

Development of white-grain pre-harvest sprouting tolerant and pyramided protein-rich leaf rust resistant wheats using molecular breeding

Tinku Gautam

Chaudhary Charan Singh University

Kuldeep Kumar

Chaudhary Charan Singh University

Priyanka Agarwal

IARI: Indian Agricultural Research Institute

Sandhya Tyagi

IARI: Indian Agricultural Research Institute

Vandana Jaiswal

CSIR-IHBT: Institute of Himalayan Bioresource Technology CSIR

Vijay Gahlaut

CSIR-IHBT: Institute of Himalayan Bioresource Technology CSIR

Sachin Kumar

Chaudhary Charan Singh University

Pramod Prasad

Directorate of Wheat Research: ICAR - Indian Institute of Wheat and Barley Research

Parveen Chhuneja

Punjab Agricultural University

Harindra Singh Balyan

Chaudhary Charan Singh University

Pushpendra Kumar Gupta (✉ pkgupta36@gmail.com)

Ch. Charan Singh University <https://orcid.org/0000-0001-6374-9148>

Research Article

Keywords: Marker-assisted selection (MAS), pyramiding, pre-harvest sprouting tolerance, grain protein content, rust resistance

Posted Date: April 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-284315/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The present study was undertaken for developing pre-harvest sprouting tolerant (PHST) wheat genotypes using marker-assisted backcross breeding (MABB). A major QTL for PHST was introgressed into an elite Indian wheat cv. Lok1 that is PHS susceptible. These PHST lines were also pyramided with one gene each for high grain protein content (*Gpc-B1*) and leaf rust resistance (*Lr24*). For introgression of PHST QTL, initially Lok1 was separately crossed with each of the two donors (PHS tolerant white-grained AUS1408 and CN19055). Backcrossing in each generation was followed by foreground and background selections using SSR markers. In advanced lines, KASP assay was also carried out for the candidate gene *TaMKK3-A* underlying the PHST QTL. The MAS derived lines homozygous for PHST QTL were screened for PHS using simulated rain chambers resulting in the selection of 10 PHST lines. For pyramiding of three QTL/genes (PHST QTL, *Gpc-B1*, and *Lr24*), MABB derived BC₄F₂ plants (from the cross Lok1/CN19055) were crossed with a MAS derived BC₂F₅ line [Lok1 (*Gpc-B1* + *Lr24*)] developed earlier by us in the same background of Lok1. After foreground MAS followed by PHS screening, four advanced lines carrying all the three QTL/genes in homozygous condition were selected. These lines exhibited high level of PHST (PHS score 2–3) associated with significant improvement in GPC with no yield penalty and resistance against leaf rust under artificial epiphytotic conditions.

Introduction

Pre-harvest sprouting (PHS) refers to precocious germination of grains within physiologically mature spikes on prolonged rains before harvesting (Groos et al. 2002). Seed dormancy (SD) has also been used as a surrogate trait for PHS tolerance (PHST) because PHST is known to depend on seed dormancy and the two traits exhibit high correlation (Li et al. 2004). PHS leads to yield losses and significant reduction in grain quality due to activation of enzymes like lipases, amylases and proteases in developing grains leading to degradation of lipids, starch, and proteins (Andreoli et al. 2006; Simsek et al. 2014). PHS also leads to reduction in market value of wheat grain, by 50%, ultimately resulting in a global estimated loss of US \$1 billion annually (Ali et al. 2019). PHS problem is common in all major wheat growing areas of the world, including Canada, Europe, Australia, China, USA, Japan, and also some parts of India (Rajjou et al. 2012; Zhou et al. 2018).

PHS is a complex trait controlled by a large number of genes and is influenced by the environment. PHST is often associated with red grain color (DePauw and McCaig, 1983; Groos et al. 2002; Fofana et al. 2009), so that grain color and seed dormancy both are used as a markers for PHST (Flintham, 2000; Mares et al. 2005). However, PHS tolerant white grain wheat genotypes are also known, which include AUS1408 (Mares, 1987) and CN19055 (Hucl and Matus-Cadiz, 2002). Following parameters are generally used for estimating the level of PHST: PHS index, falling number, germination index and alpha amylase activity (Nörnberg et al. 2015; Shorinola et al. 2016). Among cereals, in wheat alone, ~50 QTL interval mapping studies involving ~40 mapping populations have already been conducted leading to identification of as many as >250 QTLs for PHST, which also included hexaploid synthetic wheats, tetraploid durum wheats and diploid einkorn wheats (see review by Gupta et al. 2020). Relatively, more

important QTL for PHST and seed dormancy are known to be carried by chromosomes 3A, 3B, 3D, and 4A, although all the 21 chromosomes carry QTLs for this trait (see Gupta et al. 2020). MetaQTLs have also been identified for PHST in wheat (Tyagi and Gupta, 2012). Genome wide association studies (GWAS) have also been conducted for PHST and related traits leading to identification of ~250 MTAs (for details see review by Gupta et al. 2020). A number of these MTAs were located in genomic regions carrying QTLs earlier identified through interval mapping. Among known QTLs, 30 QTLs have also been shown to be stable over environments; these have been considered potentially useful for improvement of PHST in wheat. A major QTL on chromosome 3A, associated with red grain color (*QPhs.ccsu-3A.1*; Kulwal et al. 2005) and another QTL *Phs-A1*, located on chromosome arm 4AL are considered to be relatively more important for breeding (Mori et al. 2005; Mares et al. 2005; Liu et al. 2008). *Phs-A1* has also been cloned and characterized (Barrero et al. 2015; Shorinola et al. 2017). Two genes, namely *PM19-A1* and *PM19-A2* (Plasma Membrane 19 genes) were proposed as the main candidates for the QTL *Phs-A1* (Barrero et al. 2015), although on the basis of comparative analysis of synteny, as many as 10 orthologous genes were proposed as candidate genes. The QTL *Phs-A1* also overlaps the gene *TaMKK3-A* for seed dormancy (Torada et al. 2016). Recently, markers for QTL *Phs-A1* and the gene *TaMKK3-A*, were also developed (Shorinola et al. 2017). It was also shown that *TaMKK3-A* alleles are diagnostic for sprouting resistance in parents of mapping populations used for identification of the QTL *Phs-A1*.

It has been recognized that initially during green revolution, improvement in yield was a major concern, and that during the last three decades, GPC has also attracted the attention of wheat breeders to address the problem of nutritional security in developing countries (Joshi et al. 2007). Therefore, among important traits for wheat breeders, grain protein content (GPC) and resistance to three rusts including leaf rust are also important. Although, several loci affecting GPC have been identified in wheat, the most important gene affecting GPC is *Gpc-B1*, which was initially identified in wild emmer wheat (*Triticum turgidum* L. ssp. *dicoccoides*) and was subsequently cloned following map-based cloning strategy (Uauy et al. 2006). *Gpc-B1* encodes a NAC-domain transcription factor and is located on the short arm of chromosome 6B, tightly linked with genes for accelerated senescence and high grain Fe/Zn concentration (Distelfeld et al. 2007; reviewed in Tabbita et al. 2017).

Suitable RFLP, CAPS, and SSR markers are available for *Gpc-B1* (Mesfin et al. 1999; Khan et al. 2000; Olmos et al. 2003; Distelfeld et al. 2006; Uauy et al. 2006). During the last decade, *Gpc-B1* has been extensively deployed in durum and common wheats for improvement of GPC, so that most modern wheat varieties all over the world now carry *Gpc-B1* (Uauy et al. 2006; Hagenblad et al. 2012; Brevis and Dubcovsky 2010; Kumar et al. 2011; Balyan et al. 2013; Vishwakarma et al. 2014; Vishwakarma et al. 2016; Bokore et al. 2019; Gautam et al. 2020). However, it has been a challenge to develop wheat varieties with high GPC without yield penalty due to several factors including the following: negative correlation between the grain yield and GPC (range of correlation = -0.30 and -0.60), large environmental effects, genotype-by-environment interactions, and a complex genetic system governing GPC (Simmonds, 1995; DePauw et al. 2007; Klindworth et al. 2009; Brevis and Dubcovsky 2010). The backcross-derived lines containing *Gpc-B1* have also been found to have reduced kernel weight and earlier senescence, both adversely affecting grain yield (Sherman et al. 2008).

In our own research extending over two decades, we also observed that PHS tolerant wheats improved for GPC are often susceptible to leaf rust, which causes significant losses (~7-30%) in wheat yield worldwide (Bolton et al. 2008). Leaf rust resistance genes have been regularly discovered, so that ~80 Lr genes are now known in wheat (McIntosh et al. 2017). Among these Lr genes, *Lr24* is an important gene, which has been widely deployed in wheat cultivars because it provides resistance at all developmental stages. The gene *Lr24* is also linked with *Sr24* so that *Lr24* has become a gene of choice in wheat breeding programmes (Bhardwaj et al. 2019).

In view of the above, the development of PHS tolerant, protein-enriched leaf rust resistant wheats using molecular breeding became an important goal in our laboratory, since markers for all the three targeted traits are available. This goal has now been successfully achieved using wheat cv. Lok1, which has been widely grown in central India. The results of this study, extending almost a decade, are presented in this communication.

