesel *47/51,* HS507
78S84	*Yr1* (Chinese 166*), 4, 10* (Moro*), 14, 15, 24, SD,* Riebesel *47/51,* Cappelle-Desprez, *32* (Carsten V*), Sp,* HS507 *l2, 3, 6, 7, 8, 9, 19, 21, 22, 23, 25, 27, SU*
T	*Yr5, 9, 10* (Moro*), 15, 24, 32, Sp,* Riebesel *47/51,* Cappelle-Desprez**,** HS507/*1, 2, 3, 4, 6, 7, 8, 11, 17, 18, 19, 22, 23, 25, A, AvS, SD*
110S119	*Yr1* (Chinese 166*), 5, 10* (Moro*), 24, 32, Sp,* Riebesel *47/51,* Cappelle-Desprez, HS507/*2, 3, 4, 6, 7, 8, 9, 11, 14, 17, 18, 19, 21, 22, 23, 25, 27, A, AvS, SD, SU*
(c) Puccinia graminis f. sp. tritici	
11	*Sr7a, 8a, 8b, 9e, 22, 23, 24, 25, 26, 27, 31, 32, 33, 35, 37, 39, 40, 43, Tmp, Tt3l 5, 6, 7b 9a, 9b, 9c, 9d, 9f, 9 g, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 28, 29, 30, 34, 36, 38, McN*
21A-2	*Sr5, 6, 7a, 8a, 8b, 9a, 9b, 9c, 9e, 11, 12, 21, 22, 23, 24, 25, 26, 27, 29, 30, 31, 32, 33, 35, 37, 38, 39, 40, 43, Gt, Tmp, Tt3l7b, 9d, 9f, 9 g, 10, 13, 14, 15, 16, 17, 19, 28, 34, 36, McN*
34–1	*Sr6, 7a, 8a, 8b, 9a, 9e, 10, 11, 13, 17, 19, 21, 22, 23, 24, 25, 26, 27, 30, 31, 32, 33, 35, 36, 37, 39, 40,43, Tmp, Tt3l5, 7b, 9b, 9d, 9f, 9 g, 14, 15, 16, 18, 20, 21, 28, 29, 34, 38, McN*
40A	*Sr7a, 13, 21, 22, 24, 25, 26, 27, 30, 31, 32, 33, 35, 36, 37, 38, 39, 40, 43, Tmp, Tt3l5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9f, 9 g, 10, 11, 14, 15, 16, 17, 18, 19, 20, 23, 28, 29, 34, McN*
40–1	*Sr7a, 13, 21, 22, 25, 26, 27, 30, 31, 32, 33, 35, 36, 37, 38, 39, 40, 43, Tmp, Tt3l5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9f, 9 g, 10, 11, 14, 15, 16, 17, 18, 19, 20, 23, 24, 28, 29, 34, McN*
117–6	*Sr5, 8a, 8b, 9b, 22, 24, 25, 26, 27, 28, 30, 31, 32, 33, 35, 36, 37, Tmp/2, 6, 7a, 7b, 9e, 9f, 9 g, 10, 11, 12, 13, 14, 15, 16, 17, 19, 21, 23, 29, 34, McN*
122	*Sr7a, 8a, 8b, 9e, 10, 12, 14, 15, 16, 17, 18, 19, 20, 22, 24, 25, 26, 27, 28, 31, 32, 33, 35, 36, 37, 38, 39, 40, 43, Tmp, Tt3l5, 6, 7b 9a, 9b, 9c, 9d, 9f, 9 g, 11, 13, 21, 23, 29, 30, 34, McN*

MAS-derived progenies along with their recipient parents for rust resistance was carried out as described in Gautam et al. (2020). The seedlings were assayed for the infection types (ITs) against each pathotype at 15 days post-inoculation following Stakman et al. (1962). The ITs used for scoring level of resistance were as follows: (i) ITs 0;, ;⁻, ;, 1 to 2, and 2⁻ indicated resistant; (ii) IT 1 to 2⁺ indicated moderately resistant; (iii) IT 3 indicated moderately susceptible; and (iv) IT 3⁺ indicated susceptible response. Scoring of different ITs was done as follows: (i) 0;=no visible uredia or flecking; (ii) ;⁻=minute hypersensitive fleck without uredia; (iii) ;=minute hypersensitive fleck with little necrosis or chlorosis; (iv) 1 to 2=small uredia with little chlorotic or necrotic areas; (iv) 2⁻ =small uredia with more pronounced chlorotic or necrotic areas; (v) 1 to 2⁺ =similar uredia size as for IT 2⁻ but more sporulation; (vi) 3=uredia medium in size with more pronounced chlorosis without necrosis, and (vii) 3⁺ =large uredia, coalescing with minor chlorosis or necrosis.

