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RESEARCH ARTICLE

Marker-assisted introgression of leaf rust resistance from *Triticum turgidum* var. *durum* cv. Trinakria to bread wheat variety HD3086

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Abstract

Breeders have extensively used marker-assisted selection to improve the agronomically superior varieties for disease resistance. Here, the mega wheat variety of India, HD3086, has been improved for leaf rust resistance by transferring a leaf rust resistance gene *LrTrk* from *Triticum turgidum* var. *durum* cv. Trinakria (AABB, 2n = 4X = 28). Taking a tetraploid donor parent instead of a hexaploid produced partial sterility in initial generations, but fertility improved beyond BC_2F_1 generations and aided in the rapid and higher recovery of recurrent parent genomes. Leaf rust resistance gene *LrTrk* was selected in every backcross generation with the help of resistance gene linked marker *Xgwm234* and further confirmed by rust screening. Further, rigorous phenotypic selection of plants with rust resistance gene *LrTrk* for their phenotypic similarity to recurrent parent HD3086 in backcross generations helped us identify six homozygous NILs in BC_2F_3 generation. All six NILs carried more than 95% of the recurrent parent genome (RPG) when analyzed with polymorphic markers between the parents. The six NILs also showed no difference in the ago-morphological traits compared to RP HD3086. Out of six, one NIL, HD3086+*LrTrk*-2, was selected with numerically higher yielding than recurrent parent HD3086 and at par performance for all other traits. This NIL will be nominated in AICRP trials before being it to the farmer's field. The improved NIL will provide an alternative for the susceptible cultivar from the farmer's field and broaden the genetic base of wheat cultivars grown in India.

Keywords: Wheat, leaf rust, marker-assisted selection, phenotypic selection, gene transfer.

Introduction

Bread wheat (*Triticum aestivum* L.) is a staple food for millions worldwide, providing protein, carbohydrates, and other vital nutrients. Worldwide, wheat is grown on an area of 222.77 million hectares, with a global production of 788.95 million metric tons from 2023 to 2024 (USDA). India is the second-largest wheat producer in the world after China, with productivity of 3.52 tonnes per hectare (USDA). Wheat production in India is affected by several biotic and abiotic factors. Among biotic factors, rusts caused by *Puccinia* spp. have significant economic importance (Kolmer 2013; Tomar et al. 2014).

There are three types of wheat rust: leaf or brown rust caused by *Puccinia triticina*, stem or black rust caused by *Puccinia graminis tritici* and stripe or yellow rust caused by *Puccinia striiformis*. While stripe rust is mostly confined to the cooler regions of North India and stem rust occurs mainly in warmer areas of central and Peninsular India, leaf rust can occur throughout the country, wherever wheat is grown. The leaf rust accounts for 30% or even higher yield loss under favorable climatic conditions (Singh et al. 2002). Though fungicides can control this disease, it is not an economical and environment-friendly approach. The most efficient and cost-effective method is to improve the genetic resistance of

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wheat cultivars. In wheat, 83 *Lr* genes have been cataloged (McIntosh et al. 2020; Kolmer et al. 2023), comprising seedling and adult plant resistance to leaf rust.

About half of these resistance genes are derived from alien sources. Wheat wild relatives contain a significant reservoir of economically beneficial genes (Ellis et al. 2014). Wheat genetic resources are divided into primary, secondary, and tertiary gene pools. Transferring gene(s) from primary and secondary gene pools is generally easy. However, gene transfer from the tertiary gene pool is complex and involves chromosomal modification techniques (Tomar et al. 2014). *Triticum turgidum* var. *durum* cv. Trinakria (AABB, 2n = 4X = 28) is a tetraploid belonging to the primary gene pool of wheat and carries resistance to both leaf and stripe rusts (Mallick et al. 2022a,b). Identification and transfer of genes with broad-spectrum resistance to leaf rust are of enormous importance due to the knockdown of some of the crucial genes like *Lr9*, *Lr19*, *Lr24*, and *Lr28* (Nayar et al. 2003; Bhardwaj et al. 2005; Huerta-Espino et al. 2008; Bhardwaj et al. 2010) due to continuous evolution of virulent pathotypes. Though virulence to *Lr9*, *Lr19*, and *Lr28* has also been reported in India, there is no report of virulence towards *Lr24* in India.