Materials And Method

Material

Plant materials used in the present study included the following: (i) an Indian wheat cv. Lok1, (ii) two wheat genotypes, namely AUS1408 and CN19055, each carrying a major PHST QTL located on chromosome arm 4AL (Mares et al., 2005; Ogonnaya et al. 2008); these two genotypes were used as two independent donors; (iii) a MAS derived line in the background of cv. Lok1 containing *Gpc-B1+Lr24*, where *Gpc-B1* is a locus for high grain protein content, which is in close linkage with a stripe rust resistance gene (*Yr36*); this improved line was used as a donor for pyramiding of genes for GPC and resistance to leaf rust and on to PHST locus carried by the improved BC₄F₂ plants. The Lok1 cultivar is a high yielding wheat cultivar, released in 1982 for late sown irrigated conditions of central India, where it has been the most preferred wheat variety for cultivation, due to its good bread making quality and superior agronomic performance. The choice of Lok1 as the recipient cultivar was based on the fact that Lok1 was the first among the best 10 varieties, selected from 150 varieties for breeder's seed production across the country for seven consecutive years between 2004 and 2010. At present, Lok1 (63.91 Mt) has third rank after PBW343 (73.42 Mt) and HD2967 (93.38 Mt) in terms of breeder's seed indent for the year 2019-20; it is still grown in an estimated 1 mha wheat area in central India (<https://www.lokbharti.org/Wheat-Research>). Lok1 is also highly resistant to the most virulent rust pathotype *Ug-99*.

Molecular markers

The details of the markers linked with QTL/genes introgressed and pyramided during the present study are presented in Table 1. For the recovery of the recurrent parent genome (RPG) in the segregating backcross progenies during background selection, a total of ~130 polymorphic SSR markers (selected from 453 markers) covering all the 21 chromosomes were used in the following two crosses: Lok1/AUS1408 and Lok1/CN19055. In each backcross generation, the proportion of the genome from the

recipient parent was estimated using the following formula; $G = [(X+1/2Y) \times 100]/N$, where, X is the number of markers showing homozygosity for recipient parent alleles; Y is the number of markers showing heterozygosity involving the two parental alleles and N is the total number of polymorphic markers.

DNA extraction, primer synthesis and PCR analysis

DNA isolation from 30-40 days old leaves was carried out using the CTAB method following Saghai-Maroo et al. (1984), with some modifications. DNA was quantified on 1% agarose gel using agarose gel electrophoresis. Primers were synthesized by Eurofins Genomics India Pvt Ltd., Bangaluru, India. Polymerase chain reaction (PCR) was carried out in 20 μ L reaction mixture containing the following component: ~50 ng of template DNA, 1 U *Taq* polymerase (Sigma-Aldrich, USA), 0.02 mM of each dNTPs (Sigma-Aldrich, USA), 0.2 μ M primers (forward and reverse), 1X *Taq* buffer in each case. PCR was performed using Veriti™ 96-well Thermal Cycler of Applied Biosystem, on following PCR profile: initial denaturation for 4 min at 94°C, followed by 35 cycles each with 30 sec at 94°C, 30 sec at 50–60°C (specific for different primers), 1 min at 72°C, and 7 min at 72°C for final extension. Amplification products for marker SCS73₇₁₉ were resolved on 2% agarose gel and visualized on UV trans-illuminator; products of other markers were resolved on 10% PAGE gel and visualized by silver staining.

KASP assay

Kompetitive allele specific PCR (KASP) assay was also conducted to confirm the presence of PHST QTL in the improved lines developed during this study by using the primers earlier developed and used by Shorinola et al (2017). The KASP assay was carried out as described in Bansal et al (2020)

Breeding scheme for introgression and pyramiding of QTL/genes

Marker assisted backcross breeding (MABB) scheme was followed to transfer the *PHST QTL* from each of the two donor genotypes (AUS1408 and CN19055) independently into the genetic background of cv. Lok1 (Fig. 1). Recurrent parent Lok1 was used as female and crossed with each donor as male parent. F₁ plants were used for four generations of backcrossing leading to BC₄F₁. Both foreground and background selections were exercised. The MABB scheme followed in the present study is shown in Fig. 1. Foreground MAS in BC₄F₂ and BC₄F₃ was carried out for selecting plants homozygous for the PHST QTL; these plants were then screened in simulated rain chambers for PHST (more details are provided in the next section). Seed of the selected PHS tolerant plants was multiplied for conducting station yield trials for two consecutive years for evaluation of agronomic traits.

The BC₄F₂ plants (improved for PHST) derived from only one of the two crosses namely Lok1/CN19055 was utilized for pyramiding of genes for high GPC and leaf rust resistance in the background of cv. Lok1. Scheme of the breeding program for pyramiding is also depicted as a part of Fig. 1. For the purpose of pyramiding, the improved PHST plants were crossed as female parent with MAS derived BC₂F₅ line Lok1 (*Gpc-B1+Lr24*) earlier developed by us in a separate programme. The initial crossing was followed by

forward breeding for a number of generations using plant to progeny scheme. The F₄ progenies were screened for rust resistance under artificial epiphytotic conditions (for details see below). The progenies in F₄ to F₆ generations were also screened for PHS tolerance under artificial rains (for detail see below). Seed of the selected progenies was multiplied for station yield trials for two consecutive years (for details see below).

Evaluation for PHS and seed dormancy

For the PHS screening, five randomly selected physiologically mature spikes (when 75% spikes in a plot turned yellow) were harvested from each selected progenies and immediately kept in specialized simulated rain chambers at room temperature providing artificial rain every 6 h for seven consecutive days, when data on PHS was scored on a scale of 1-9; score of 1 represented no visible sprouting and a score of 9 represented complete sprouting (Kumar et al. 2010). Mean PHS scores were used for selection of PHS tolerant genotypes.

Data was also recorded on seed dormancy of MAS derived lines following Knox et al (2012). In brief 50 seed were placed in a Petri dish with the crease of each seed facing down to make it easier to observe the germination. Seed with white radicle or plumule protruding with a visible tear in the seed coat were treated as germinated seeds and removed from the plate. Seed counts were recorded daily for 21 days and germination index (GI) was calculated.

Screening of MAS-derived lines for rust resistance

Screening of the pyramided lines (containing *QPhs.dpi.vic.4A.2*, *Gpc-B1* and *Lr24*) were carried out against a total of 16 pathotypes of the three rusts reported from different regions of India (for the details of pathotypes used see Table 2). The procedure for screening of rust resistance was described in an earlier study (Gautam et al. 2020). In brief, seedlings were assayed for the infection against each pathotype at 15 days post-inoculation (dpi) following Stakman et al (1962).

Yield trials of MAS-derived lines

The yield trials included the following 18 lines; (i) 10 lines with only PHST QTL, (ii) 4 lines containing *QPhs.dpi.vic.4A.2+Gpc-B1+Lr24* and (iii) one recipient and three donor parental genotypes [Lok1, Lok1 (*Gpc-B1+Lr24*), AUS1408, and CN19055]. Randomized block design with two replications was used at Meerut during 2018-19 and 2019-20 crop seasons. Each line in a replication was evaluated in 6 m² plots, each having six rows of six meter with a row-to-row distance of 20 cm. Phenotypic data were recorded on the following agronomic and grain quality traits: plant height (PH in cm), tillers per meter (TPM), grain number per spike (GNPS), 1000-grain weight (TGW in g), grain yield (GY in g), grain protein content (GPC in %), iron (Fe in ppm), and zinc (Zn in ppm) contents in grains. The GPC (%) at 12% grain moisture content was estimated using FOSS InfratecTM 1241 Grain Analyzer (Denmark).

Statistical analysis

For statistical analyses, mean values of the two replications for each trait in each trial were used. The descriptive statistics were obtained using Excel. The analysis of variance (ANOVA) for the eight traits was carried out using the SPSS Statistics for Windows, version 16.0 (SPSS Inc., Chicago, Ill., USA); significance in ANOVA was tested for the following sources of variation: replications, genotypes, environments, genotype-by-environment interactions.

Results And Discussion

MABB for PHST involving two donors (AUS1408/CN19055): For introgression of PHST QTL separately from each of the two donors, namely AUS1408 and CN19055 into Lok1, two separate MABB programmes were followed. In each case, F_1 plants were subjected to four backcrosses to recipient parent cv. Lok1 involving foreground and background MAS. For foreground selection of PHST QTL, separate pairs of polymorphic SSR markers were used as follows: (i) *Xgwm397* and *Xbarc170* in case of AUS1408, and (ii) *Xgwm637* and *Xgwm894* in case of CN19055. Similarly, for background selection a set ~130 polymorphic SSR markers were used in each cross. The amplification profiles of markers used for foreground selection in two crosses are shown in Fig. 2 (for details of SSRs used for background selection see Supplementary Table 1). In $BC_4F_{2/3}$, homozygous plants were selected and were then subjected to phenotypic selection for PHS in subsequent generations (BC_4F_4 - BC_4F_5). KASP assay involving SNPs associated with PHST was also carried out in BC_4F_6 lines to confirm the presence of PHST QTL and the seed dormancy gene *TaMKK-3* (Fig. 3). Only 62 of the 115 progenies carried the SNP allele associated with PHST in homozygous condition. It appears that during the foreground selection for PHST QTL, recombination occurred in the large intervals (14.2 cM in case of AUS1402 and 17.8 cM in case of CN19055) between the markers flanking the PHST QTL, leading to the loss of PHST QTL allele in some progenies or to the selection of heterozygous allele at the PHS locus. Therefore, in future, closely linked markers need to be used. Eventually, 10 lines (8 involving AUS1408 and 2 involving CN19055) were selected, each carrying the marker associated with the desired QTL in homozygous condition, associated with 90-96% RPG, PHS score <3 and germination index (G17 at 7 days for testing seed dormancy) ranging from 0.2-0.6.