Field screening against leaf rust and stripe rust Screening of MAS-derived progenies in the

backgrounds of three recipient cvs. PBW550 (*Yr15*), DBW17, and PBW621 was done in the field under artificial rust epidemics created by spraying the experimental material with the mixture of uredinospores of prevalent races of *P. striiformis* f. sp. *tritici* (78S84, 49S119 196, and 110S119) and *P. triticina* (077–5, 104–2 + unknown races collected from farmer's field). Details of races and their virulence formulae are available elsewhere (Sharma et al. 2021; also listed in Table 1).

Screening for rust resistance under field conditions was undertaken on the progenies and the parents raised in a non-replicated augmented block design in paired rows of 1 m. The distance within the paired rows was 22 cm and between two paired rows was 30 cm. The planting of the seed material was done in the first fortnight of November each year. The susceptible genotypes including PBW621, PBW550, DBW17, PBW343, Agra local (for stripe rust and leaf rust), and C306 (for leaf rust) were planted as infector rows (at every 7th paired row) and in spreader rows (perpendicular to the 1-m paired rows) surrounding each plot to allow development of sufficient inoculum. In order to ensure uniform disease distribution, pots with rust-infected plants were also placed in fields between the experimental materials.

For creating stripe rust epidemics, repeated inoculations through spray with uredinospores of *P. striiformis* f. sp. *tritici* were carried out. The inoculation was carried out in the evening with an ultralow volume sprayer on alternate days beginning from the end of December to the end of January till stripe rust appeared on the susceptible checks/parents. The infected leaves of susceptible host (which were pre-inoculated to multiply the pathogen) were collected and immersed in water for extracting uredinospores. The inoculum was prepared in 10 l of water using 5–7 drops of Tween-20, by suspending rust uredinospores @5.6 g/ha, which is equivalent to 1000 spores per plant (Imtiaz et al. 2003).

The response to rusts in the field was recorded at the reproductive stage using disease severity (DS) and infection response (IR) following the modified Cobb scale (Peterson et al. 1948). DS was measured as an estimation of percentage coverage (0, 5, 10, 18, 20, 40, 60, 80, and 100) of rust pustules (uredinia) seen on the flag leaf. IR was scored as a reaction of the host to rust infection and was categorized as

0 = immune; R = resistant, MR = moderately resistant; MS = moderately susceptible, and S = susceptible. Data were recorded three times at equal intervals (starting mid-January) when the flag leaves of the susceptible check cultivars showed a disease score of 80S (DS: 80; IR: S). Out of these three scores of a test line, the highest score toward susceptibility was used for the subsequent analysis.

Statistical analysis

Means and ANOVA The means were calculated using Excel. The analysis of variance (ANOVA) was carried out using SPSS Statistics for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA).

Estimation of the recovery of recipient genome (RPG) The recovery of RPG was estimated using the following formula: $RPG = [(X + 1/2Y)/N] \times 100$, where X is the number of homozygous marker loci for recurrent parent allele, Y is the number of heterozygous marker loci for the parental alleles, and N is the total number of parental polymorphic markers used for screening.

Results and discussion

Marker-assisted selection (MAS) is routinely used now to supplement conventional plant breeding (Collard and Mackill 2008; Gupta et al. 2010; Jiang 2013). Among different MAS-related breeding approaches, MABB is the most widely used approach for improving an existing elite cultivar/line for traits like disease resistance, grain quality, and sometimes also for abiotic stress tolerance (such as submergence tolerance in rice and pre-harvest sprouting tolerance in wheat). MABB has mostly been used to improve traits controlled by major genes and is not considered suitable for improvement of a trait that is controlled by a large number of minor genes (Hospital et al. 2003; Francia et al. 2005; Collard and Mackill, 2008; Kumar et al. 2010; Tyagi et al. 2014; Giaever et al. 2017; Gautam et al. 2021). During the present study, MABB was used for improving GPC for all 11 cultivars, rust resistance for five cultivars, and gluten strength for only one cultivar, namely NI5439.

