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

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Abstract

A widely grown but rust susceptible Indian wheat variety HD2932 was improved for multiple rust resistance by marker-assisted transfer of genes *Lr*19, *Sr*26 and *Yr*10. 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 *Lr*19, *Sr*26 and *Yr*10 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 *Lr*19, *Sr*26 and *Yr*10. In the BC_2F_2 generation, NILs were intercrossed and two gene combinations of *Lr*19+*Yr*10, *Sr*26 + *Yr*10 and *Lr*19+*Sr*26 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. *tritici* Eriks. & Henn., leaf rust (brown rust) caused by *Puccinia triticina* Eriks. (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 zones characterized by different ranges of temperature regimes, only if it possesses multiple rust resistance. Genes such as *Lr*19, *Sr*25, *Sr*26 and *Yr*10 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 agronomical 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 *Lr*19/*Sr*25 (genes for leaf and stem rust resistance linked on the same alien translocation), *Sr*26 (stem rust resistance) and *Yr*10 (stripe rust resistance) was undertaken by employing marker-assisted backcross breeding procedure for product development in just 3 years by shuttling 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 *Lr*19/*Sr*25 (HD2687+ *Lr*19/*Sr*25), Eagle (*Sr*26) and a NIL of exotic variety Avocet with stripe rust resistance gene *Yr*10 (Avocet+ *Yr*10). To improve HD2932 for rust resistance, marker-assisted backcross breeding was initiated. HD2932 was crossed with HD2687+ *Lr*19/*Sr*25, Eagle and Avocet+*Yr*10 containing rust resistance genes *Lr*19/*Sr*25, *Sr*26 and *Yr*10, respectively, in 2009–2010. F_1 plants were backcrossed with recurrent parent HD2932 twice to raise BC_1F_1 and BC_2F_1 generations. Marker-selected plants in the BC_2F_1 generation were selfed. In the BC_2F_2 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 *Lr*19/*Sr*25, *Sr*26 and *Yr*10 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 F_1 and BC_2F_1 crosses and to evaluate BC_1F_1 and BC_2F_2 generations. Plants were grown during the off season (summer) at Wellington to generate the BC_1F_1 crosses and evaluate F_1 and BC_2F_1 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 *P. triticina* and *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 *Puccinia triticina* pathotype 121R63-1 and two pathotypes of *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. Infector rows 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 urediospore 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 *P. striiformis*) and boot initiation stage (=Z41) for *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 urediniospores 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 BC_1F_1 and BC_2F_2 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 *Puccinia* pathway in India (Nagarajan and Joshi 1980). The selections were also scored in the field for their rust resistance phenotype to confirm the efficacy 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 $MgCl_2$, 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 (BC_1F_1, BC_2F_1 and BC_2F_2) 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+*Lr*19/*Sr*25, Eagle and Avocet+*Yr*10 with 793 SSR markers resulted in the identification of 65 polymorphic markers between HD2932

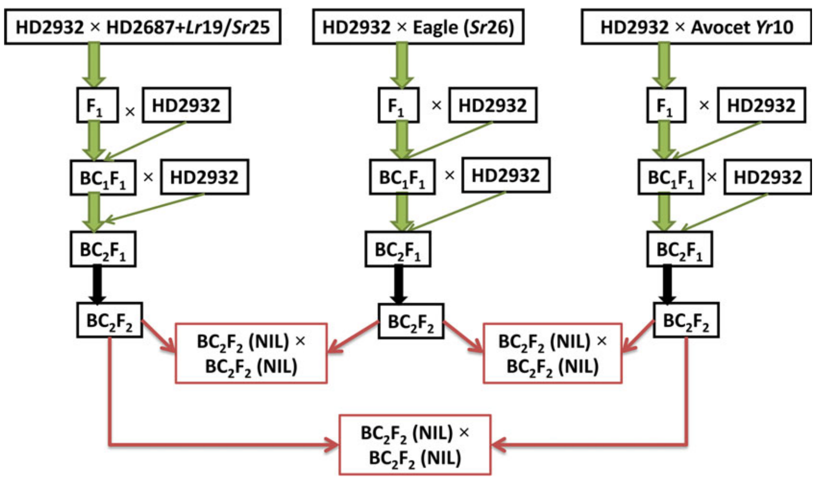

Fig. 1: Breeding scheme for transfer of multiple rust resistance in wheat variety HD 2932 (Foreground and background markers were scored from BC_1F_1 generation onwards)