RPG recovery in the two crosses in the present study did not differ from several earlier wheat breeding programmes where MABB was used for introgression of genes/QTL for biotic and abiotic stresses in wheat (Yadav et al. 2015; Mallick et al. 2015; Vishwakarma et al. 2016; Rai et al. 2018; Randhawa et al. 2019; Todkar et al. 2020; Gautam et al. 2020a). This confirmed the utility of MABB for effective introgression of genes/QTL into the agronomically superior genotypes associated with recovery of recurrent parent genome in a relatively short time as also reported earlier in wheat (Mallick et al. 2015), rice (Divya et al. 2015; Ellur et al. 2016; Ramalingam et al. 2020; Jamaloddin et al. 2020), and maize (Sureshkumar et al. 2014; Kaur R et al. 2020).

Pyramiding of QTL/genes for PHST, high GPC and resistance to leaf rust

MAS derived BC₄F₂ plants containing the PHST QTL from the donor CN19055 in the background of cv. Lok1 were used for the pyramiding of *Gpc-B1*, and *Lr24*, on to *QPhs.dpivic.4A.2* (already introgressed in BC₄F₂ plants). Markers utilized during pyramiding are listed in Table 1. In F₂ generations, foreground selection was carried out for three QTL/genes (*Gpc-B1*, *Lr24* and *QPhs.dpi.vic.4A.2*) and plants homozygous/heterozygous for the three QTL/genes were selected (Fig. 4). Further foreground selection and phenotypic selection was exercised for PHS and four advanced F₅ lines were selected, which carried the above three QTL/genes in homozygous conditions and PHS score 2-3 (Fig. 5). These lines were subjected to rust screening and evaluation in yield trials.

Screening of the pyramided lines for rust resistance

The original cv. Lok1 was only moderately resistant to most of the pathotypes of the three rusts except one pathotype for each (Table 2). However, the MAS-derived parental genotype Lok1 (*Gpc-B1+Lr24*) used for gene pyramiding, was resistant against three pathotypes (238S119, 78S84, and 47S103) of stripe rust, all the five pathotypes (43232, 77-5, 77-8, 77-9, and 104-2) of leaf rust, and four pathotypes (21A-2, 34-1, 40-1 and 117-6) of stem rust (Table 2). Similar was the resistance pattern of the pyramided lines containing *Gpc-B1+Lr24+PHST QTL QPhs.dpivic.4A.2* (Table 2). Therefore, it is obvious that the pyramiding approach used in the present study was successful to produce improved version of cv. Lok1 with improved grain quality and rust resistance. In India, *Lr24* (derived from *Agropyron elongatum*) continues to provide effective resistance, although virulence against *Lr24* has been reported in South America, North America, and South Africa (Tomar et al. 2014). Therefore, introgression of *Lr24* using MAS has also been reported in several earlier studies in India (Samsampour et al. 2009; Revathi et al. 2010; Chhuneja et al. 2011; Kaur et al. 2020). However, for maximum protection, it is recommended that *Lr24* should be used in combination with other Lr genes (Bolton et al. 2008).

The *Lr24* gene is also linked with *Sr24*, a stem rust resistance gene effective even against the deadliest race TTKSK (Ug99) (Singh et al. 2006). According to some estimates, the wheat cultivars carrying *Lr24/Sr24* occupied a total of about 15 Mha over a period of 20 years (Tomar et al. 2014). In addition to the linked genes *Lr24/Sr24*, use of other resistance genes, namely *Sr31* and *Sr36* for stem rust and *Lr9*, *Lr19*, and *Lr34* for leaf rust has also been suggested for breeding resistant varieties (Bhardwaj et al. 2019). In India, some of the important genes conferring resistance against the prevalent pathotypes of three rusts include the following: *Lr24*, *Lr37*, *Lr76* for leaf rust; *Sr24*, *Sr38* for stripe rust; and *Yr17*, *Yr36*, *Yr70* for yellow rust (Prasad et al. 2019). However in recent years, new virulent races have emerged for some of these genes, of which 77 is the most important race of leaf rust and pathotype 77-9 is the most prevalent. In leaf rust samples of cultivated wheat collected from India and Nepal, pathotype 77-9 was identified in 149 (51.1%), 77-13 in 20.2% and 77-5 in 15.1% rust samples; *Lr24* provided resistance against the pathotype 77-9. Using gene pair *Lr24/Sr24*, 18 wheat varieties for different ecological conditions ranging from moisture stress to irrigated environments, and timely sown to late sown conditions have been released for commercial cultivation in India (Tomar et al. 2014). The presence of this gene pair in our pyramided lines was also confirmed using two stem rust pathotypes (34-1 and 40-1),

where all the derived pyramided lines were found to be completely resistant to the above two pathotypes (Table 2).

Performance of MAS-derived lines

The results of ANOVA for two yield trials involving 14 MAS derived lines along with parents [Lok1, Lok1 (*Gpc-B1+Lr24*), AUS1408, and CN19055] are presented in Table 3. Differences between genotypes were significant for five (TGW, GY, GPC, Fe, and Zn) of the eight traits. Environment effects were also significant for four of the six traits (except TGW and GY). Genotype-by-environment ($g \times e$) interactions were significant for only three (PH, GY, and GPC) traits suggesting significant effect of environment on the expression of these three traits.

Phenotypic evaluation of the PHS tolerant lines

(a) Progenies containing only PHST QTL

The ten (10) improved wheat lines obtained during the present study following MABB involving two crosses (8 from Lok1/AUS1408 and 2 from Lok1/CN19055) and subsequent phenotypic selection for PHST were also evaluated for important agronomic traits. All the 10 derived lines were similar to the recurrent parent cv. Lok1 for four of the eight traits (PH, TPM, GNPS, and GPC), possibly as a result of the 90-96% recovery of the RPG in these lines following four backcrosses along with background selection (Table 4). However only one line (CCSU-78) showed higher TGW, and only three lines (CCSU-84, -97, and -109) showed higher GY relative to the recipient cv. Lok1. Interestingly, seven lines also had improved grain Fe content (36.5-52.6 ppm) and three lines also had higher grain Zn content (28.7-35.0 ppm) relative to the grain Fe/Zn contents of the recurrent parent and each of the two donors (Table 4). Since there is no known relationship between the PHS tolerance and grain mineral contents, we speculate that the observed biofortification may be due to the possible accumulation of complementary genes for high grain Fe and Zn distributed in the recipient parent cv. Lok1 and each of the two donor genotypes (AUS1408 and CN19055). Future studies may be planned to understand the basis of associated biofortification in PHS tolerant lines.

(b) Pyramided progenies containing genes/QTLs for GPC, leaf rust, and PHST

Four (4) improved Lok1 lines with *QPhs.dpivic.4A.2+Gpc-B1+Lr24* (PHS score=2-3) were also evaluated for five agronomic traits and three grain quality traits. These lines resembled the recipient cv. Lok1 for all the five agronomic traits, but carried higher GPC (12.3% to 12.5%), and higher Fe; one line (CCSU-35) also had higher Zn (Table 4). Interestingly, the higher GPC in these lines had no yield penalty despite the known negative association of GPC with grain yield and the effect of genotype, environment and epistatic interactions on GPC (Davies et al. 2006; Brevis and Dubcovsky 2010; Kumar et al. 2011). Wheat lines with high GPC and no yield penalty were also developed earlier through introgression of *Gpc-B1* gene following MAS (Kade et al. 2005; Brevis and Dubcovsky 2010; Kumar et al. 2011; Vishwkarma et al. 2016). Using the above *Gpc-B1* gene, two commercial bread wheat cv. Lassik and Farnum were also developed earlier

(Brevis and Dubcovsky 2010; <http://variety.wsu.edu/extensionpubs/Farnumtrifold.pdf>, <http://uvdavis.edu/files2/57360.pdf>; for review see Balyan et al. 2013). The high GPC gene *Gpc-B1* has also been exploited in breeding (mostly following MAS) for improvement of grain Fe and Zn associated with GPC in wheat (for reviews see Tabbita et al. 2017; Gupta et al. 2020). These results suggest that while improving PHS tolerance, pyramiding with *Gpc-B1* can be used not only for protein enrichment but also for biofortification with Fe and Zn through MAS associated with judicious phenotypic selection.

In summary, a combination of marker-assisted foreground/background and phenotypic selection is a successful strategy for development of improved lines for the trait like PHST. The present study is yet another study of its kind, where MAS was utilized for the improvement of PHS in wheat. The two earlier studies were also conducted in our own laboratory for improvement of wheat cultivars for PHST (Kumar et al. 2010; Tyagi et al. 2014). However in both these earlier studies, we used a major PHS QTL located on 3A which was associated with red grain color, which is not preferred by the consumers. During this study, we not only developed PHS tolerant wheat lines with white grains, but also pyramided the PHS tolerance with high GPC and rust resistance. These improved lines should serve as new genetic resources for breeders in future wheat breeding programs.