Gireesh et al. 2014, reported the presence of a single dominant leaf rust resistance gene (*LrTrk*) in durum wheat genotype Trinakria and further mapped it to the short arm of chromosome 5B with the closest marker *Xgwm*234 at 6.3cM for marker-assisted selection. Mallick et al.2022**,** used marker-assisted backcrossing to transfer this gene from Trinakria to wheat varieties HD2967 and HD2932. Along with the leaf rust resistance gene *LrTrk*, the stripe rust resistance gene *YrTrk* was also transferred into these varieties.

HD3086 is a high-yielding wheat variety released in 2013 for commercial cultivation in India's North Western Plain Zone (NWPZ). Farmers and seed producers prefer this variety because of its high yield and bold grains. However, this variety became susceptible to leaf rust over time, though it still possesses resistance to pathotypes of stripe rust, which are most prevalent and virulent in India. Efforts have been made to improve the leaf rust resistance of HD3086 by transferring the leaf rust resistance gene *Lr24* through marker-assisted selection (Sunilkumar et al. 2022). The availability of virulence to *Lr24* in other parts of the world necessitates the availability of diverse resistance in HD3086 for long-term cultivation in India. Leaf rust resistance gene *LrTrk*, a broad-spectrum resistance gene from tetraploid donor parent Trinakria, will provide an alternate source of leaf rust resistance to HD3086 while enabling recovery of the entire D genome of HD3086 without any recombination. Thus, the aim of the current work was to develop an NIL with the best genotypic recovery and matching phenotypic performance by transferring the leaf rust resistance gene *LrTrk* in the wheat variety HD3086.

Materials and methods

Plant material

In the present study, the mega wheat variety of India HD3086 was used as a recurrent parent (RP), whereas *Triticum turgidum* var. *durum* cv. Trinakria, a durum wheat genotype, was used as a donor parent (DP) to transfer the leaf rust resistance gene *LrTrk* through integrated phenotypic and marker-assisted selection. RP HD3086 has been a high-yielding variety of India's North Western Plain Zone (NWPZ) since its release. It is very popular among farmers, but in the recent past, it has become susceptible to leaf rust. The tetraploid DP Trinakria is highly resistant to leaf rust and stripe rust and was earlier used by Mallick et al. 2022a, b to improve wheat varieties HD2967 and HD2932.

Backcross breeding scheme

The parental lines HD3086 and Trinakria were crossed to produce the F_1 generation. Here, RP HD3086 was used as a female parent as it has a higher ploidy level (hexaploidy), and DP Trinakria was used as a male parent as it has a lower ploidy level (tetraploid). The SSR marker *Xgwm234*, linked to the leaf rust resistance gene *LrTrk*, was used to confirm the hybridity of F_1 plants (Gireesh et al. 2014). True F_1 plants were backcrossed to RP to produce BC_1F_1 generation. Foreground selection of leaf rust resistance gene *LrTrk* was conducted in BC_1F_1 generation with linked SSR marker *Xgwm234.* The BC_1F_1 plants were also evaluated against *P. triticina* pathotype 77-5 at the seedling stage to discard the marker-positive but susceptible plants due to recombination between the resistance gene and the linked marker. Phenotypically selected BC_1F_1 plants carrying *LrTrk* were backcrossed to RP to produce BC_2F_1 generation.

Phenotypic selection for leaf rust resistance, resemblance to RP and foreground selection for *LrTrk* was also carried out in BC_2F_1generation. BC_2F_1 plants carrying the *LrTrk* gene and most similar to RP were selfed to develop the BC_2F_2 generation. The BC_2F_2 plants having resistance were further selected based on phenotypic similarity with RP. The 20 selected BC_2F_2 plants were selfed to generate 20 BC_2F_3 families. The 20 BC_2F_3 families were evaluated against leaf rust by sowing 20 plants from each family. The families found to be homozygous through rust phenotyping were also confirmed through foreground marker *Xgwm*234. The families found to be homozygous for leaf rust resistance gene *LrTrk* were further examined for their background recovery using polymorphic markers between RP and DP. The NILs with higher RPG% were evaluated for different agro-morphological traits. The crossing scheme to transfer the leaf rust resistance gene *LrTrk* in wheat variety HD3086 is given in Fig. 1.

Molecular marker analysis

The CTAB approach was used to extract DNA from one-month-old seedlings (Murray and Thompson 1980).