S. no.	Target gene	Linked marker	Size of fragment (bp)	Dominant/Codominant	Reference
1	*Lr*19/*Sr*25	*Xwmc221*	190	Codominant	Gupta et al. (2006)
2	*Sr*26	*Sr26#43* (presence)	207	Dominant	Mago et al. (2005)
		BE518379 (absence)	303	Dominant	
3	*Yr*10	*Xpsp3000*	285/240	Codominant	Wang et al. (2002)

Table 1: Molecular markers employed for foreground selection of rust resistance genes

Table 2: Number of gene-positive plants in BC_1F_1, BC_2F_1 and BC_2F_2 generations and maximum recurrent parent genome (RPG) recovery

Breeding cross	Generation	No. of plants screened	No. of gene-positive plants		No. of gene negative plants	Maximum RPG recovery[1] (%)
			Homozygous	Heterozygous		
(HD2932 × HD2687+ *Lr*19/*Sr*25) × HD2932	BC_1F_1	258		159	99	90.0
(HD2932 × HD2687+ *Lr*19/*Sr*25) × HD2932*^{2}	BC_2F_1	70		31	39	96.15
(HD2932 × HD2687+*Lr*19/*Sr*25) × HD2932*^{2}	BC_2F_2	87	42	38	7	98.46
(HD2932 × Eagle) × HD2932	BC_1F_1	250		117	133	82.58
(HD2932 × Eagle) × HD 2932*^{2}	BC_2F_1	48		25	23	89.88
(HD2932 × Eagle) × HD 2932*^{2}	BC_2F_2	78	5	39	34	94.38
(HD2932 × Avocet + *Yr*10) × HD2932	BC_1F_1	108		23	85	85.97
(HD2932 × Avocet+ *Yr*10) × HD2932*^{2}	BC_2F_1	61		29	32	93.29
(HD2932 × Avocet+*Yr*10) × HD2932*^{2}	BC_2F_2	114	28	48	38	96.95

[1]Maximum RPG recovery (%) of plants carrying targeted genes.

and HD2687+*Lr*19/*Sr*25, 89 polymorphic markers between HD2932 and Eagle (*Sr*26) and 82 polymorphic markers between HD2932 and Avocet+*Yr*10. These markers were employed for selecting the BC_1F_1 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 BC_1F_1 generation derived from the cross (HD2932 × HD2687+*Lr*19/*Sr*25) × HD2932, 159 plants were identified to carry leaf rust resistance gene *Lr*19/*Sr*25 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 BC_1F_1 generation produced from the cross (HD2932 × Eagle) × HD2932, two dominant markers *Sr26#43* (coupling phase) and *BE518379* (repulsion phase) were used to detect the heterozygous plants carrying the donor allele of resistance for the *Sr*26 locus. A total of 117 plants were identified as possessing stem rust resistance gene *Sr*26. Similarly, 23 plants were identified as *Yr*10 positive with codominant marker *Xpsp3000* in the BC_1F_1 population developed from the cross (HD2932 × Avocet+*Yr*10) × HD2932 (Table 2, Fig. 2).

Background selection was carried out using polymorphic markers in plants carrying targeted genes in respective backcross generations for the recurrent parent-specific allele at each locus. Plants showing high recovery of the recurrent parent (HD2932) genome were identified and backcrossed to get BC_2F_1 seeds. In the BC_1F_1 generation of (HD2932 × HD2687+*Lr*19/*Sr*25) × HD2932 plants with up to 90% genome of HD2932 were recovered. Similarly, in the remaining two BC_1F_1 populations, plants exhibiting genome recovery up to 82.58% and 85.97%, respectively, of HD2932 were recovered (Table 2). Three plants which were foreground marker positive and with high levels of morphological similarity and recurrent parent genome similarity were backcrossed to get BC_2F_1 seeds to enhance the HD2932 genome recovery percentage. In the BC_2F_1 generation, foreground analysis identified 31 plants carrying *Lr*19/*Sr*25, 25 plants carrying *Sr*26 and 29 plants carrying the *Yr*10 gene in a population of 70, 48 and 61 plants respectively (Table 2). All of the gene-positive plants were heterozygous for the targeted genes in BC_1F_1 and BC_2F_1 as could be seen by the codominant or pair of dominant markers for the different tagged loci.