Declarations

Acknowledgements

The work was carried out, when TG held JRF/SRF position under a research project funded by DBT, Government of India. PKG was awarded Hony Scientist position and HSB was awarded Senior Scientist position both from Indian National Science Academy (INSA). Head, Department of Genetics and Plant Breeding, CCS University, Meerut, provided the necessary infrastructure.

Funding

Funds received from DBT (File No. DBT/PR11695/AGR/02/643/2008), New Delhi, India for carrying out this study.

Authors' contributions

PKG and HSB designed the experiment. TG carried out phenotyping, data analysis and prepared first draft of the MS. PA, ST, VJ, VG, SK performed the initial breeding work. KK assisted TG in phenotypic data recording. PP carried out rust screening of the pyramided lines. PC provided facilities for KASP assay.

Ethics declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare no competing interests.

References

Ali A, Cao J, Jiang H et al (2019) Unraveling molecular and genetic studies of wheat (*Triticum aestivum* L.) resistance against factors causing pre-harvest sprouting. *Agronomy* 9:117.

<https://doi.org/10.3390/agronomy9030117>

Andreoli C, Bassoi MC, Brunetta D (2006) Genetic control of seed dormancy and pre-harvest sprouting in wheat. *Sci Agric* 63:564-566. <https://doi.org/10.1590/S0103-90162006000600009>

Balyan HS, Gupta PK, Kumar S et al (2013) Genetic improvement of grain protein content and other health-related constituents of wheat grain. *Plant Breed* 132:446–457. <https://doi.org/10.1111/pbr.12047>

Barrero JM, Cavanagh C, Verbyla KL et al (2015) Transcriptomic analysis of wheat near-isogenic lines identifies *PM19-A1* and *A2* as candidates for a major dormancy QTL. *Genome Biol* 16:93.

<https://doi.org/10.1186/s13059-015-0665-6>

Bhardwaj SC, Singh GP, Gangwar OP, Prasad P, Kumar S (2019) Status of wheat rust research and progress in rust management-Indian context. *Agronomy*

9:892. <https://doi.org/10.3390/agronomy9120892>

Bansal M, Adamski NM, Toor PI et al (2020) *Aegilops umbellulata* introgression carrying leaf rust and stripe rust resistance genes *Lr76* and *Yr70* located to 9.47-Mb region on 5DS telomeric end through a combination of chromosome sorting and sequencing. *Theor Appl Genet* 133:903–915.

<https://doi.org/10.1007/s00122-019-03514-x>

Bokore FE, Knox RE, DePauw RM, et al (2019) Validation of the effects of the *Gpc-B1* high grain protein concentration locus from Lillian hard red spring wheat (*Triticum aestivum* L.) using locus specific

markers. *Euphytica* 215:2. <https://doi.org/10.1007/s10681-018-2322-0>

- Bolton MD, Kolmer JA, Garvin DF (2008) Wheat leaf rust caused by *Puccinia triticina*. Mol Plant Pathol 9:563–575. <https://doi.org/10.1111/j.1364-3703.2008.00487.x>
- Brevis JC, Dubcovsky J (2010) Effects of the chromosome region including the locus on wheat grain and protein yield. Crop Sci 50:93. <https://doi.org/10.2135/cropsci2009.02.0057>
- Chhuneja P, Vikal Y, Kaur S et al (2011) Marker assisted pyramiding of leaf rust resistance genes *Lr24* and *Lr28* in wheat (*Triticum aestivum*). Indian J Agric Sci 81: 214-218.
- Davies J, Berzonsky WA, Leach GD (2006) A comparison of marker-assisted and phenotypic selection for high grain protein content in spring wheat. Euphytica 152:117-134. <https://doi.org/10.1007/s10681-006-9185-5>
- De Pauw RM, Mccaig TN (1983) Recombining dormancy and white seed color in a spring wheat cross. Can J Plant Sci 63:581-519. <https://doi.org/10.4141/cjps83-074>
- DePauw RM, Knox RE, Clarke FR et al (2007) Shifting undesirable correlations. Euphytica 157:409-415. <https://doi.org/10.1007/s10681-007-9379-5>
- Distelfeld A, Cakmak I, Peleg Z et al (2007) Multiple QTL-effects of wheat *Gpc-B1* locus on grain protein and micronutrient concentrations. Physiol Plant 129:635–643. <https://doi.org/10.1111/j.1399-3054.2006.00841.x>
- Distelfeld A, Uauy C, Fahima T, Dubcovsky J (2006) Physical map of the wheat high-grain protein content gene *Gpc-B1* and development of a high-throughput molecular marker. New Phytol 169:753-763. <https://doi.org/10.1111/j.1469-8137.2005.01627.x>
- Divya D, Naik B, Sundaram RM, Laha GS, Bentur JS (2015b) Marker-assisted pyramiding of bacterial blight and gall midge resistance genes in samba Mahsuri and study of their interactions. IJSR 4:2277–8179.
- Ellur RK, Khanna A, Yadav A et al (2016) Improvement of Basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding. Plant Sci 242:330-341. <https://doi.org/10.1016/j.plantsci.2015.08.020>
- Flintham JE (2000) Different genetic components control coat imposed and embryo-imposed dormancy in wheat. Seed Sci Res 10:43-50. <https://doi.org/10.1017/S0960258500000052>
- Fofana B, Humphreys DG, Rasul G et al (2009) Mapping quantitative trait loci controlling pre-harvest sprouting resistance in a red · white seeded spring wheat cross. Euphytica 165, 509-521. <https://doi.org/10.1007/s10681-008-9766-6>
- Gautam T, Amardeep, Saripalli G et al (2020a) Introgression of a drought insensitive grain yield QTL for improvement of four Indian bread wheat cultivars using marker assisted breeding without background

selection. *J Plant Biochem Biotechnol* 30:172–183. <https://doi.org/10.1007/s13562-020-00553-0>

Gautam T, Dhillon GS, Saripalli G et al (2020b) Marker-assisted pyramiding of genes/QTL for grain quality and rust resistance in wheat (*Triticum aestivum* L.). *Mol Breed* 40:49. <https://doi.org/10.1007/s11032-020-01125-9>

Groos C, Gay G, Perretant MR et al (2002) Study of the relationship between preharvest sprouting and grain color by quantitative trait loci analysis in a white × red grain bread-wheat cross. *Theor Appl Genet* 104:39-47. <https://doi.org/10.1007/s001220200004>

Gupta PK, Balyan HS, Sharma S, Kumar R (2020) Genetics of yield, abiotic stress tolerance and biofortification in wheat (*Triticum aestivum* L.). *Theor Appl Genet* 133:1569-1602. <https://doi.org/10.1007/s00122-020-03583-3>

Hucl P, Matus-Cadiz M (2002) W98616, a white seeded spring wheat with increased preharvest sprouting. *Can J Plant Sci* 82:129-131. <https://doi.org/10.4141/P01-041>

Jamaloddin M, Durga Rani CV, Swathi G et al (2020) Marker Assisted Gene Pyramiding (MAGP) for bacterial blight and blast resistance into mega rice variety “Tellahamsa”. *PLoS One* 15: e0234088. <https://doi.org/10.1371/journal.pone.0234088>

Joshi AK, Mishra B, Chatrath R, Ortiz Ferrara G, Singh RP (2007) Wheat improvement in India: present status, emerging challenges and future prospects. *Euphytica* 157:43-446. <https://doi.org/10.1007/s10681-007-9385-7>

Kade M, Barneix AJ, Olmos S, Dubcovsky J (2005) Nitrogen uptake and remobilization in tetraploid ‘Langdon’ durum wheat and a recombinant substitution line with the high grain protein gene *Gpc-B1*. *Plant Breed* 124:343-349. <https://doi.org/10.1111/j.1439-0523.2005.01110.x>

Kaur R, Kaur G, Vikal Y et al (2020) Genetic enhancement of essential amino acids for nutritional enrichment of maize protein quality through marker assisted selection. *Physiol Mol Biol Plants* 26, 2243-2254. <https://doi.org/10.1007/s12298-020-00897-w>

Khan IA, Procnier JD, Humphreys DG et al (2000) Development of PCR-based markers for a high grain protein content gene from *Triticum turgidum* ssp. transferred to bread wheat. *Crop Sci* 40:518. <https://doi.org/10.2135/cropsci2000.402518x>

Klindworth DL, Hareland GA, Elias EM, Faris JD, Chao S, Xu SS (2009) Agronomic and quality characteristics of two new sets of Langdon durum-wild emmer wheat chromosome substitution lines. *J Cereal Sci* 50:29-35. <https://doi.org/10.1016/j.jcs.2009.02.003>

Knox RE, Clarke FR, Clarke JM, Fox SL, DePauw RM, Singh AK (2012) Enhancing the identification of genetic loci and transgressive segregants for preharvest sprouting resistance in a durum wheat population. *Euphytica* 186, 193-206. <https://doi.org/10.1007/s10681-011-0557-0>