Grain protein content (GPC) and grain yield

The introgression of *Gpc-B1* (high GPC gene) through MABB (involving foreground and background selections; Fig. 2a, b, d) into 11 Indian wheat cultivars was straight-forward and led to the successful development of improved lines despite significant genotype x environment interactions for GPC (Tables S2-S8) that was also reported in several earlier studies (Kumar et al. 2011; Carter et al. 2012; Singh et al. 2018). The number of progenies with improved GPC ranged from 1 to 16 for individual recipients (Supplementary Table S9). GPC level in these lines ranged from 12.4% (against 11.6% in recipient parent UP2338) to 17.2% (13.9% in recipient parent NI5439) (Table 2). This amounted to an increase of 0.8% (in the background of UP2338) to 3.3% (in the background of NI5439) (Table 2), which is not

very different from the results of some earlier studies (Khan et al. 2000; Kumar et al. 2011; Balyan et al. 2013; Tyagi et al. 2014; for reviews, see Tabbita et al. 2017; Gautam et al. 2020).

Two of the 11 recurrent parents, namely MACS2496 and NI5439, and their derived progenies evaluated at Pune location (located in the Peninsular zone) had relatively higher GPC than the rest of the recurrent parents and their progenies. It is well known that the GPC is generally higher in varieties grown in the peninsular zone and also that the high GPC is associated with higher 1000-grain weight (Mohan et al. 2017). This is contrary to the reported negative relationship between 1000-grain weight and GPC in most Indian wheat material (Nagarajan et al. 2007). Therefore, it appears that the negative relationship has been broken in the varieties grown in the Peninsular zone (Mohan et al. 2017).
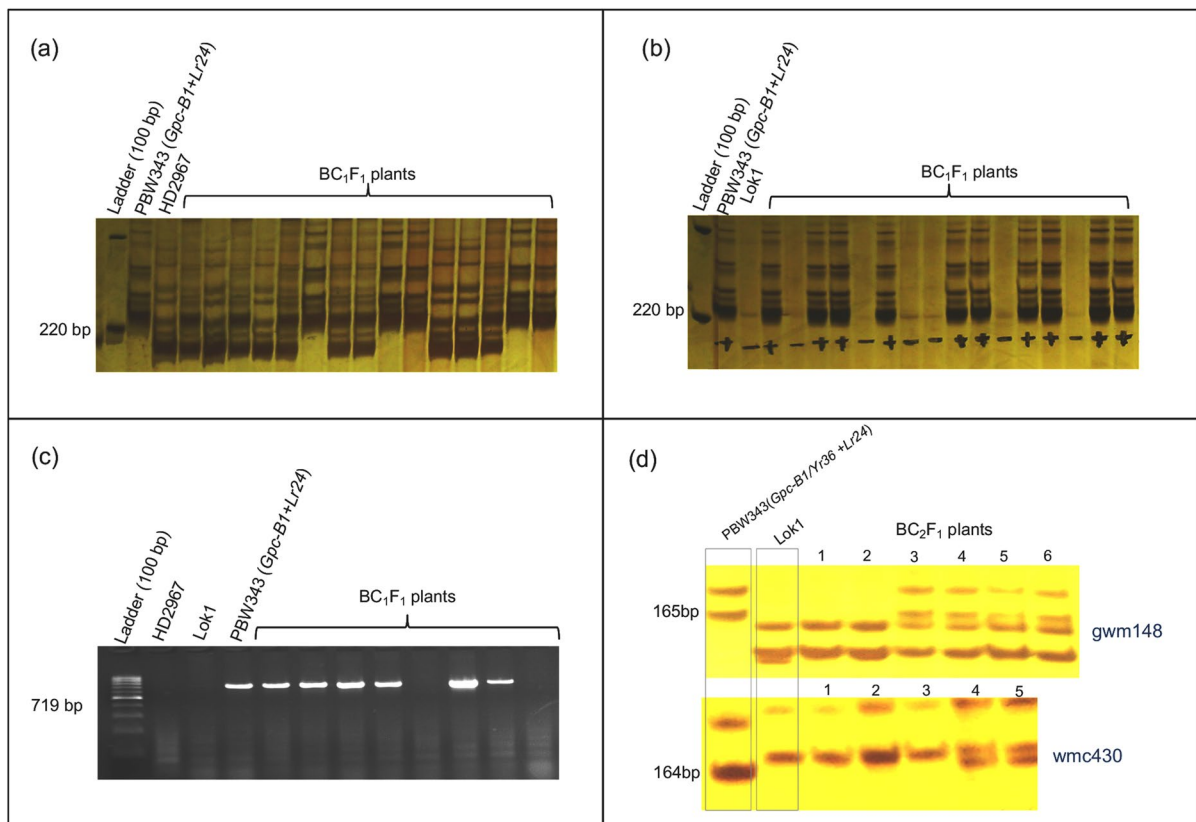