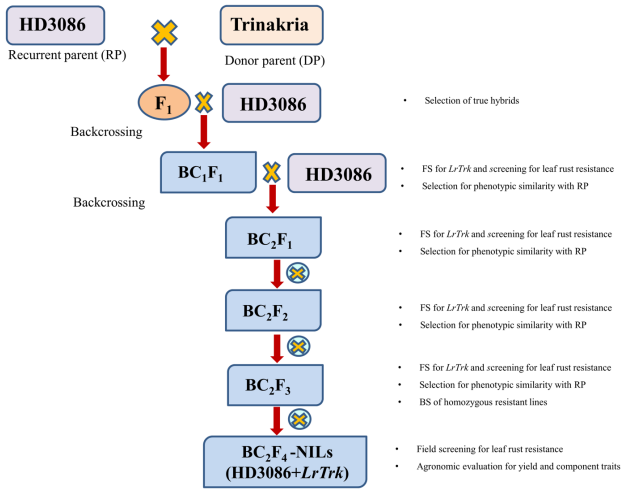

Fig. 1. Schematic representation of backcross plan to transfer leaf rust resistance gene *LrTrk* in wheat variety HD3086. FS = Foreground selection; BS = Background selection

Extracted DNA was estimated for its quality and quantity using a NanoDrop™ spectrophotometer. The DNA samples were diluted up to a concentration of 25 ng/µL and then stored at a -20°C refrigerator. A PCR reaction for SSRs was carried out in a 10 µL reaction volume comprising 2 µL of 25 ng/µL gDNA (50 ng), 3 µL of 2× GoTaq PCR Master Mix (Promega, #M7122), 3 µL of nuclease-free water, and 1-µL of each primer (5 pmol/ul) in a thermally sealed PCR plate using Eppendorf thermal cycler. A thermal profile of initial denaturation: 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 seconds, primer annealing at 50 to 60°C for 30 seconds, and primer extension at 72°C for 30 seconds with a final extension for 10 minutes at 72°C were used for amplification of the corresponding SSR markers. The amplicon was resolved on a gel containing 3.5% agarose and visualized using a UV Gel Documentation System (G: Box, Syngene). The Recurrent Parent Genome (RPG%) recovery was calculated using the formula used by Mallick et al. 2015 and visualized using the Graphical GenoTypes (GGT) v.2.0 package (Berloo, 2008).

Screening of NILs for leaf rust resistance

The six selected NILs from the BC_2F_3 families were screened with the five most prevalent and virulent *P. triticina* pathotypes (77-5, 77-9, 104-2, 106 and 12-5) at the seedling stage under glasshouse conditions. Inoculums of *P. triticina* pathotypes were initially obtained from the ICAR-IIWBR, Flowerdale, Shimla and were multiplied on the susceptible wheat cultivar, Agra Local, in the glasshouse at IARI, New Delhi. Parents, HD3086 and Trinakria, susceptible check Agra Local and 6 NILs were raised in aluminium trays (4×10×3 inches) in the glasshouse for screening. Inoculation mixture (Urediospores) of individual pathotypes was prepared separately in sterile water with a drop of tween-20 and sprayed manually on the 7 to 10 days old seedlings. After inoculation, all trays were placed in a humid glass chamber for the next 48 hours, which were subsequently moved to glass house benches under ambient growth temperature and light conditions (Rani et al. 2020; Raghunandan et al. 2022). After 10 to 12 days of inoculation, the first leaf of each seedling displaying a specific leaf rust disease response (infection types) was recorded following a 0-4 scale (Stakman et al. 1962).

Field evaluation of HD3086 + LrTrk NILs for agro-morphological traits

The six NILs (HD3086+*LrTrk*) and their RP HD3086 were evaluated for their agronomic performance in a randomized complete block design (plot size 7.7 m^2)with three replications at the experimental farms, IARI, New Delhi, following all the recommended agricultural practices. Data on agro-morphological traits such as plant height (PH), spike length (SL), number of spikelets per spike (SPS), number of grains per spike (GPS), thousand kernel weight (TKW) were taken from 5 random plants for each entry, whereas data for days to heading (DTH), grain yield (GY) and days to maturity (DTM) were recorded on plot basis for each replication. The collected data were analyzed for their statistical significance using WASP-Web Agri Stat Package 2.0 (Jangam et al. 2004).