- Kulwal P, Kumar N, Kumar A, Gupta RK, Balyan HS, Gupta PK (2005) Gene networks in hexaploid wheat: interacting quantitative trait loci for grain protein content. *Funct Integr Genom* 5:254–259. <https://doi.org/10.1007/s10142-005-0136-3>
- Kulwal PL, Kumar N, Gaur A et al (2005) Mapping of a major QTL for pre-harvest sprouting tolerance on chromosome 3A in bread wheat. *Theor Appl Genet* 111:1052-1059. <https://doi.org/10.1007/s00122-005-0021-4>
- Kumar J, Jaiswal V, Kumar A et al (2011) Introgression of a major gene for high grain protein content in some Indian bread wheat cultivars. *Field Crop Res* 123:226–233. <https://doi.org/10.1016/j.fcr.2011.05.013>
- Kumar J, Mir RR, Kumar N et al (2010) Marker assisted selection for pre-harvest sprouting tolerance and leaf rust resistance in bread wheat. *Plant Breed* 129:617–621. <https://doi.org/10.1111/j.1439-0523.2009.01758.x>
- Li C, Ni P, Francki M, Hunter A et al (2004) Genes controlling seed dormancy and pre-harvest sprouting in a rice-wheat-barley comparison. *Func Integr Genomic* 4:84-93. <https://doi.org/10.1007/s10142-004-0104-3>
- Liu S, Cai S, Graybosch R, Chen C, Bai G (2008) Quantitative trait loci for resistance to pre-harvest sprouting in US hard white winter wheat Rio Blanco. *Theor Appl Genet* 117:691-699. <https://doi.org/10.1007/s00122-008-0810-7>
- Mallick N, Vinod, Sharma JB, Tomar RS, Sivasamy M, Prabhu KV (2015) Marker assisted backcross breeding to transfer multiple rust resistance in wheat. *Plant Breed* 134:172–177. <https://doi.org/10.1111/pbr.12242>
- Mares D, Mrva K, Cheong J, Williams K, Watson B, Storlie E et al (2005) A QTL located on chromosome 4A associated with dormancy in white and red grained wheats of diverse origin. *Theor Appl Genet* 111:1357-1364. <https://doi.org/10.1007/s00122-005-0065-5>
- Mares DJ (1987) Preharvest sprouting tolerance in white-grained wheat. In: Mares DJ (eds) *Proceedings of 4th International Symposium. Pre-harvest sprouting in cereals*, Westview Press, Boulder, 64.
- McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Xia XC (2017) Catalogue of gene symbols for wheat: 2017 supplement (<https://shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp>).
- Mesfin, A, Froberg RC, Anderson JA (1999) RFLP Markers associated with high grain protein from *Triticum turgidum* L. var. *dicoccoides* introgressed into hard red spring wheat. *Crop Sci* 39:508-513. <https://doi.org/10.2135/cropsci1999.0011183X003900020035x>
- Mori M, Uchino N, Chono M, Kato K, Miura H (2005) Mapping QTLs for grain dormancy on wheat chromosome 3A and the group 4 chromosomes, and their combined effect. *Theor Appl Genet* 110:1315-

1323. <https://doi.org/10.1007/s00122-005-1972-1>

Nörnberg R et al (2015) Tolerance to preharvest sprouting and yield of wheat genotypes from different breeding programs. *Pesq Agropec Bras* 50, 698-706. <http://dx.doi.org/10.1590/S0100-204X2015000800008>

Ogbonnaya FC, Imtiaz M, Ye G et al (2008) Genetic and QTL analyses of seed dormancy and preharvest sprouting resistance in the wheat germplasm CN10955. *Theor Appl Genet* 116:891-902. <https://doi.org/10.1007/s00122-008-0712-8>

Olmos S, Distelfeld A, Chicaiza O, Schlatter AR, Fahima T, Echenique V, Dubcovsky J (2003) Precise mapping of a locus affecting grain protein content in durum wheat. *Theor Appl Genet* 107:1243-51. <https://doi.org/10.1007/s00122-003-1377-y>

Prasad P, Gangwar OP, Bharadwaj SC, Kumar S (2019) Monitoring pathotype distribution of *Puccinia* species on wheat and barley. *Mehtaensis* 39:5–9. 10.13140/RG.2.2.35390.72007

Rai N, Bellundagi A, Kumar PKC, Thimmappa RK, Rani S, Sinha N et al (2018) Marker-assisted backcross breeding for improvement of drought tolerance in bread wheat (*Triticum aestivum* L. em Thell). *Plant Breed* 137:514-526. <https://doi.org/10.1111/pbr.12605>

Rajjou L, Duval M, Gallardo K et al (2012) Seed germination and vigor. *Annu Rev Plant Biol* 63:507–533. <https://doi.org/10.1146/annurev-arplant-042811-105550>

Ramalingam J, Raveendra C, Savitha P et al (2020) Gene pyramiding for achieving enhanced resistance to bacterial blight, blast, and sheath blight diseases in rice. *Front Plant Sci* 11:591457. <https://doi.org/10.3389/fpls.2020.591457>

Randhawa MS, Bains NS, Sohu VS et al (2019) Marker assisted transfer of stripe rust and stem rust resistance genes into four wheat cultivars. *Agronomy* 9:497. <https://doi.org/10.3390/agronomy9090497>

Revathi P, Tomar SMS, Vinod, Singh NK (2010) Marker assisted gene pyramiding of leaf rust resistance genes *Lr24*, *Lr28* along with stripe rust resistance gene *Yr15* in wheat (*Triticum aestivum* L.). *Indian J Genet* 70:349-354.

Saghai-Marouf MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc Natl Acad Sci USA* 81:8014-8018. <https://doi.org/10.1073/pnas.81.24.8014>.

Samsampour D, Maleki Zanjani B, Singh A Pallavi JK, Prabhu KV (2009) Marker assisted selection to pyramid seedling resistance gene *Lr24* and adult plant resistance gene *Lr48* for leaf rust resistance in wheat. *Indian J Genet* 69:1-9.

- Sherman JD, Lanning SP, Clark D, Talbert LE (2008) Registration of near-isogenic hard- textured wheat lines differing for presence of a high grain protein gene. *J Plant Regist* 2:162-164.
<https://doi.org/10.3198/jpr2007.09.0528crg>
- Shorinola O, Balcárková B, Hyles J et al (2017) Haplotype analysis of the pre-harvest sprouting resistance locus *Phs-A1* reveals a causal role of *TaMKK3-A* in global germplasm. *Front Plant Sci* 8:1555.
<https://doi.org/10.3389/fpls.2017.01555>
- Simmonds N (1995) The relation between yield and protein in cereal grain. *J Sci Food Agric* 67:309–315.
<https://doi.org/10.1002/jsfa.2740670306>
- Simsek S, Ohm JB, Lu H et al (2014) Effect of pre-harvest sprouting on physicochemical changes of proteins in wheat. *J Sci Food Agric* 94:205-12. <https://doi.org/10.1002/jsfa.6229>
- Singh RP, Hodson DP, Jin Y et al (2006) Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 1:54.
<https://doi.org/10.1079/PAVSNNR20061054>
- Stakman EC, Stewart DM, LoegeringWQ (1962) Identification of physiologic races of *Puccinia graminis* var *tritici*. *Agric Res Serv* 617:53
- Sureshkumar S, Tamilkumar P, Thangavelu AU et al (2014) Marker-assisted introgression of *lpa2* locus responsible for low-phytic acid trait into an elite tropical maize inbred (*Zea mays* L.). *Plant Breed* 133:566-578. <https://doi.org/10.1111/pbr.12185>
- Tabbita F, Lewis S, Vouilloz JP et al (2013) Effects of the *Gpc-B1* locus on high grain protein content introgressed into argentinean wheat germplasm. *Plant Breed* 132:48-52. <https://doi.org/10.1111/pbr.12011>
- Tabbita F, Pearce S, Barneix AJ (2017) Breeding for increased grain protein and micronutrient content in wheat: ten years of the *Gpc-B1* gene. *J Cereal Sci* 73:183-191. <https://doi.org/10.1016/j.jcs.2017.01.003>
- Todkar L, Harikrishna, Singh GP, Jain N, Singh PK, Prabhu KV (2020) Introgression of drought tolerance QTLs through marker assisted backcross breeding in wheat (*Triticum aestivum* L.). *Indian J Genet* 80:209-212. <https://doi.org/10.31742/IJGPB.80.2.12>
- Tomar SMS, Singh SK, Sivasamy M, Vinod (2014) Wheat rusts in India: resistance breeding and gene deployment - A review. *Indian J Genet* 74:129-156. <https://doi.org/10.5958/0975-6906.2014.00150.3>
- Torada A, Koike M, Ogawa T, Takenouchi Y, Tadamura K, Wu J (2016) A causal gene for seed dormancy on wheat chromosome 4A encodes a MAP kinase kinase. *Current biol* 26:782-787.
<https://doi.org/10.1016/j.cub.2016.01.063>