Fig. 2 Representative amplification profiles of the markers (a and b) *UCW108* linked with *Gpc-B1*, (c) SCS73$_{719}$ linked with *Lr24* in recipient and donor parental lines and BC$_1$F$_1$ plants, and (d) Segregation for SSR markers (gwm148 and wmc430)

used in background selection in BC$_2$F$_1$ population involving recipient genotype Lok1 and the donor genotype PBW343 (*Gpc-B1/Yr36 + Lr24*)

Table 2 Mean and range values for five traits of the recipient parents and the MABB-derived progenies with significantly higher GPC

Recipient parent/derived progenies	Mean 1000-GW (g) (range)	Mean GY (q/ha) (range)	Mean GPC (%) (range)	Mean Zn content (ppm) (range)	Mean Fe content (ppm) (range)
Lok1 (R)	51.9	40.20	12.02	19.83	29.07
Progenies (n = 5)	48.7 (44.0–55.1)	40.6 (38.5–42.8)	13.1 (12.9–13.3)	21.50 (20.7–22.2)	30.9 (29.6–32.5)
HD2967 (R)	42.9	50.60	12.90	21.50	30.80
Progenies (n = 1)	47.5 (-)	48.7 (-)	14 (-)	22.43 (-)	32.5 (-)
MACS2496 (R)	40.2	32.60	14.90	NA	NA
Progenies (n = 12)	38.4 (35.1–43.4)	28.9 (23.00–38.30)	16.50 (15.90–16.80)	NA	
NI5439 (R)	36.9	46.09	13.87	NA	NA
Progenies (n = 16)	39.9 (32.4–46.9)	37.3 (24.0–53.8)	15.8 (14.8–17.2)	NA	
DBW17(R)	37.2	55.60	11.20	NA	NA
Progeny (n = 1)	36.3 (-)	53.2 (-)	13.80 (-)	NA	NA
PBW550 (R)	43.3	56.30	11.10	35.20	35.13
Progenies (n = 3)	40.87 (40.1–42.1)	51.36 (50.40–53.10)	13.6 (13.20–14.20)	37.78 (31.5–46.6)	37.12 (31.2–43.37)
PBW621 (R)	39.6	50.10	10.30	36.40	39.60
Progenies (n = 01)	35.4 (-)	47.30 (-)	13.70* (-)	40.70* (-)	35.40 (-)
UP2338 (R)	31.4	44.50	11.6	NA	NA
Progenies (n = 6)	37.0 (34.7–41.0)	50.90 (48.2–52.5)	13.1 (12.4–13.3)	NA	
UP2382 (R)	31.8	46.60	11.60	NA	NA
Progenies (n = 7)	41.1 (40.20–43.0)	51.20 (48.4–53.1)	12.70 (12.1–13.2)	NA	
HUW234 (R)	36.5	45.30	11.70	NA	NA
Progenies (n = 2)	36.40 (36.2–36.6)	45.60 (45.1–46.0)	13.70 (13.6–13.8)	NA	
HUW468 (R)	32.6	40.5	11.1	NA	NA
Progenies (n = 2)	35.7 (35.2–36.2)	42.5 (42.2–42.8)	13.75 (13.7–13.8)	NA	

GW, grain weight; GY, grain yield; GPC, grain protein content; Zn, zinc; Fe, iron; R, recipient parent; *n*, number of progenies; q/ha, quintals per hectare; NA, not available

Despite reports of a general negative correlation between GPC and grain yield (O'Brien and Panozzo 1988; Simmonds 1995; Brevis and Dubcovsky 2010) and reported negative effect of *Gpc-B1* gene on grain yield (Tabbita et al. 2017), several progenies during the present study showed improved GPC with no yield penalty (Table 3, Table S9). Following are the other desirable attributes in the improved lines

obtained during the present study: (i) seven progenies with high GPC also had high 1000-grain weight and no yield penalty; progenies in the background of three recipients (MACS2496, NI5439, and HUW468) showed this unique combination of traits (Table 3), and (ii) 12 progenies had high GPC along with significantly higher 1000-grain weight and higher grain yield; these progenies belonged to the four recipients,

Table 3 MAS-derived progenies (a) having significantly higher GPC, 1000-grain weight and no yield penalty in comparison to the respective recipient parents and (b) having significantly higher GPC, 1000-grain weight and grain yield in comparison to the respective recipient parents