Results

Development of near-isogenic lines (NILs) of HD3086 carrying LrTrk

Crossing between the tetraploid donor parent (DP), Trinakria and the recurrent parent (RP), HD3086, was done to develop the F_1 generation. The hybridity test using the co-dominant SSR marker *Xgwm234,* linked with the targeted leaf rust resistance gene *LrTrk* confirmed the heterozygosity of the F_1 plants. Ten F_1 plants were backcrossed with recurrent parent HD3086 to produce the BC_1F_1 generation. The BC_1F_1 seeds were a mixture of shriveled and normal-filled seeds. A total of 93 normal-filled BC_1F_1 seeds were raised in the glass house and phenotyped for leaf rust resistance (Table 1). Out of 93, 47 plants were found to be resistant to *P. triticina* pathotype 77–5. Of the 47 BC_1F_1 plants, 13 were selected based on their phenotypic similarity with RP HD3086 and were backcrossed with RP HD3086 to produce 58 BC_2F_1 seeds. These BC_2F_1 seeds were well-filled and normal compared to the BC_1F_1 generation. Fifty-eight BC_2F_1 plants were again screened for rust resistance with virulent pathotype 77–5, out of which 31 were found resistant. Out of 31 plants, five plants looking phenotypically most similar to the RP HD3086 were selected and selfed. Twenty-five selfed seeds from each selected BC_2F_1 plant were used for rust screening in BC_2F_2 generation. About 125 BC_2F_2 plants were screened with *P. triticina* pathotype 77-5, out of which 94 plants were found to be resistant and 31 were found to be susceptible (Table 1).

Out of 94 BC_2F_2 plants, again, 20 plants were selected based on agro-morphological resemblance with RP. Twenty plants were selfed to produce 20 BC_2F_3 families. Rust screening of 20 BC_2F_3 families identified six homozygous resistant lines (NILs) carrying *LrTrk* in the homozygous state.

The six HR NILs also showed the presence of linked marker *Xgwm234* in the homozygous state (Fig. 2). Parental polymorphism survey between HD3086 and Trinakria using 738 SSR markers identified 84 (8.79%) polymorphic markers. These 84 markers distributed well across the 14 chromosomes of A and B genomes of wheat and subsequently were used for background analysis of six NILs (Fig. 3). All the NILs showed high background recovery with RPG of more than 95% (Table 3). The Graphical representation of the six NILs revealing recovery of the RP genome with some residual segments from the donor parentis depicted in Fig. 4.

Screening of NILs for leaf rust resistance

The six homozygous resistant near-isogenic lines carrying the *LrTrk* gene were selected from 20 BC_2F_3 families based on their initial leaf rust screening and maximum phenotypic similarity with the RP HD3086. The lines were also found to have a very high level of recurrent parent genome recovery. These 6 BC_2F_3 NILs and the parental lines were screened for the five most common virulent *P. triticina* pathotypes at the seedling stage, along with Agra Local as a susceptible check. Donor parent Trinakria exhibited high seedling resistance against all the five pathotypes of *P. triticina* with IT ";". In contrast, recurrent parent HD3086 showed a high level of susceptibility with IT "33+". Congruently, all the six NILs tested were found to be resistant to all the *P. triticina* pathotypes with a response of IT of ";", except a NILs (HD3086+*LrTrk*-5) that displayed a response with IT ";1-" for races 77-5 and 77-9 (Table 2). The response of disease reaction in a NIL-2 against pathotype 77-5, along with parental lines, is presented in Fig. 5.

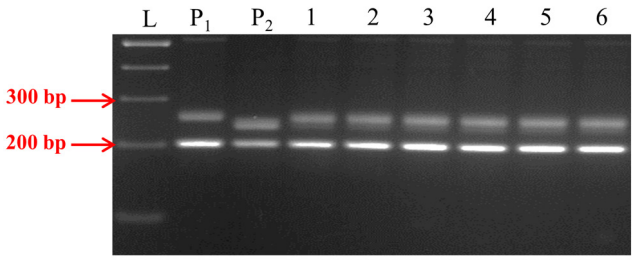

Fig. 2. Representative gel picture of foreground selection for *LrTrk* in BC_2F_3 generation. Here, L: 100 bp ladder; P1: Trinakria; P2: HD3086; 1-6: HD3086+*LrTrk*-NILs (1-6)

Evaluation of NILs for yield and yield-contributing traits

Evaluation of six NILs (HD3086+*LrTrk*-1 to 6) for agro-morphological traits, like days to heading (DTH), plant height (PH), spike length (SL), grains per spike (GPS), days to maturity (DTM) and grain yield per plot (GY) showed no significant difference to recurrent parent HD3086. Only the NIL, HD3086+*LrTrk*-5, showed a significantly higher number of spikelets per spike (SPS) but a lower thousand kernel weight (TKW) than recurrent parent HD3086.