- Tyagi S, Gupta PK (2012) Meta-analysis of QTLs involved in pre-harvest sprouting tolerance and dormancy in bread wheat. *Triticeae Genom Gene* 3:9-24. <https://doi.org/10.5376/tgg.2012.03.0002>
- Tyagi S, Mir RR, Kaur H et al (2014) Marker-assisted pyramiding of eight QTLs/genes for seven different traits in common wheat (*Triticum aestivum* L.). *Mol Breed* 34:167-175. <https://doi.org/10.1007/s11032-014-0027-1>
- Uauy C, Distelfeld A, Fhima T, Blechl A, Dubcovsky J (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* 314:1298-1301. <https://doi.org/10.1126/science.1133649>
- Vishwakarma MK, Arun B, Mishra VK, Yadav PS, Kumar H, Joshi AK (2016) Marker-assisted improvement of grain protein content and grain weight in Indian bread wheat. *Euphytica* 208:313–321. <https://doi.org/10.1007/s10681-015-1598-6>
- Vishwakarma MK, Mishra VK, Gupta PK, Yadav PS, Kumar H, Joshi AK (2014) Introgression of the high grain protein gene *Gpc-B1* in an elite wheat variety of Indo-Gangetic Plains through marker assisted backcross breeding. *Curr Plant Biol* 7:292-300. <https://doi.org/10.1016/j.cpb.2014.09.003>
- Yadav PS, Mishra VK, Arun B, Chand R, Vishwakarma MK, Vasistha NK et al (2015) Enhanced resistance in wheat against stem rust achieved by marker assisted backcrossing involving three independent Sr genes. *Curr Plant Biol* 2:25-33. <https://doi.org/10.1016/j.cpb.2015.05.001>
- Zhou Y, Tang H, Cheng MP et al (2018) Genome-wide association study for pre-harvest sprouting resistance in a large germplasm collection of Chinese wheat landraces. *Front Plant Sci* 8:401. <https://doi.org/10.3389/fpls.2017.00401>

Tables

Table 1 List of QTL/genes, chromosomal location, linked molecular markers and the size of PCR product in parental lines.

Trait	QTL/genes (chromosome)	Marker (product size bp)	Reference
SD	<i>Phs-A1</i> (4A)	<i>Xbarc170</i> (197)	Mares et al. 2005
SD	<i>Phs-A1</i> (4A)	<i>Xgwm397</i> (190)	Mares et al. 2005
SD/PHST	<i>QPhs.dpi.vic.4A.2</i>	<i>Xgwm637</i> (160)	Ogbonnaya et al. 2008
SD/PHST	<i>QPhs.dpi.vic.4A.2</i>	<i>Xgwm894</i> (180)	Ogbonnaya et al. 2008
SD	<i>TaMKK3-A</i> (4A)	KASP assay (C/A)	Shorinola et al. 2017
GPC	<i>Gpc-B1/ Yr36</i> (6B)	<i>Xucw108</i> (220)	Uayu et al. 2006
Leaf rust	<i>Lr24</i> (3D)	SCS73 ₇₁₉ (719)	Prabhu et al. 2004

SD, seed dormancy; PHST, pre-harvest sprouting tolerance; GPC, grain protein content

Table 2 A summary of the results of screening of MAS-derived pyramided lines, a MAS-derived parental genotype, and a check cv. Lok1 against different pathotypes of the three rusts under glasshouse conditions.

Rust	Pathotype	Distribution in India	Status	Lok1	Lok1 (<i>Gpc-B1+Lr24</i>)	Pyramided lines
Stripe rust	238S119	NHZ and NWPZ	Predominant	3 ⁺	0;	0;
	78S84		Predominant	0;	0;	0;
	47S103		High virulence	3 ⁺	;	0;
	110S119		Predominant	3 ⁺	3 ⁺	3 ⁺
Leaf rust	12-5	Uniform distribution in all the zones	Predominant	-	;	0;
	77-5		Predominant	3 ⁺	;	0;
	77-8		Predominant	3 ⁺	;	0;
	77-9		Predominant	0;	0;	0;
	104-2		High virulence	3 ⁺	;	0;
Stem rust	11	PZ	Predominant	2 ⁻	2 ⁻	2 ⁻
	21A-2	NHZ and NWPZ	Predominant	;	;	;
	34-1	Restricted to Leh Ladakh area	For checking presence of Lr24/Sr24	-	0;	0;
	40A	CZ, PZ and SHZ	Predominant	3 ⁺	2 ⁻	2 ⁻
	40-1	CZ, PZ and SHZ	For checking presence of Lr24/Sr24	2 ⁻	;	;
	117-6	CZ, PZ and SHZ	High virulence	2 ⁻	;	;
	122	CZ, PZ and SHZ	High virulence	2 ⁻	2 ⁻	2 ⁻

ITs ; and 0; indicating resistant; 2⁻, 2⁺ indicating moderately resistant; 3 indicating moderately susceptible; and 3+ indicating susceptible; plus (+), and minus (-) signs indicate variation within a given infection type.

Table 3 Analysis of variance (ANOVA) for seven traits involving parental genotypes and MAS-derived lines based on data collected from trials over two years.

Source of Variation	Environments	Replications	Genotypes	G*E	Error
DF	1	1	17	17	35
Traits					
PH	33.3**	2.3	1098.7	2.1**	0.7
TPM	6469.5**	5451.4	636.2	301.2	406.1
GNPS	31920.0*	187.0	1555.0	557.0	1180.0
TGW	5.0	2.7	82.4*	41.7	39.4
GY	0.3	0.0	0.8**	0.7**	0.3
GPC	2.2**	0.3	5.8**	1.3**	0.2
Fe (ppm)	-	2.5	199.7**	-	6.4
Zn (ppm)	-	11.3	67.3**	-	17.5

*Significantly higher at 5% level of significance; **significantly higher at 1% level of significance; G, genotype; E, environment; PH, plant height; TPM, tillers per meter; GNPS, grain per spike; TGW, 1000-grain weight; GY, grain yield, GPC, grain protein content, Fe, iron; Zn, zinc

Table 4 Mean values for seven different traits of the 10 MABB derived PHS tolerant and 4 pyramided lines and their parental genotypes based on pooled data over two years.

Genotypes	PH (cm)	TPM	GNPS	TGW (g)	GY (t/ha)	GPC (%)	Fe (ppm)	Zn (ppm)
Parents								
Lok1	96.0	104.0	160.0	57.0	3.2	9.9	25.6	18.5
Lok1 (<i>Gpc-B1+Lr24</i>)	95.8	111.5	165.0	57.6	3.1	12.4	29.5	19.9
AUS1408	151.0	113.7	144.5	50.9	3.1	11.7	24.4	19.3
CN19055	142.0	128.0	114.5	52.0	2.5	12.1	29.9	23.4
MAS derived PHST lines								
CCSU-55	96.3	83.9	162.7	62.5	3.7	10.0	32.9	17.4
CCSU-63	95.8	94.9	182.2	60.2	3.8	9.6	50.3*	35.0*
CCSU-64	94.8	104.9	179.5	59.8	3.9	10.3	52.6*	27.2
CCSU-68	95.3	86.4	157.2	64.9	3.7	9.7	31.4*	16.7
CCSU-73	95.0	82.1	166.7	60.7	3.7	9.5	24.2	15.3
CCSU-77	94.7	104.6	177.2	64.9	3.4	9.5	38.3*	23.4
CCSU-78	95.3	98.1	160.2	66.6*	3.8	10.2	21.7	14.5
CCSU-84	95.3	80.7	199.5	57.7	4.0*	10.2	45.0*	28.7*
CCSU-97	95.0	94.1	152.0	57.1	4.3*	10.6	36.5*	25.7
CCSU-109	97.0	93.6	132.7	57.9	4.0*	9.9	46.6*	30.9*
Pyramided lines								
CCSU-25 (<i>Gpc-B1+Lr24+QPhs.dpivic.4A.2</i>)	95.8	106.7	146.2	62.1	3.8	12.5*	31.1*	16.7
CCSU-31 (<i>Gpc-B1+Lr24+QPhs.dpivic.4A.2</i>)	96.3	85.4	176.7	56.0	3.2	12.5*	35.6*	20.8
CCSU-34 (<i>Gpc-B1+Lr24+QPhs.dpivic.4A.2</i>)	95.5	94.7	155.0	52.4	3.2	12.3*	31.8*	19.9
CCSU-35 (<i>Gpc-B1+Lr24+QPhs.dpivic.4A.2</i>)	97.0	89.4	174.7	54.9	3.5	12.3*	51.4*	27.3*
LSD	1.2	28.9	49.3	9.0	0.7	0.7	5.3	8.8

*Significantly higher at 5% level of significance; PH, plant height; TPM, tillers per meter; GNPS, grain per spike; TGW, 1000-grain weight; GY, grain yield, GPC, grain protein content, Fe, iron; Zn, zinc; LSD, least significant difference