Recipient parent/ progeny	1000-GW	GY (q/ha)	GPC(%)
(a) Significantly higher GPC, 1000-GW and no yield penalty			
MAC2496 (R)	42.0	32.6	14.9
MACS2496-22	47.2*	26.0	16.8*
MACS2496-45	43.4*	28.1	16.5*
NI5439 (R)	36.9	46.1	13.1
NI5439-2	41.2*	44.3	15.0*
NI5439-6	42.2*	50.1	14.9*
HUW468 (R)	32.6	40.5	11.0
HUW468-358	35.5*	46.6	12.2*
HUW468-234	39.8*	46.5	14.0*
HUW468-258	40.3*	45.9	14.0*
(b) Significantly higher GPC, 1000-GW and grain yield			
NI5439 (R)	36.9	46.1	13.9
NI5439-6	39.6*	53.8*	16.0*
UP2338 (R)	31.4	44.5	11.2
UP2338-14–1	34.9*	52.5*	13.0*
UP2338-14–2	34.9*	52.1*	12.7*
UP2338-14–3	35.4*	51.6*	13.3*
UP2338-14–4	35.9*	53.1*	13.1*
UP2382 (R)	31.8	46.6	11.6
UP2382-14–1	40.4*	49.9*	13.2*
UP2382-14–2	40.2*	50.7*	12.4*
UP2382-14–3	40.8*	50.7*	12.4*
UP2382-14–4	41.9*	52.4*	12.4*
UP2382-14–6	40.5*	52.0*	13.0*
HUW468 (R)	32.6	40.5	11.0
HUW468-358	35.5*	42.8*	13.7*
HUW468-375	36.2*	42.2*	13.8*

R, recipient parent; GW, grain weight; GY, grain yield; GPC, grain protein content; q/ha, quintals per hectare; *, significantly higher at 5% level

namely NI5439, UP2338, UP2382, and HUW468 (Table 3).

Progenies containing higher GPC with no yield penalty due to *Gpc-B1* were also reported in our earlier study (Kumar et al. 2011). It appears that the negative association between GPC and grain yield in such progenies could be successfully broken allowing simultaneous improvement of both these important traits. These progenies (developed during the present study) will need more extensive evaluations so that they may be included in the ongoing quality improvement breeding programs and may also be considered for testing in the varietal development trials for direct release as varieties.

High GPC with improved concentration of grain Zn and Fe

The improved progenies also had higher grain Zn and/or Fe concentration, a trait that is associated with GPC due to the *Gpc-B1* gene (Distelfeld et al. 2006; Uauy et al. 2006). This is illustrated in high GPC representative lines derived from four genotypes (Lok1, HD2967, PBW550, and PBW621), where grain Zn and Fe were estimated and found to be improved (Table 3, Table S9). We believe that the progenies with high GPC due to *Gpc-B1* in the backgrounds of the remaining seven recipients should also contain higher grain Zn and/or Fe concentrations. These results are in agreement with earlier reports (for a review, see Tabbita et al. 2017). Suitable explanations for this association of *Gpc-B1* with high Zn and high Fe are based on the fact that *Gpc-B1* encodes a NAC transcription factor (NAM-B1), which is known to accelerate senescence and also increases nutrient remobilization from leaves to grains (Uauy et al. 2006; Distelfeld et al. 2007). The *Gpc-B1* gene has also been shown to regulate other genes, particularly those belonging to ZIP and YSL categories of transporters in the early senescence stage, and these transporters are eventually helpful in the export of Zn and Fe from the cytoplasm into the phloem and also for the biosynthesis of chelators that facilitate the phloem-based transport of Zn and Fe to the grains (Pearce et al. 2014). Therefore, the gene *Gpc-B1* may be deployed for biofortification of Zn and Fe in wheat grains as shown in the present study and also as recently proposed by us (Gupta et al. 2021).