Discussion

The yield potential of agronomically superior varieties can be improved by reducing the losses caused due to diseases. The mega wheat variety PBW343, once ruled in the farmland of India, has to be withdrawn due to its susceptibility to wheat rust. During 2017, this variety was again released into the farmers' field as Unnat PBW343 after the successful pyramiding of linked resistance genes, *Lr37/Yr17/Sr38* and *Lr76/Yr70* for leaf, stem, and stripe rusts (Sharma et al. 2021). Soon after its release, it received massive enthusiasm from wheat growers in India. Similarly, HD3406 (Unnat HD2967) was released in 2023 for cultivation in the farmer's field after its recurrent parent HD2967, a mega wheat variety of India, became susceptible to stripe rust. Wheat variety HD3406, a NIL, carries the leaf and stripe rust resistance gene *LrTrk/YrTrk* from Trinakria (Tetraploid donor) with a maximum background of recurrent parent HD2967. In addition to genetic improvement, the NILs can also serve as excellent material for fine mapping of *R* gene (Sun et al. 2010**),** and will also help in deciphering the underlying molecular pathways associated with the resistance mechanism (Bhurta et al. 2022; Hurali et al. 2022; Tyagi et al. 2022). Owing to the broad-spectrum resistance of leaf rust resistance gene *LrTrk* (Gireesh et al. 2014; Mallick et al. 2022), it has been decided to transfer this gene to HD3086, another mega variety of India, which became susceptible to leaf rust in due course of time (Sunilkumar et al. 2022).

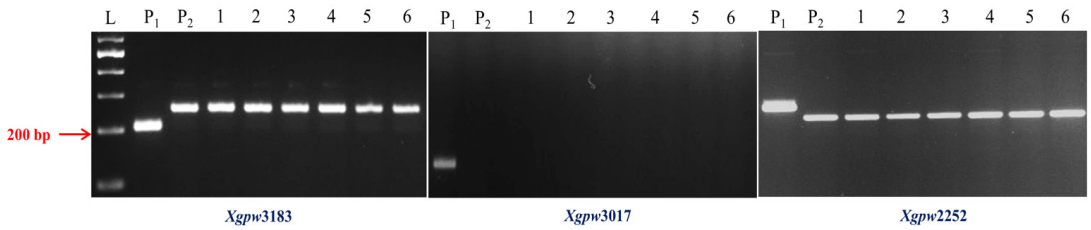

Fig. 3. Representative gel picture of polymorphic markers used in background analysis for NILs of HD3086 + *LrTrk* in BC_2F_3 generation. Here, L: 100 bp ladder; P1: Trinakria; P2: HD3086; 1-6: HD3086 + *LrTrk*-NILs (1-6)

Table 1. Details of *LrTrk* gene-positive plants screened during backcross breeding and evaluation

S. No.	Recipient parent	Target gene	Generation	No. of plants (P)/families (F) screened	No. of resistant plants (P)/ families (F)	No. of susceptible plants (P)	Number of plants (P)/families (F) selected for generation advancement/evaluation
1			F_1	10 P	10 P	0 P	10 P
2			BC_1F_1	93 P	47 P	46 P	13 P
3			BC_2F_1	58 P	31 P	27 P	5 P
4	HD3086	*LrTrk*	BC_2F_2	125 P	94 P	31 P	20 P
5			BC_2F_3	20 F	6 (HR, F)	-	6 (homozygous resistant (HR) families)
6			BC_2F_4	6 F	6 (HR, F)	-	6 (HR families for agronomic evaluation)

