Marker-assisted backcross breeding to combine multiple rust resistance in wheat

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With 3 figures and 4 tables
Received July 31, 2012/Accepted October 24, 2014
Communicated by K. Gill

Abstract
A widely grown but rust susceptible Indian wheat variety HD2932 was improved for multiple rust resistance by marker-assisted transfer of genes Lr19, Sr26 and Yr10. Foreground and background selection processes were practised to transfer targeted genes with the recovery of the genome of HD2932. The near-isogenic lines (NILs) of HD2932 carrying Lr19, Sr26 and Yr10 were individually produced from two backcrosses with recurrent parent HD2932. Marker-assisted background selection of NILs with 94.38–98.46% of the HD2932 genome facilitated rapid recovery of NILs carrying Lr19, Sr26 and Yr10. In the BC2 generation, NILs were intercrossed and two gene combinations of Lr19+Yr10, Sr26 + Yr10 and Lr19+Sr26 were produced. A total of 16 progeny of two gene combinations of homozygous NILs of HD2932 have been produced, which are under seed increase for facilitating the replacement of the susceptible HD2932 with three of the sixteen improved backcross lines with resistance to multiple rusts.

Key words: marker-assisted selection — foreground and background selection — genome recovery — leaf rust — stem rust and stripe rust

Stem rust (black rust) caused by Puccinia graminis Pers.f. sp. triticci Erks. & Henn., leaf rust (brown rust) caused by Puccinia triticina Erks. (Syn: Puccinia recondita) and stripe rust (yellow rust) caused by Puccinia striiformis Westend are known to cause significant damage to wheat throughout the world (Line and Chen 1995, Singh et al. 2005). Judicious deployment of rust resistance genes has been adopted by breeders to control the losses due to rust diseases. Evolution of new virulent races of rust pathogens is a continuing phenomenon which renders resistance genes ineffective and thus necessitates deployment of different genes. In India, the rust pathogens proliferate under different environmental conditions. Leaf rust occurs in all of the wheat-growing zones in India at various intensities depending on the growth stage of crop and environmental conditions. The impact of leaf rust on yield reduction in wheat is well documented globally, with yield losses ranging from 10% under moderate infection to 65% under intense infection (Saari and Prescott 1985). Stripe rust in India is generally confined to the cooler areas of the Indo-Gangetic Plains, Himachal Pradesh, Uttarakhand, Punjab, Haryana and Uttar Pradesh covering nearly 9 million hectares with a potential of moving over to cooler high plain regions of central and peninsular India facilitated by unprecedented climate change phenomenon being observed. Stem rust prevails under warmer conditions of temperature regimes between 20 and 35°C. In India, central and peninsular zones are particularly prone to stem rust where favourable environmental conditions prevail.

A wheat variety is considered suitable for cultivation across regions characterized by different ranges of temperature regimes, only if it possesses multiple rust resistance. Genes such as Lr19, Sr25, Sr26 and Yr10 have been known to provide a high degree of resistance in the subcontinent (Tomar and Menon 2001), which, however, are not present in the high-yielding variety HD2932. Screening genotypes for multiple rust resistance is often difficult and requires multilocation testing and availability of virulent discriminatory races. However, with the advent of molecular markers linked to rust resistance genes, it is possible to incorporate multiple rust resistance genes even in the absence of discriminating virulence. Moreover, with the availability of molecular markers such as simple-sequence repeat microsatellites (SSR) mapped with high density across all the chromosomes of wheat, it is possible to transfer rust resistance genes in the genotypic background of an otherwise agronomically superior cultivar by adopting an accelerated backcross breeding procedure using both foreground and background selections. Marker-assisted background selection can result in rapid recovery of recurrent parent genome in a short span of 2–3 backcross generations (Ribaut et al. 2002).