Background selection in the gene-positive plants in the BC_2F_1 generation was completed using polymorphic SSR markers which were heterozygous in the BC_1F_1 generation. In the BC_2F_1 generation recovery of the recurrent parent genome increased to 96.15%, 89.88% and 93.29% for the *Lr*19/*Sr*25, *Sr*26 and *Yr*10 genes, respectively. Plants with the highest genomic similarity in the BC_2F_1 generation were selfed to produce BC_2F_2 seed. In the BC_2F_2 generation, foreground analysis using linked markers was completed to identify the plants carrying targeted alleles in the homozygous and heterozygous states (Fig. 3). Forty-two plants were identified as homozygous for *Lr*19/*Sr*25, five were homozygous for *Sr*26, and 28 plants were homozygous for *Yr*10. Background analysis resulted in the highest genomic recovery of 98.46% for the *Lr*19/*Sr*25 population, whereas the maximum genomic similarity was 94.38% for the *Sr*26 population. In the BC_2F_2 population for *Yr*10, maximum genomic similarity with variety HD2932 was 96.95%.

Homozygous progeny of the BC_2F_1 plants carrying *Lr*19/*Sr*25, *Sr*26 and *Yr*10 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 *Lr*19/*Sr*25 + *Yr*10, seven plants with *Sr*26+*Yr*10, while only one plant showed gene combination *Lr*19/*Sr*25 + *Sr*26 (Table 3). Eight plants with the combination of *Lr*19/*Sr*25 + *Yr*10 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+*Lr*19/*Sr*25+*Yr*10.

Discussion

Rust resistance genes *Lr*19/*Sr*25, *Sr*26 and *Yr*10 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