High GPC, gluten strength, and HMW-GS 5 + 10

Four high GPC progenies also had gluten strength that was comparable to that of the recipient parent NI5439, a popular cultivar of the Peninsular zone (data not presented). Screening for HMW-GS showed that two of these four lines had the following HMW-GS composition: 17 + 18 at *Glu-B1*

and 5 + 10 at *Glu-D1* and no subunit at *Glu-A1* loci (Fig. 3a). It is known that the high GPC donor parent genotype PBW343 (*Gpc-B1/Yr36 + Lr24*) contained 5 + 10 subunits whereas NI5439 contained 2 + 12 at *Glu-D1*. Therefore, HMW-GS 2 + 12 at the *Glu-D1* locus in the background of recipient genotype NI5439 were successfully replaced by HMW-GS 5 + 10. Micro-baking quality test showed that all the four MABB-derived progenies had > 43 ml micro-loaf volume, which was significantly higher than the 38.5-ml micro-loaf volume of NI 5439 (Fig. 3b). The higher loaf volume in two of the four MABB-derived progenies may be attributed to both the high GPC and HMW-GS 5 + 10, as reported earlier (Brevis et al. 2010). However, the higher loaf volume in the remaining two progenies which carried HMW-GS 2 + 12 at *Glu-D1* loci is intriguing. We speculate that this may be resolved through future studies of the gliadin content, gliadin:glutenin ratio, grain hardness, etc. in these progenies. However, the above findings suggest simultaneous improvement

in GPC and baking quality could be achieved as in case of NI5439. The high GPC MABB-derived progenies from other seven recipient parents [where PBW343 (*Gpc-B1/Yr36 + Lr24*) was used as a donor parent] could be subjected to HMW-GS allele analysis and micro-baking test in future studies to select the progenies with improved baking quality.

Rust resistance in derived progenies

Progenies in the backgrounds of cvs. Lok1 and HD2967 It may be recalled that the donors used for improvement of GPC were not the same for all 11 recipients. The donor for *Gpc-B1* was PBW343 (*Gpc-B1/Yr36 + Lr24*) in case of eight of the 11 recipient genotypes (excluding PBW550, PBW621, and DBW17) and Glupro (*Gpc-B1/Yr36*) for the remaining three recipients (Table S1). Among the eight recipients, where PBW343 (*Gpc-B1/Yr36 + Lr24*) was used as the donor, Lok1 and HD2967 were susceptible to moderately resistant to most of the pathotypes

Fig. 3 (a) SDS-PAGE analysis of HMW-GS in the recipient cultivar NI5439 and the corresponding representative MABB-derived progeny containing HMW-GS 5 + 10 combination. (b) Loaf volume of the bread prepared from recipient cultivar NI5439 and the corresponding representative MABB-derived progeny with high GPC and HMW-GS 5 + 10 combination
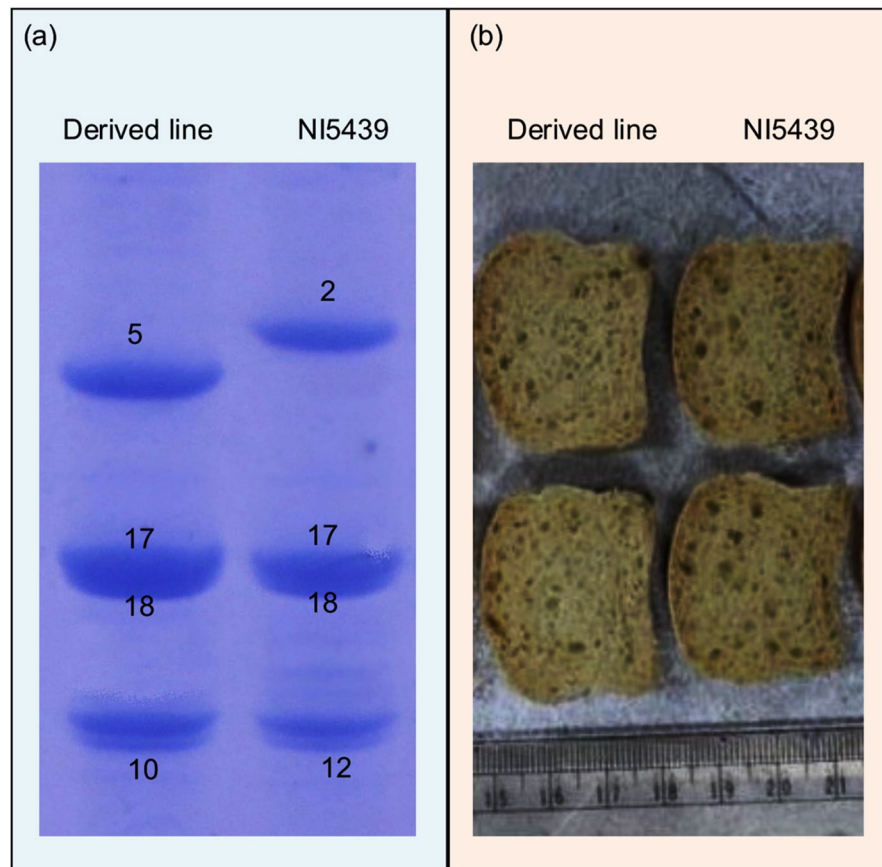
of the *P. triticina* and *P. striiformis* f. sp. *tritici* used during the present study, although these were resistant to several pathotypes of *P. graminis* f. sp. *tritici* (Table 4; Figure S2). However, the donor genotype PBW343 (*Gpc-B1/Yr36 + Lr24*) containing *Yr36* gene for resistance to stripe rust and *Lr24* gene for leaf rust was resistant to all the five pathotypes of *P. triticina* and all the seven pathotypes of *P. graminis* f. sp. *tritici* used for screening in the present study, but was resistant to only one (T pathotype) of the four pathotypes of *P. striiformis* f. sp. *tritici* (Table 4). Similar was the resistance pattern of the other derived progenies containing *Yr36 + Lr24* genes against the five *P. triticina* pathotypes (Table 4). However, disease reaction differed for *P. striiformis* f. sp. *tritici* and *P. graminis* f. sp. *tritici* pathotypes in each derived line that was predominantly resistant type.