Wheat variety HD2932 developed by the Indian Agricultural Research Institute, New Delhi, is a high-yielding variety suitable for late sowing conditions with good adaptability. During first year of testing (crop year 2004–2005), variety HD2932 was significantly superior in yield in four different agro-ecological zones making up 28 of the 30 million hectare area in India. Subsequently, on the basis of 3 years of testing, the variety was released for only two zones occupying about 10 million hectares representing the central and peninsular India for late sown irrigated conditions (Anonymous 2005, Shoran et al. 2011) for reasons of susceptibility to leaf and stripe rusts in the other zones. Considering the agronomical superiority of HD2932 and its wide adaptability, the process of improving the variety by transferring rust resistance genes Lr19/Sr25 (genes for leaf and stem rust resistance linked on the same alien translocation), Sr26 (stem rust resistance) and Yr10 (stripe rust resistance) was undertaken by employing marker-assisted backcross breeding procedure for product development in just 3 years by shutting the breeding materials with two or more seasons per year.

Materials and Methods
Plant materials and marker-assisted backcrossing scheme: Wheat variety HD2932 was used as the recurrent parent in backcross breeding. Donor genotypes included a near-isogenic line (NIL) of Indian variety HD2687 with the rust resistance genes Lr19/Sr25 (HD2687+ Lr19/Sr25), Eagle (Sr26) and a NIL of exotic variety Avocet with stripe rust resistance gene Yr10 (Avocet+ Yr10). To improve HD2932 for rust resistance, marker-assisted backcross breeding was initiated. HD2932 was crossed with HD2687+ Lr19/Sr25, Eagle and Avocet+Yr10 containing rust resistance genes Lr19/Sr25, Sr26 and Yr10, respectively, in 2009–2010. F1 plants were backcrossed with recurrent parent HD2932.
Marker assisted backcross breeding

twice to raise BC1F1 and BC2F1 generations. Marker-selected plants in the BC1F1 generation were selfed. In the BC2F2 generation, plants homozygous for markers linked to rust resistance genes and background markers for recurrent parent alleles were selected and intercrossed for resistance gene combinations to produce near-isogenic lines of HD2932 carrying rust resistance genes Lr19/Sr25, Sr26 and Yr10 individually and in two gene combinations. The details of the breeding scheme are given in Fig. 1.

To accelerate the improvement of wheat variety HD2932, two generations were grown each year: one at IARI, New Delhi, and the other at IARI Regional Station, Wellington (South India). The main season (winter) in Delhi was used to generate F1 and BC1F1 crosses and to evaluate BC1F1 and BC2F1 generations. Plants were grown during the off season (summer) at Wellington to generate the BC1F1 crosses and evaluate F1 and BC2F1 generations in each targeted gene combination.

Rust inoculation: Although Delhi is not a natural hot spot for the occurrence of rusts, under artificial inoculation, successful infection by 

\[ \text{P. triticina} \text{ and } \text{P. striiformis} \]
can be created. However, the location is not conducive for stem rust infection and spread in the field. Single-spore culture increased inoculum of the most virulent and predominant 

\[ \text{Puccinia triticina} \text{ pathotype 121R63-1 and two pathotypes of} \]

\[ \text{P. striiformis, 78S84 and 46S119, were obtained from the Directorate} \]

of Wheat Research, Regional Station in Flowerdale, Shimla, and were used for disease phenotyping. The test material was space-planted with plant to plant distance of 10 cm in plots containing 2-m-long rows spaced 30 cm apart. Infectors were planted after every 10 rows as well as around the population. Spores were sprayed as a suspension in water with Tween-20 (0.75 µl/ml) using a ureidospore concentration of 30 mg/l of water at boot-2 leaf stage (~Z48, a growth stage on cereal development scale proposed by Zadoks et al. (1974) for \text{P. striiformis}) and boot initiation stage (~Z61) for \text{P. triticina} infection at the Delhi location. The disease levels were recorded at adult plant stage (~Z80) for selected plants of the targeted \( Lr \) and \( Yr \) genes as the per cent of leaf area covered with urediospores according to modified Cobb scale (Peterson et al. 1948) by which severity of rust is recorded on 0–100 scale combined with the type of infection response (Loegering 1959, Joshi et al. 1982) as follows: R (resistant; necrotic areas with or without uredia present); MR (moderately resistant; small uredia present surrounded by necrotic areas); MS (moderately susceptible; medium uredia without necrosis but with or without chlorosis) and S (susceptible; large uredia without necrosis and chlorosis). The selected plants possessing the targeted \( Lr, Sr \) and \( Yr \) genes were evaluated for the diseases in BC1F1 and BC2F2 generations.