Figures

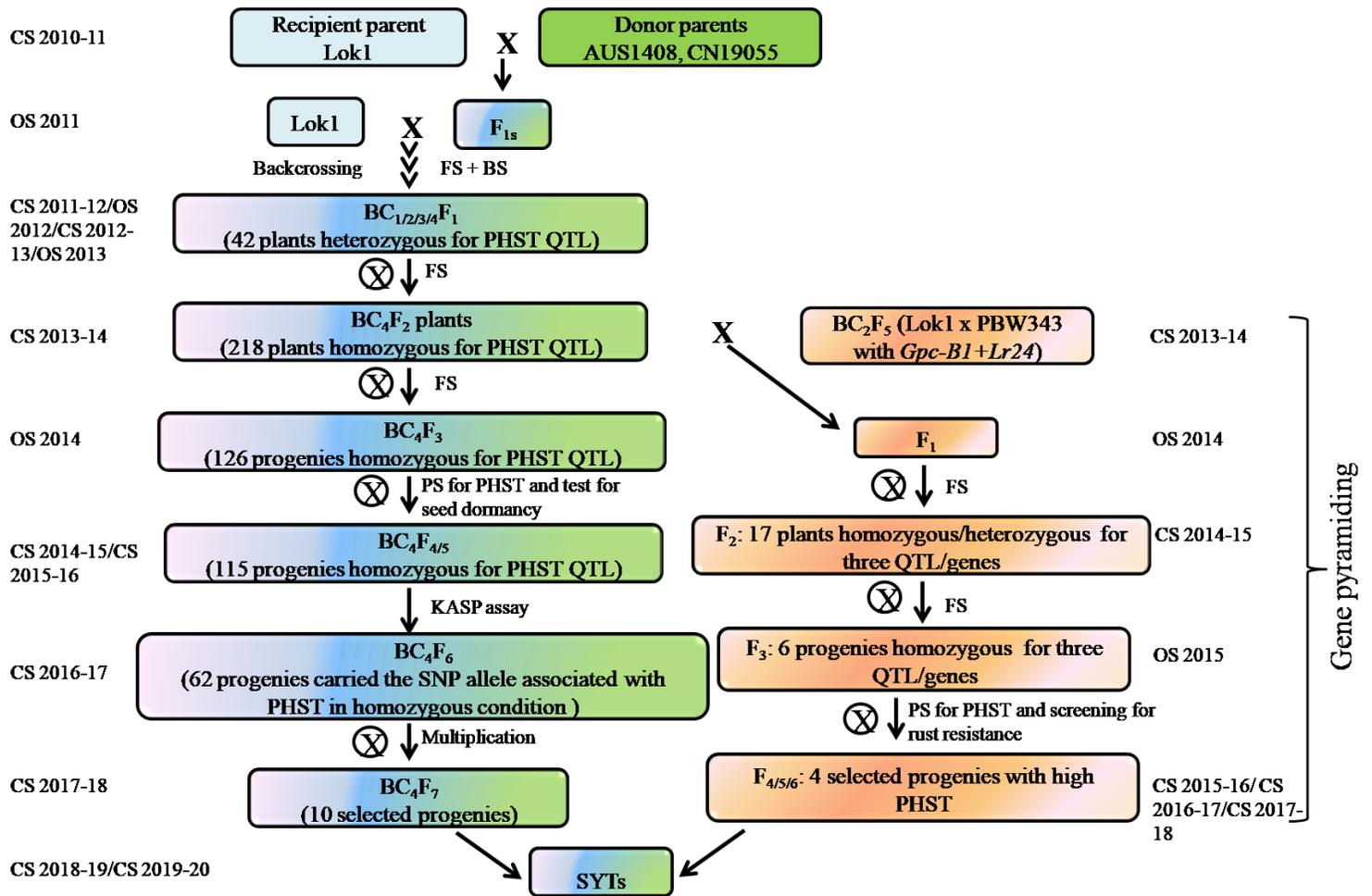


Figure 1

Marker assisted breeding scheme used in the present study for introgression of a PHST QTL (involving two different donors, AUS1408 and CN19055) and pyramiding QTL/genes for PHST, GPC and leaf rust resistance: PHST=pre-harvest sprouting tolerances; GPC= grain protein content; CS = crop season; OS = off season; FS = foreground selection; BS = background selection; PS = phenotypic selection; SYT = station yield trial.

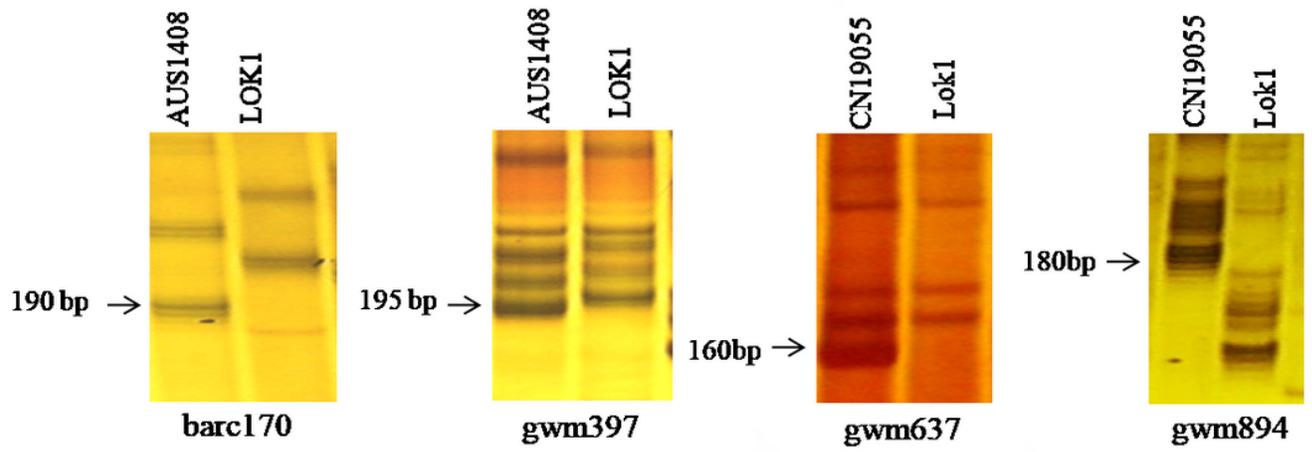


Figure 2

Amplification profiles of markers associated with PHST QTL in parental lines.

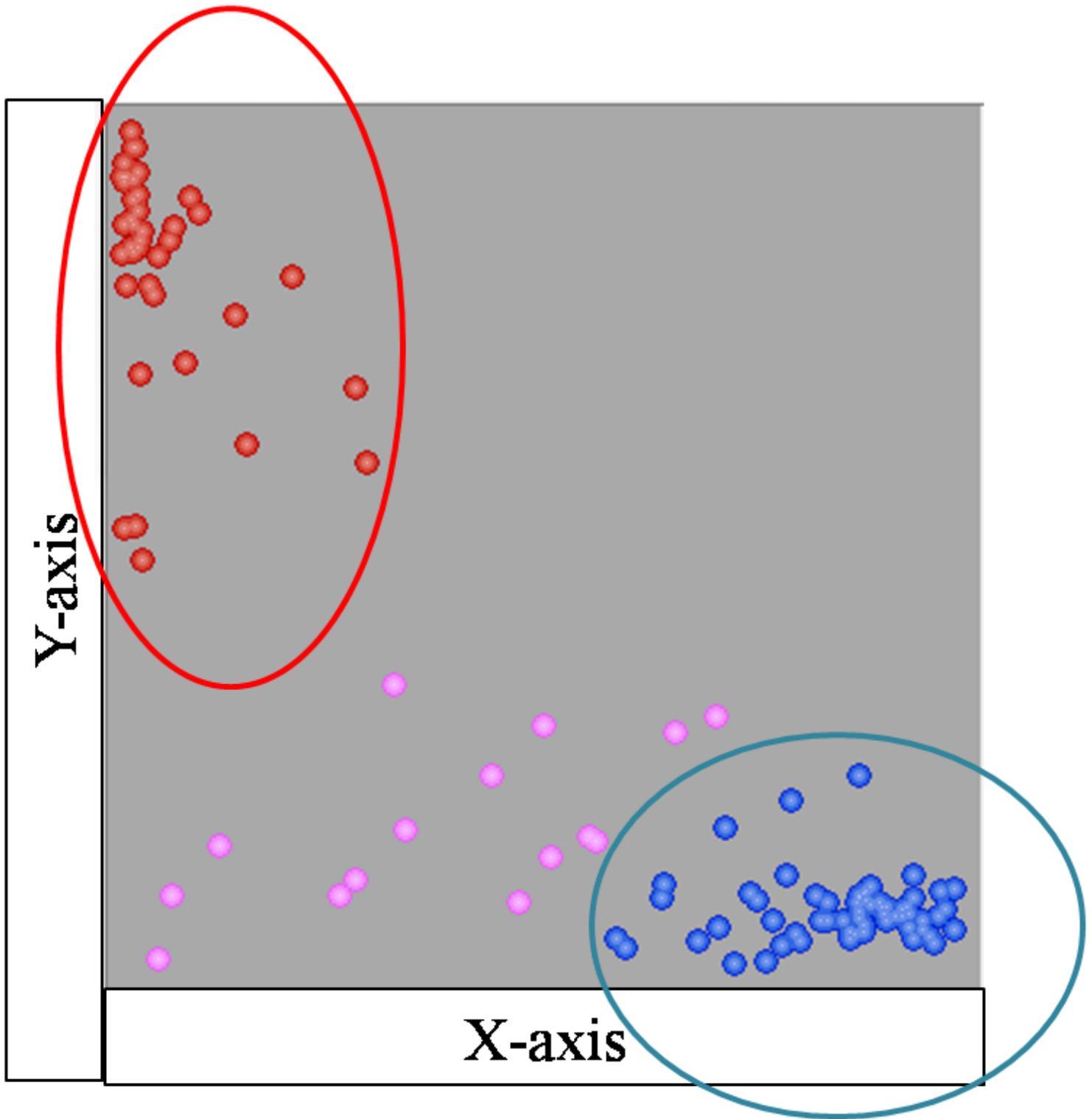


Figure 3

KlusterCaller output view of segregation of KASP marker associated with PHST gene TaMKK3-A in BC4F6 population. Co-dominant KASP marker segregating for FAM tailed TaMKK3-A-snp1-res_FAM (blue colour) and VIC tailed TaMKK3-A-snpA1-sus_VIC (red colour) alleles on X- and Y-axes of the plot, respectively, and heterozygous individuals on mid-axis (pink colour).