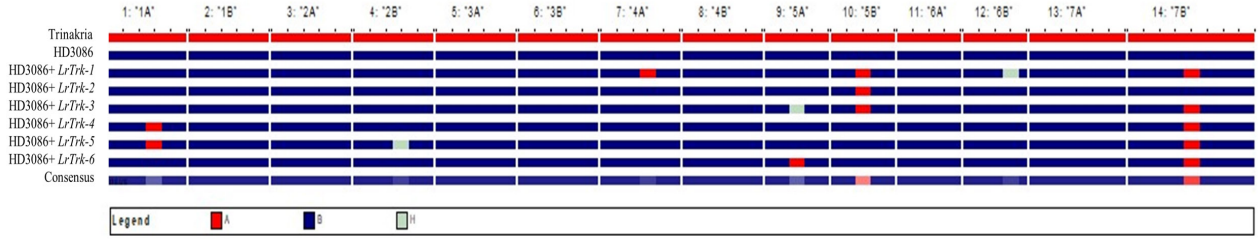

Fig. 4. Graphical Genotypes (GGT) depicting recurrent parent genome (RPG) recovery across 14 chromosomes of A and B genomes of the six selected NILs with their parents

There are many ways by which genes can be transferred from donor to recurrent parent by keeping the maximum genome of the recurrent parent intact. One way is backcrossing to the recurrent parent based solely on the phenotypic selection, the second is exclusively based on marker-assisted selection (marker-assisted foreground and background selection), and the third is integrating both phenotypic and marker-assisted selection. While the first method is cumbersome and requires more time, the second method requires more resources. The integrated phenotypic and marker-assisted selection proved efficient and cost-effective in developing near-isogenic lines (NILs) (Mallick et al. 2015, 2022a, b; Ellur et al. 2016). Here, while transferring leaf rust resistance gene *LrTrk* to HD3086, marker-assisted foreground selection and phenotypic selection for rust resistance were carried out in every backcross generation to avoid the identification of plants that are marker-positive but rust-susceptible. The distance between the linked marker *Xgwm234* and leaf rust resistance gene *LrTrk* is 6.3 cM (Gireesh et al. 2014), which can lead to identifying recombinants with foreground positive but having susceptible reactions. For the identification of plants with maximum recovery of recurrent parent HD3086, the rigorous phenotypic selection was carried out in BC_1F_1, BC_2F_1, $BC_2F_2$and BC_2F_3 generations, and with the help of SSR markers, background analysis was carried out in BC_2F_3 generation for lines homozygous resistant for leaf rust resistance gene *LrTrk*. A total of 20 plants resistant to leaf rust and marker positive for *Xgwm234* were identified in the BC_2F_2 generation, from which six homozygous NILs were identified in the BC_2F_3 generation. Although marker-assisted background selection was not carried out in initial backcross generations, meticulous phenotypic selection in every backcross generation helped in retrieving more than 95% RPG in all 6 NILs. The background recovery of 6 NILs is represented by graphical GenoTypes (GGT) w.r.t. "A"

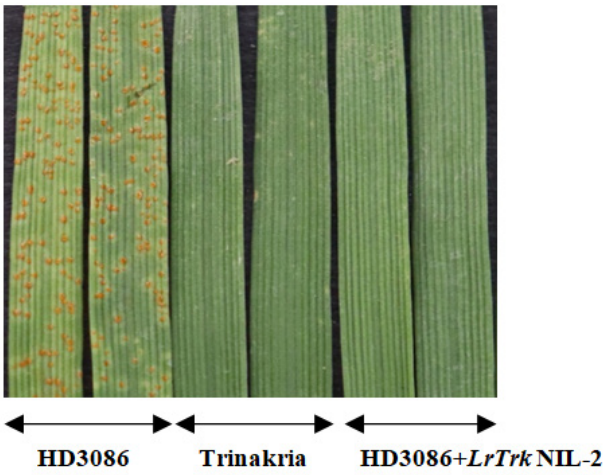

Fig. 5. Seedling reaction for leaf rust response of BC_2F_3 NIL (HD3086+ *LrTrk*-2) and the parents Trinakria and HD3086 against prevalent *P. triticina* pathotype 77-5

Table 2. Screening of HD3086, Trinakria and NILs (HD3086 + *LrTrk*) against the five most prevalent *P. triticina* pathotypes