The alien gene *Lr24* belonging to *Agropyron elongatum* introgressed during the present study (Fig. 2c) is known to confer resistance throughout the life of the plant, starting from seedling up to adult stage. Since 1993, a number of leaf rust resistant wheat varieties containing *Lr24* gene, which is still effective, have been released in India, although virulence against this gene has been reported from other parts of the world (Tomar et al. 2014). It has been recommended that *Lr24* should be used in combination of other Lr genes to prolong the life of this important gene for leaf rust resistance. *Lr24* is also tightly linked to a stem

rust resistance gene *Sr24*, which is effective even against one of the deadliest race (TTKSK) of Ug99 (Singh et al. 2006). The presence of this gene pair in our derived progenies was confirmed using two *P. striiformis* f. sp. *tritici* pathotypes (34–1 and 40–1). Almost all the derived lines containing this gene pair were found to be completely resistant to the above two pathotypes (Table 4). The chromosomal segment of *Ag. elongatum* carrying the gene combination *Lr24/Sr24*, besides providing resistance, has also been recently shown to be associated with improvement of most of the quality traits needed for making bread and other leavened products (Rai et al. 2021). Therefore, this gene pair could be safely utilized in wheat breeding program aimed at improving the grain quality and rust resistance, as demonstrated in this study.

Two derived progenies (Lok1-729–12 and Lok1-729–13) containing *Yr36 + Lr24/Sr24* were resistant against all the 16 pathotypes of the three rusts including *P. graminis* f. sp. *tritici* pathotypes 238S11 and 78S84 for which the donor genotype PBW343 (*Gpc-B1/Yr36 + Lr24*) was susceptible (Figure S2). The availability of resistance in two derived progenies, despite susceptible donor, may be attributed to disease escape. It has been argued that the following gene combinations are effective in providing resistance against the prevalent pathotypes of the three rusts in India: (i) leaf rust (*Lr24 + Lr37 + Lr76*), (ii) stem rust (*Sr24 + Sr38*), and (iii) stripe rust

Table 4 Summary of the results of screening of the parental genotypes and MAS-derived progenies (in the backgrounds of cvs. Lok1 and HD2967) against different pathotypes of the three rusts under glasshouse conditions

Genotype	Leaf rust pathotypes					Stripe rust pathotypes				Stem rust pathotypes						
	12–5	77–5	77–8	77–9	104–2	238S119	78S84	T	110S119	11	21A-2	34–1	40A	40–1	117–6	122
Lok1 (R)	;	3^+	3^+	0;	3^+	3^+	0;	3^+	3^+	2^-	;	-	3^+	;$^-$	-	;
Lok1-729–4	;	0;	0;	0;	;$^-$	0;	0;	0;	0;	;$^-$	;$^-$	;$^-$	2^-	3	0;	;
Lok1-729–11	-	0;	0;	0;	12^+	0;	2^-	0;	3^+	2^-	;$^-$	;	3+	3	;	3^+
Lok1-729–12	;	0;	;$^-$	0;	;$^-$	0;	0;	;	0;	;$^-$	;$^-$	;$^-$	;$^-$	-	;	;$^-$
Lok1-729–13	0;	0;	;$^-$	0;	;$^-$	0;	2^-	0;	0;	2^-	;$^-$	;$^-$	2^-	12	;	;$^-$
Lok1-729–14	;$^-$	3^+	0;	0;	;$^-$	0;	0;	;	0;	;$^-$	0;	;$^-$	0;	3^+	;	2^-
HD2967 (R)	12	3^+	0;	3+	3^+	3^+	0;	3^+	3^+	3^+	0;	12	0;	0;	0;	;$^-$
HD2967-756	;$^-$	0;	0;	0;	0;	3	0;	0;	3^+	;$^-$	;	;	0;	;$^-$	0;	0;
PBW343(*GpcB1/Yr36+Lr24*) (D)	;	0;	0;	0;	0;	3^+	3	0;	3^+	;	;$^-$	0;	0;	;$^-$	;	;$^-$