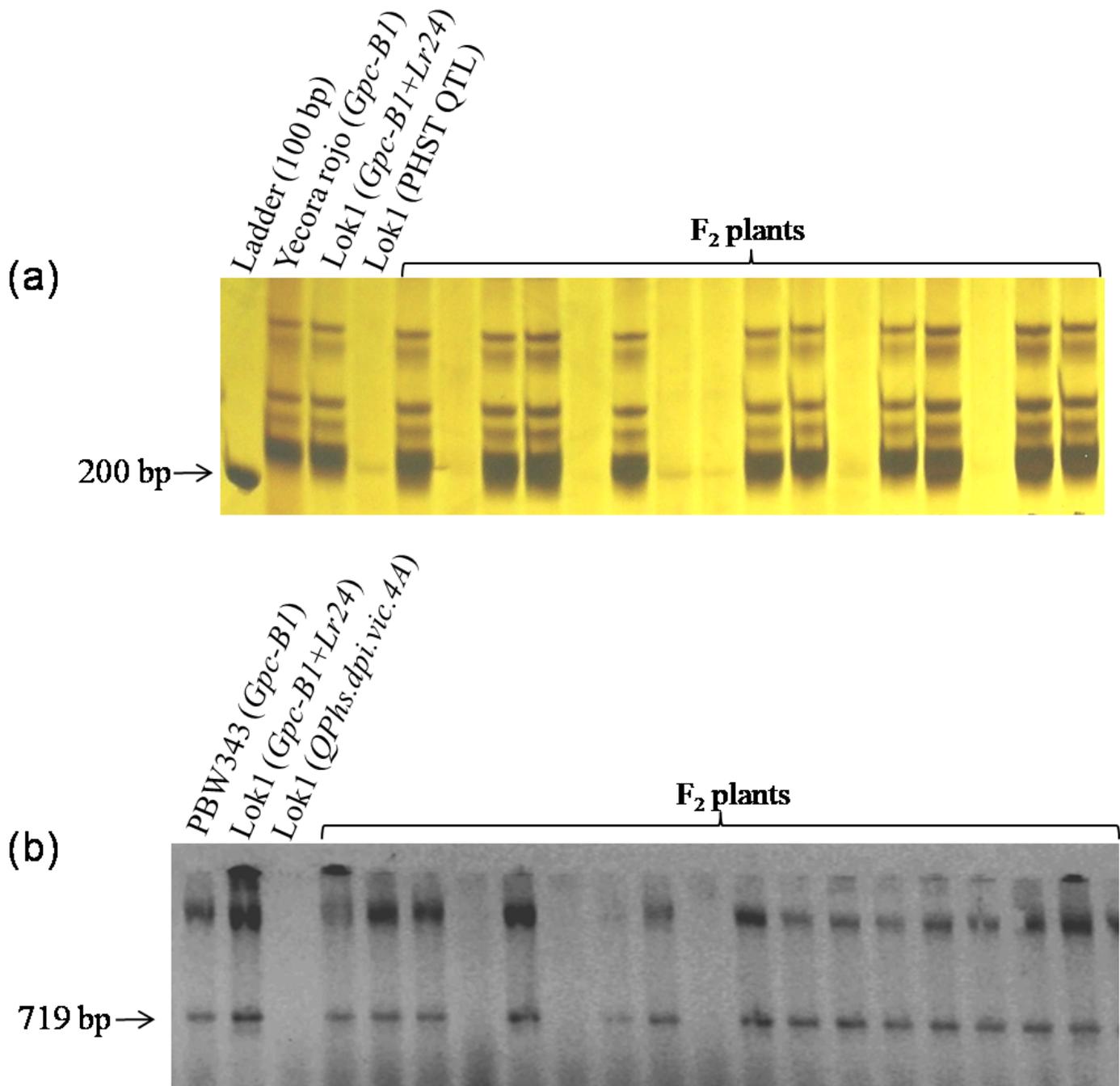


Figure 4

Amplification profiles of markers associated with (a) *Gpc-B1* and (b) *Lr24* in parental lines and F₂ plants.

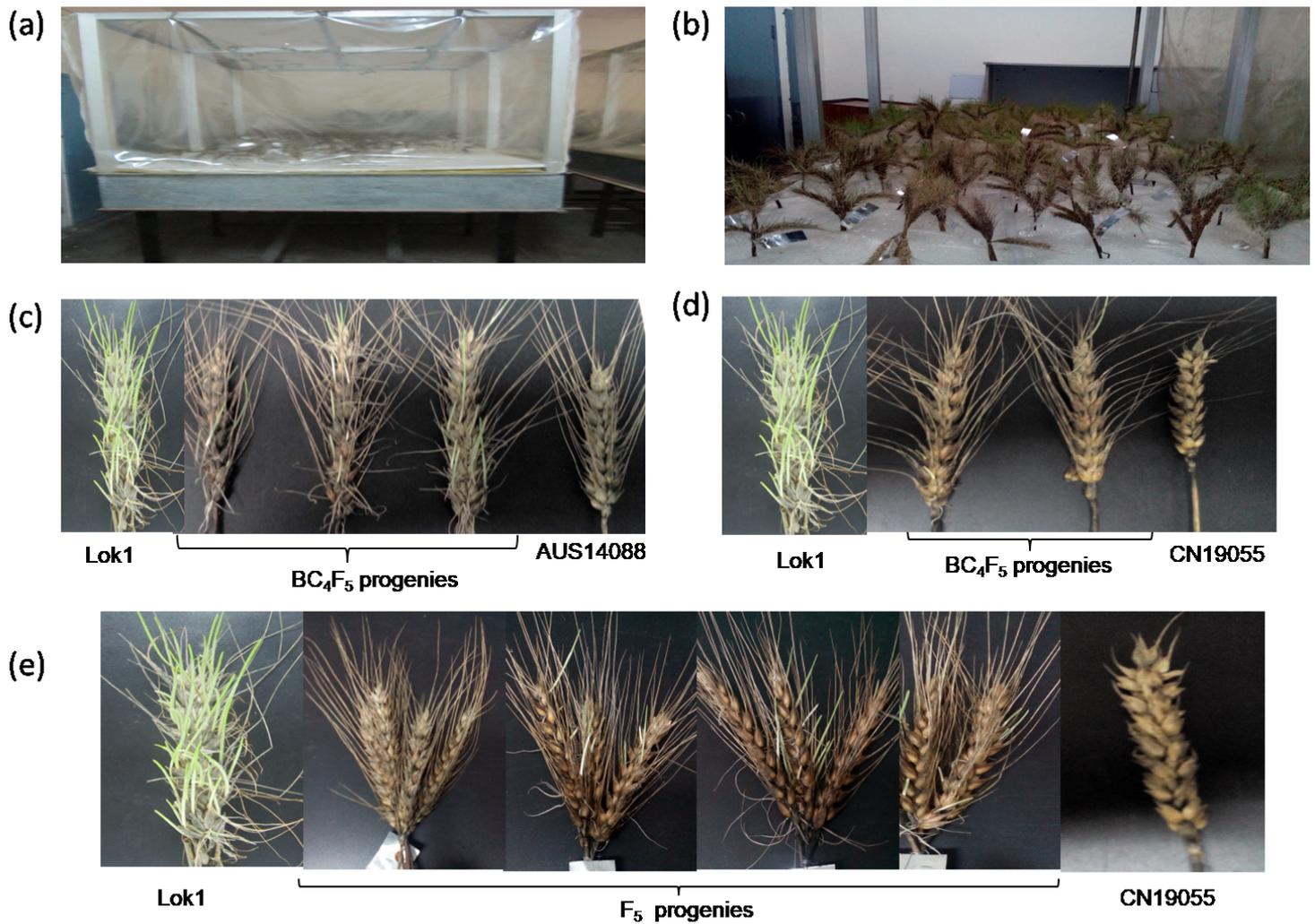


Figure 5

Screening for PHS under laboratory conditions: (a) Rain simulating chambers designed for the screening of PHS of wheat genotypes, (b) Spikes arranged in a rain simulating chamber for screening of PHS, (c) and (d) Phenotypes of spikes following testing of PHS of the two PHS tolerant donor genotypes (AUS1408 and CN19055), a PHS sensitive recipient genotype (Lok1) and the BC₄F₅ progenies. (e) Phenotypes of spikes following testing of PHS of the PHS tolerant donor genotype (CN19055) and PHS sensitive recipient genotype (Lok1) and four PHS tolerant QTL/gene pyramided F₅ lines.