P. triticina pathotypes	HD3086	Trinakria	Agra local	HD3086+ LrTrk -1	HD3086+ LrTrk -2	HD3086+ LrTrk -3	HD3086+ LrTrk -4	HD3086+ LrTrk -5	HD3086+ LrTrk -6
77-5	33+	;	33+	;	;	;	;	;1-	;
77-9	33+	;	33+	;	;	;	;	;1-	;
104-2	33+	;	33+	;	;	;	;	;	;
106	33+	;	33+	;	;	;	;	;	;
12-5	33+	;	33+	;	;	;	;	;	;

Table 3. Field evaluation of HD3086 NILs carrying leaf rust resistance *LrTrk* gene for yield and yield-contributing traits

NILs	DTH	PH	SL	SPS	GPS	TKW	GY	DTM	RPG%
HD3086+ *LrTrk-1*	93.3 ± 0.3	102.5 ± 0.2	11.81 ± 0.01	17.8 ± 0.1	54.3 ± 1.6	40.73 ± 0.13	5.75 ± 0.06	138.7 ± 0.3	95.83
HD3086+ *LrTrk-2*	93.7 ± 0.3	102.2 ± 0.3	11.84 ± 0.00	18.5 ± 0.4	58.5 ± 3.2	39.94 ± 0.28	5.90 ± 0.05	138.7 ± 0.3	98.88
HD3086+ *LrTrk-3*	94.3 ± 0.3	103.0 ± 0.1	11.82 ± 0.02	18.0 ± 0.2	56.8 ± 2.2	40.61 ± 0.83	5.85 ± 0.11	139.7 ± 0.9	97.02
HD3086+ *LrTrk-4*	93.3 ± 0.7	103.0 ± 0.3	11.81 ± 0.02	17.9 ± 0.1	58.9 ± 5.1	39.53 ± 0.89	5.87 ± 0.04	139.0 ± 0.0	97.61
HD3086+ *LrTrk-5*	93.7 ± 0.7	102.5 ± 0.3	11.86 ± 0.01	19.7 ± 0.2*	60.9 ± 2.2	36.26 ± 0.72*	5.73 ± 0.05	139.0 ± 0.6	97.02
HD3086+ *LrTrk-6*	93.7 ± 0.3	102.9 ± 1.1	11.81 ± 0.01	18.6 ± 0.2	56.1 ± 1.3	38.47 ± 0.77	5.69 ± 0.07	140.3 ± 0.3	97.61
HD3086	94.3 ± 0.3	102.6 ± 0.3	11.80 ± 0.01	18.1 ± 0.2	55.4 ± 1.0	39.925 ± 1.01	5.81 ± 0.04	139.7 ± 0.3	-
CD (0.05)	1.5035	1.4941	0.0422	0.6446	6.8216	2.2172	0.2004	1.2479	-

Data representing mean ± SD of five independent plants for each entry. DTH- Days to heading; PH- Plant Height in cm; SL- Spike length in cm; SPS- Number of spikelets per spike; GPS- Number of grains per spikelets; TKW- Thousand Kernel Weight in gm; GY- Per plot grain yield in kg; DTM- Days to maturity; RPG% - percent of recurrent parent genome recovered;* represent NILs significantly different as compared to Recurrent parent HD3086 at $p > 0.05$

and "B" genome only as the donor lacks the "D" genome, and the maximum recovery "D" genome happens in BC_1F_1 itself (Mallick et al. 2022a). The higher recovery of RPG in NILs can also be attributed to the lack of D genome in DP. Multi-pathotype testing with five pathotypes showed resistance reaction in all the 6 NILs. The effect of rigorous phenotypic selection can also be assessed by yielding contributing traits of six NILs, which showed almost identical performance to that of recurrent parent HD3086 (Table 3). The NIL HD3086+*LrTrk*-5 though showed significantly lower TKW; there is no difference in GY due to a significantly higher number of spikelets per spike. The result also suggests a negative correlation between TKW and SPS or GPS. The NIL HD3086+*LrTrk*-2 with numerically higher GY and at par performance for other traits as well as maximum background recovery with 98.88% of RP genome is selected for nomination in All India Co-ordinated Research Project (AICRP) trials for multilocation testing. The improved NILs of HD3086 with leaf rust resistance will be a substitute for the susceptible cultivar. Also, the deployment of diverse rust resistance genes in HD3086 background will broaden the genetic base of Indian wheat varieties and pose a hurdle to the continuous evolution of rust pathotypes.