The other generations which were routed through the off-season site Wellington were naturally exposed to all three rusts on a regular basis because the Nilgiri Hills where Wellington is located is a natural hot spot of infection of rusts in the southern Indian part of the annual \text{Puccinia} pathway in India (Nagarajan and Joshi 1980). The selections were also scored in the field for their rust resistance phenotype to confirm the efficiency of the targeted \( Lr, Sr \) and \( Yr \) genes.

DNA extraction: Leaf tissues were collected from either 7- to 10-day-old seedlings or 3- to 4-week-old plants. Genomic DNA was isolated following the CTAB method (Murray and Thompson 1980). DNA samples were quantified by comparison with 100 ng/200 ng of Lambda uncut DNA on 0.8% agarose gel. The DNA was diluted in TE buffer so that final concentration of DNA was approximately 25 ng/µl before PCR amplification.

PCR amplification and electrophoresis: PCR amplification was performed in 20 µl reaction volumes containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 2 mM MgCl2, 200 µM of each dNTP (MBI Fermentas, Schwerte, Germany), 1 unit Taq DNA Polymerase (Bangalore Genei Pvt Ltd, Bengaluru, India), 0.2 µM of primer and 25–30 ng of genomic DNA. PCR amplification was achieved in an Eppendorf thermal cycler (Eppendorf AG, Hamburg, Germany) with the following thermal profile: one 4-min cycle at 94°C (initial denaturation), followed by 35 cycles of 30 s at 94°C (denaturation), 30 s at 60°C (vary according to primer annealing) and 30 s at 72°C and concluding with 10 min at 72°C. PCR products were resolved on 3% MetaPhor (Lonza, Rockland, ME USA) gels for SSR markers and on 2% agarose gels for SCAR markers at 120 V for 3.5 h. Gels were visualized under UV and photographed using a gel documentation system (Syngene G-Box, Cambridge, UK).

Marker-assisted selection: Marker-assisted foreground and background selection technique was used to incorporate rust resistance genes in variety HD2932. Validated molecular markers linked to targeted rust resistance genes (Table 1) were used for foreground selection. A total of 793 SSR markers covering all the chromosomes and chromosome arms in a genetic and physical consensus SSR map of wheat (Sourdille et al. 2004) were surveyed for polymorphism. Markers which were polymorphic between HD2932 and the three donor parents were used for background selection in backcross (BC1F1, BC2F1 and BC2F2) populations generated from the three crosses. Per cent genomic similarity was calculated as number of homozygous loci corresponding to recurrent parent allele + half the number of heterozygous loci divided by the total number of polymorphic SSR markers used.

Results

Analysis of parental polymorphism between recurrent parent HD2932 and respective donor parents such as HD2687+Lr19/Sr25, Eagle and Avocet+Yr10 with 793 SSR markers resulted in the identification of 65 polymorphic markers between HD2932

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Fig. 1: Breeding scheme for transfer of multiple rust resistance in wheat variety HD 2932 (Foreground and background markers were scored from BC1F1 generation onwards)
and HD2687+Lr19/Sr25, 89 polymorphic markers between HD2932 and Eagle (Sr26) and 82 polymorphic markers between HD2932 and Avocet+Yr10. These markers were employed for selecting the BC1F1 plants which were also verified for their phenotypic resemblance with the recurrent parent to minimize the difference in the introgression lines from HD2932.

In the BC1F1 generation derived from the cross (HD2932 × HD2687+Lr19/Sr25) × HD2932, 159 plants were identified to carry leaf rust resistance gene Lr19/Sr25 by the presence of the marker allele at the Xwmc221 locus (Fig. 2). All the selected plants were resistant in the field with no visible leaf rust infection, while the recurrent parent was susceptible with infection response of 60S to 70S. In the BC1F1 generation produced from the cross (HD2932 × Eagle) × HD2932, two dominant markers S26#43 (coupling phase) and BE518379 (repulsion phase) were employed for detecting the heterozygous plants carrying the donor allele of resistance in both the BC1F1 population and HD2932. A total of 117 plants were identified as possessing stem rust resistance gene donor allele of resistance for the Lr26 locus. Maximum genomic similarity with the recurrent parent to minimize the difference in the introgression lines from HD2932 was 96.15%, 89.88% and 93.29% for the Lr26 and Yr10 genes individually and with high genomic similarity with the recurrent parent genotype to HD2932 were intercrossed to produce two gene combinations that were homozygous for Lr26, and 28 plants were homozygous for Yr10. Background selection in the gene-positive plants in the BC2F1 generation was selfed to produce BC2F2 seed. In the BC2F2 generation, foreground analysis using linked markers was performed to identify the plants carrying targeted alleles in the homozygous and heterozygous states (Fig. 3). Forty-two plants were identified as homozygous for Lr26/Sr25, five were homozygous for Sr26, and 28 plants were homozygous for Yr10.