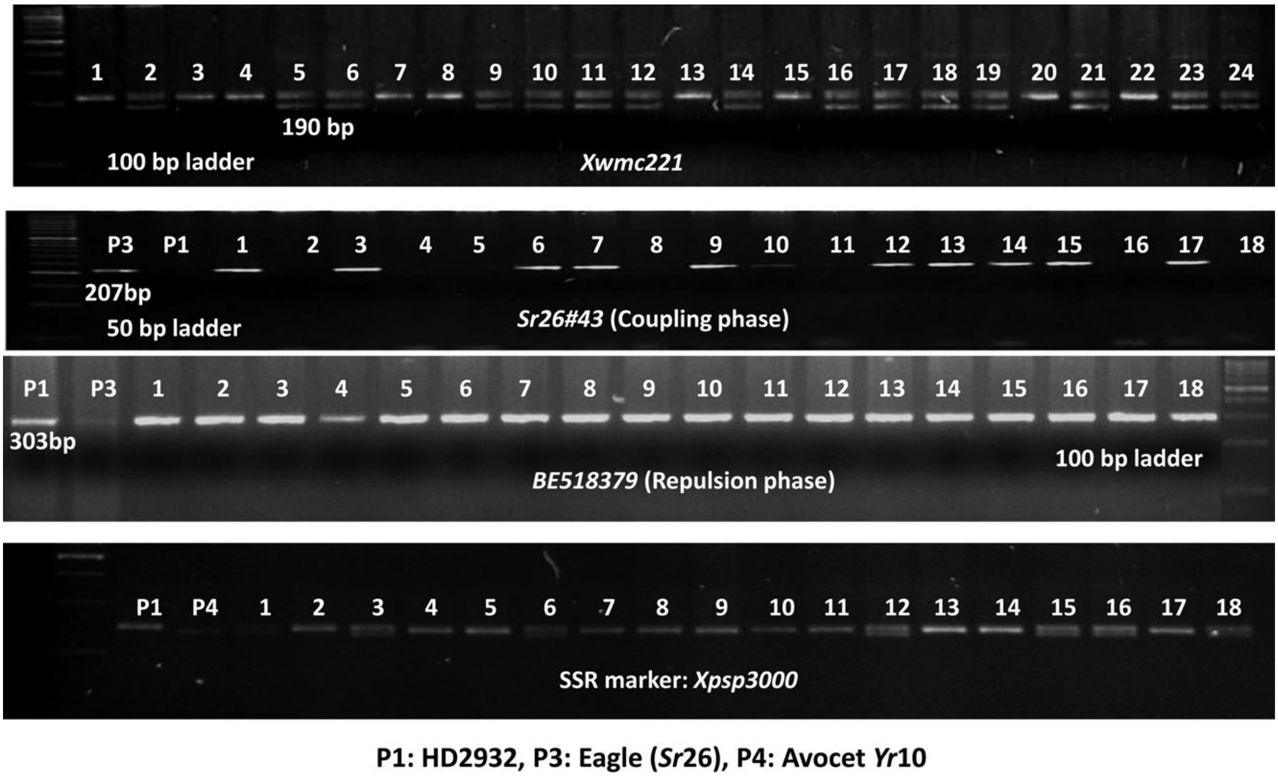

Fig. 2: Foreground selection for genes *Lr19, Sr26* and *Yr10* in BC_1F_1 generations of respective crosses involving wheat variety HD 2932 as recurrent parent

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

Cross	Total no of plants screened	No of plants with *Sr*26 and *Lr*19/*Sr*25	No of plants with *Sr*26 and *Yr*10	No of plants with *Lr*19/*Sr*25 and *Yr*10
(HD2932 + *Lr*19/*Sr*25) × (HD2932 + *Sr*26)	9	1		
(HD2932 + *Sr*26) × (HD2932 + *Yr*10)	58		7	
(HD2932 + *Lr*19/*Sr*25) × (HD2932 + *Yr*10)	43			8

can provide resistance against the three wheat rusts (Tomar and Menon 2001). The *Agropyron*-derived leaf rust resistance gene *Lr*19 (Sharma and Knott 1966) is linked with *Sr*25, 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 *Sr*26, 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 *Yr*10 has been reported from Europe, North America and Mediterranean region (Beaver and Powelson 1969, Stubbs 1985), the gene *Yr*10 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 (Basavaraj 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 *Lr*19/*Sr*25, *Sr*26 and *Yr*10 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 BC_1F_1 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 BC_1F_1 generation derived from the cross (HD2932 × HD2687+*Lr*19/*Sr*25) × HD2932, a plant with 90% genomic similarity with HD2932 was identified. In the BC_2F_1 generation, the genomic similarity increased to 96.15%. Genomic similarity further increased to 98.46% in BC_2F_2 generation. Thus, NILs possessing *Lr*19/*Sr*25 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