The ITs 0;,;$^-$,;, 1 to 2; and 2^- indicated resistant; ITs 1 to 2^+ indicated moderately resistant; ITs 3 indicated moderately susceptible; and ITs 3^+ indicated susceptible response; –, data not available; R, recipient genotype; D, donor genotype.

(*Yr17+Yr36+Yr70*). However, in recent years, new virulent races of leaf rust have emerged (Prasad et al. 2019). Among these new races, group 77 pathotypes are the most predominant and the pathotype 77–9 has been the most prevalent in wheat leaf rust samples collected from India and Nepal. However, the pathotype 77–9 that is virulent to *Lr37* could not overcome the resistance due to *Lr24* (Table 4). Thus, the transfer and pyramiding of *Yr36* and *Lr24* genes along with *Gpc-B1* gene (using the donor genotype PBW343+*Gpc-B1/Yr36*+*Lr24*) should prove useful for simultaneous improvement of grain quality and rust resistance (Fig. 1).

Progenies in the background of cvs. PW550+*Yr15*, PBW621, and DBW17 When the present study was started, the recipient cvs. PBW 621 and DBW17 were susceptible or only moderately resistant to most of the pathotypes of *P. triticana* and *P. striiformis* f. sp. *tritici,* although these recipients were resistant to several *P. graminis* f. sp. *tritici* pathotypes. However, the recipient parent PBW550 contained *Yr15* gene that was already introgressed (this was later released for commercial cultivation in Punjab as PBW761 alias *Unnat* PBW550). Introgression of *GpcB1/Yr36* gene in combination with *Yr15* gave very strong field resistance to all prevalent stripe rust races in all progenies derived in the background of PBW550. Progenies having *GpcB1/Yr36* in the backgrounds of recipient cvs. PBW621 and DBW17 showed resistance to stripe rust owing to *Yr36* gene under natural field conditions. The progenies derived from all the three recipient genotypes were moderately resistant (10S to 20S disease score; rarely 30S) to leaf rust under natural field conditions. This level of resistance of the derived progenies was similar to the level of resistance to leaf rust of the recurrent parents, although the presence of Lr genes for resistance to leaf rust in these genotypes is yet to be confirmed.

Reconstitution of genotypes of recipients

In the derived lines, the genome of the recurrent parents was reconstituted to the level of 90 to 96% (detailed data not presented), which is fairly high (Fig. 2d). RPG recovery with foreground selection followed by background selection and that with phenotypic selection following foreground selection was nearly similar suggesting no significant advantage of the background selection during MABB. The high RPG recovery in the present study matched with the recovery of phenotypic traits of the recipient parents, such that the introgressed lines and the recipient genotypes exhibited comparable values for agronomic traits including 1000-grain weight and grain yield.

Conclusions

The present study, spread over 15 years, was undertaken jointly by five institutions located in the North, North-East, and Central regions of India. All the recipient cultivars used in the present study are popular; some of them are mega-varieties adapted to various wheat growing agro-climatic zones of India, such that their contribution to national food security was immense. The improvement of these cultivars for grain quality and disease resistance through MABB is one of the major molecular breeding efforts undertaken in India. The derived progenies have the potential of developing into newer and improved cultivars for commercial cultivation or as pre-bred material for use in future wheat breeding programs.

Author contribution PKG, HSB, PC, JPJ, ST, VKM conceived and designed the experiment. HSB prepared first draft of the MS. NSB, RC, AKJ, SK, HK, GSM, MO, AS, PS, VSS supervised the experiments at different centres. PA, MA, SB, RC, VG, RPG, TG, VJ, RSK, SK, MS, AS, ST, NKV, MKV carried out genotyping and phenotyping work at different centres. PP carried out rust screening of the pyramided lines.

Data availability Not applicable.

Declarations

Ethics declarations Not applicable.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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