Background selection resulted in the highest genomic recovery of 98.46% for the Lr26/Sr25 population, whereas the maximum genomic similarity was 94.38% for the Sr26 population. In the BC2F2 population for Yr10, maximum genomic similarity with variety HD2932 was 96.95%.

Homoygous progeny of the BC2F2 plants carrying Lr19/Sr25, Sr26 and Yr10 genes individually and with high genomic similarity to HD2932 were intercrossed to produce two gene combinations in the genetic background of HD2932. Eight plants were identified with the gene combination Lr19/Sr25 + Yr10, seven plants with Sr26+Yr10, while only one plant showed gene combination Lr19/Sr25 + Sr26 (Table 3). Eight plants with the combination of Lr19/Sr25 + Yr10 were compared at the Delhi location for their yield and morphological similarity with the background variety HD2932 as a preliminary indicator for initiating bulking for seed production of the gene combination HD2932+Lr19/Sr25+Yr10.

**Discussion**

Rust resistance genes Lr19/Sr25, Sr26 and Yr10 were selected for incorporation in the high-yielding rust susceptible variety HD2932. The selected genes are effective in India and have not yet been deployed commercially and in combinations that
can provide resistance against the three wheat rusts (Tomar and Menon 2001). The Agropyron-derived leaf rust resistance gene \( Lr19 \) (Sharma and Knott 1966) is linked with \( Sr25 \), which is also highly effective to stem rust race Ug99. Singh et al. (2006) observed that the 7D:7Ag translocation had a favourable effect on yield, increasing grain yield potential by 10–15% in a range of genotypes. Stem rust resistance gene \( Sr26 \), also an Agropyron-derived gene (Knott 1961, 1968), is one of the most effective stem rust resistance genes with no known virulence globally. Although virulence for stripe rust resistance gene \( Yr10 \) has been reported from Europe, North America and Mediterranean region (Beaver and Powelson 1969, Stubbs 1985), the gene \( Yr10 \) is effective in India with no known virulence (Kumar 2011). A judicious use of both phenotypic and marker-assisted selection was made to rapidly recover the genotypic background of HD2932 as was achieved in the case of bacterial blight resistance breeding in rice (Bassavaraj et al. 2009). Plants which obviously looked agronomically inferior or not resembling the recurrent parent were rejected even if these plants were identified as possessing the targeted gene. Background MAS was used in these selected plants only with the aim of identifying plants homozygous at the highest number of loci for the recurrent parent genome (Table 2).

### Marker-assisted selection

Foreground selection using linked molecular markers was employed for selection of plants carrying \( Lr19/Sr25 \), \( Sr26 \) and \( Yr10 \) individually in the respective backcross generations. Molecular markers have been effectively used for identification of rust resistance genes in segregating populations (Prabhu et al. 2009, Samsampour et al. 2009). In the \( BC1F1 \) populations, selected plants were analysed for similarity to the recurrent parent genome using polymorphic markers. Marker-assisted background selection resulted in the rapid recovery of recurrent parent genome. In the \( BC1F1 \) generation derived from the cross (HD2932 × HD2687 + \( Lr19/Sr25 \)) × HD2932, a plant with 90% genomic similarity with HD2932 was identified. In the \( BC2F1 \) generation, the genomic similarity increased to 96.15%. Genomic similarity further increased to 98.46% in \( BC2F2 \) generation. Thus, NILs possessing \( Lr19/Sr25 \) and \( Yr10 \) were obtained with two backcrosses followed by one generation of selfing. This demonstrated the effectiveness of background selection in reducing the number of backcross generations. Randhawa et al. (2009) obtained more than 97% of the recurrent parent genome in just two backcross generations. In the remaining two backcross generations for transfer of