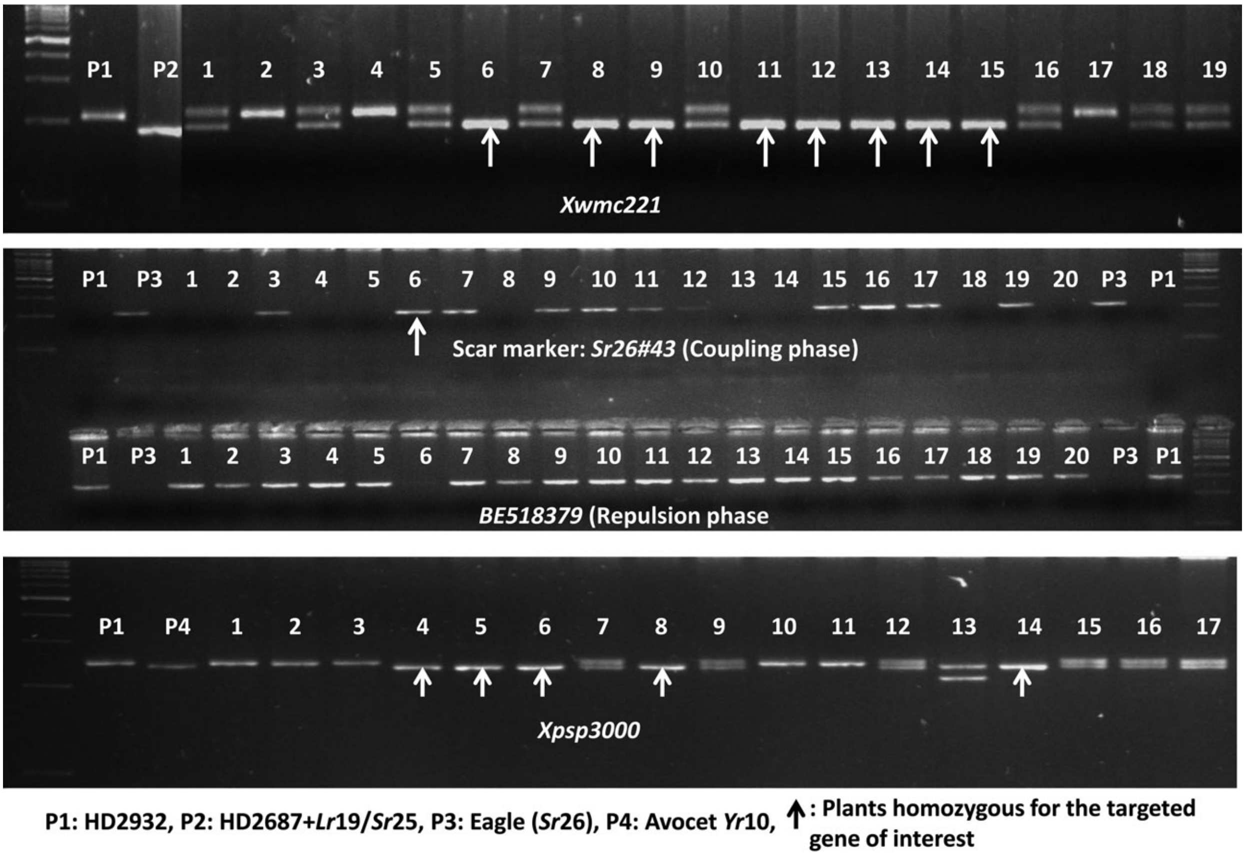

Fig. 3: Foreground selection for genes *Lr19, Sr26* and *Yr10* in BC_2F_2 generations of respective crosses involving wheat variety HD 2932 as recurrent parent

*Sr*26 and *Yr*10 in the background of HD2932, plants with high recovery of HD2932 genome were identified in BC_1F_1, BC_2F_1 and BC_2F_2 generations (Table 2). Thus, near-isogenic lines of HD2932 carrying genes *Lr*19/*Sr*25, *Sr*26 and *Yr*10 were developed in the BC_2F_2 generation with maximum HD2932 genome recovery reaching 98.46%, 94.38% and 96.95%, respectively. In the BC_1F_1 and BC_2F_1 generations, phenotypic selection was practised in addition to marker-assisted background selection.

The selected NILs carry the targeted genes *Lr*19/*Sr*25, *Sr*26 and *Yr*10 in homozygous state and could be used in future gene pyramiding programmes. Higher recovery of the HD2932 genome in the BC_2F_2 generation also provided an opportunity for intercrossing *Lr*19/*Sr*25, *Sr*26 and *Yr*10 carrying plants to produce two gene combinations in homozygous states in derived selfed progeny of crosses. In intercrossed BC_2F_2 products between two NIL combinations (F_1s), eight plants carrying *Lr*19/*Sr*25+*Yr*10, seven plants carrying *Sr*26+*Yr*10 and one plant with *Lr*19/*Sr*25+*Sr*26 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.

Table 4: Maximum rust disease levels recorded in the recurrent parent HD 2932 and selected introgressed (BC_2F_2) NILs

Variety	Level of infection		
	Stripe rust	Leaf rust	Stem rust
HD2932	60S	60S	30MS
HD2932 +*Lr*19/*Sr*25+*Yr*10	0	0	30MS
HD2932+*Lr*19/*Sr*25+*Sr*26	50S	0	0
HD2932+ *Sr*26+*Yr*10	0	40S	0

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.

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