### Table 3: Near-isogenic lines in HD2932 genetic background to be used for two gene homozygous combinations with resistance to the targeted rusts

<table>
<thead>
<tr>
<th>Cross</th>
<th>Total no of plants screened</th>
<th>No of plants with ( Sr26 ) and ( Lr19/Sr25 )</th>
<th>No of plants with ( Sr26 ) and ( Yr10 )</th>
<th>No of plants with ( Lr19/Sr25 ) and ( Yr10 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(HD2932 + ( Lr19/Sr25 )) × (HD2932 + ( Sr26 ))</td>
<td>58</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(HD2932 + ( Sr26 )) × (HD2932 + ( Yr10 ))</td>
<td>43</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(HD2932 + ( Lr19/Sr25 )) × (HD2932 + ( Yr10 ))</td>
<td>9</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2: Foreground selection for genes \( Lr19, Sr26 \) and \( Yr10 \) in \( BC1F1 \) generations of respective crosses involving wheat variety HD 2932 as recurrent parent.
Sr26 and Yr10 in the background of HD2932, plants with high recovery of HD2932 genome were identified in BC1F1, BC2F1 and BC2F2 generations (Table 2). Thus, near-isogenic lines of HD2932 carrying genes Lr19/Sr25, Sr26 and Yr10 were developed in the BC2F2 generation with maximum HD2932 genome recovery reaching 98.46%, 94.38% and 96.95%, respectively. In the BC1F1 and BC2F1 generations, phenotypic selection was practised in addition to marker-assisted background selection. The selected NILs carry the targeted genes Lr19/Sr25, Sr26 and Yr10 in homozygous state and could be used in future gene pyramiding programmes. Higher recovery of the HD2932 genome in the BC2F2 generation also provided an opportunity for intercrossing Lr19/Sr25, Sr26 and Yr10 carrying plants to produce two gene combinations in homozygous states in derived selfed progeny of crosses. In intercrossed BC3F2 products between two NIL combinations (F18), eight plants carrying Lr19/Sr25+Yr10, seven plants carrying Sr26+Yr10 and one plant with Lr19/Sr25+Sr26 were identified (Table 3). Progeny of these plants are not expected to segregate for genetic background and each introgressed selection recorded obvious resistance against the targeted rusts compared to the susceptible reaction of the recurrent parent (Table 4).

Only limited information is available on efficacy of background selection in wheat (Randhawa et al. 2009, Bhawar et al. 2011), although background as well as foreground selection has been effectively used in rice (Joseph et al. 2004, Gopalakrishnan et al. 2008, Basavaraj et al. 2009, 2010, Sundaram et al. 2009). Two different approaches are normally followed to transfer two or more effective resistance genes into an adapted cultivar. In the first approach, targeted genes of all the donor parents (two or more) are assembled first in a single gene combination line followed by backcrossing to a recurrent parent. In the second approach, individual target genes are transferred first to develop backcross lines in the genetic background of the recipient variety followed by intercrossing of these backcross lines to assemble the desired combination of targeted genes. Ishii et al. (2008) demonstrated that the second approach where backcross lines are developed first which was employed in the present study is superior to first approach. With the same number of generations and cost of genotyping, the second approach produces a much higher recovery of recurrent parent genome.

The first product in India of multiple rust disease gene pyramiding in wheat is now available for use as replacements of the variety HD2932 for the large number of farmers in the targeted area of about 19 million hectares covering the north-western, central and peninsular zones of the subcontinent, who have been using the rust susceptible HD2932 wheat in the winter season
following the rainy season crop of rice, maize, pearl millet or cotton.

Acknowledgements: The authors are grateful to the Department of Biotechnology, Government of India for sponsoring the project under Accelerated Crop Improvement Programme and to the University Grants Commission, Government of India for fellowship to NM. The authors are grateful to Directorate of Wheat Research, Flowerdale, Shimla, for providing pure inoculums of the leaf rust pathogen and the Indian Agricultural Research Institute, New Delhi, for facilitating the experiments.