Authors' contribution

Conceptualization of research (V, SKJ, NM); Designing of the experiments (SKJ, V, NM); Contribution of experimental materials (NM, SKJ, V, RK, MN); Execution of field/lab experiments and data collection (SY, AKC, PA, ST, MKC, SB, AK, HS); Analysis of data and interpretation (SY, AKC, PA, ST, NRSKJ, V); Preparation of the manuscript (SY, AKC, SA, NR, SKJ, NM, V).

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References

Berloo R. Van 2008. GGT 2.0: versatile software for visualization and analysis of genetic data. J. Hered., **99**(2): 232-236.

Bhardwaj S. C., Prashar M., Jain S. K., Kumar S., Sharma Y. P., Sivasamy M., and Kalappanavar I. K. 2010. Virulence of Puccinia triticina on *Lr28* in wheat and its evolutionary relation to prevalent pathotypes in India. Cereal Res. Commun., **38**: 83-89.

Bhardwaj S. C., Prashar M., Kumar S., Jain S. K., and Datta D. 2005. *Lr19* resistance in wheat becomes susceptible to *Puccinia triticina* in India. Plant Dis., **89**(12): 1360-1360.

Bhurta R., Hurali D. T., Tyagi S., Sathee L., Adavi B., Singh D., Mallick M., Chinnusamy V., Vinod and Jha S. K. 2022. Genome-wide identification and expression analysis of the thioredoxin (*Trx*) gene family reveals its role in leaf rust resistance in wheat (*Triticum aestivum* L.). Front. Genet., **13**: 563.

Ellis J. G., Lagudah E. S., Spielmeyer W., and Dodds P. N. 2014. The past, present and future of breeding rust resistant wheat. Front. Plant Sci., **5:** 641.

Ellur R. K., Khanna A., Yadav A., Pathania S., Rajashekara H., Singh V. K., Krishnan S. G., Bhowmick P. K., Nagarajan M., Vinod K. K. and Prakash G. 2016. Improvement of Basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding. Plant Sci., **242**: 330-341.

Gireesh C., Vinod, Sharma J.B. and Prabhu K.V. 2014. Inheritance and molecular mapping of leaf rust resistance in *Triticum turgidum* var. *durum* cv. Trinakria. Indian J. Genet., **74**: 10-15.

Huerta-Espino J., Singh R. P., and Reyna-Martinez J. 2008. First report of virulence to leaf rust resistance genes *Lr9* and *Lr25* in Mexico. Plant Dis., **92**: 311.

Hurali D. T., Bhurta R., Tyagi S., Sathee L., Sandeep A. B., Singh D., Mallick N., Vinod and Jha S. K. 2022. Analysis of NIA and GSNOR family genes and nitric oxide homeostasis in response to wheat-leaf rust interaction. Sci. Rep., **12**(1): 803.

Jangam A.K., Thali P. 2004. WASP-Web Agri Stat Package. ICAR Research Complex for Goa, Ela, Old Goa, Goa. 403 402. India

Kolmer J. 2013. Leaf Rust of Wheat: Pathogen Biology, Variation and Host Resistance. Forests, **4**(1): 70-84.

Kolmer J. A., Bajgain P., Rouse M. N., Li J., and Zhang P. 2023. Mapping and characterization of the recessive leaf rust resistance gene *Lr83* on wheat chromosome arm 1DS. Theor. Appl. Genet., **136**(5): 115.

Mallick N., Jha S.K., Agarwal P., Kumar S., Mall A., M N., Chaudhary M.K., Chandra A.K., Bansal S., Saharan M.S., Sharma J.B. and Vinod. 2022b. Marker-assisted transfer of leaf and stripe rust resistance from *T. turgidum* var. durum cv. Trinakria to wheat variety HD2932. Front. Genet.,**13**: 941287.

Mallick N., Jha S.K., Agarwal P., Mall A., Kumar S., Choudhary M.K., Bansal S., Saharan M.S., Sharma J.B. and Vinod. 2022a. Marker-assisted improvement of bread wheat variety HD2967 for leaf and stripe rust resistance. Plants, **11**(9): 1152.

Mallick N., Vinod, Sharma J.B., Tomar R. S., Sivasamy, M. and Prabhu K.V. 2015. Marker-assisted backcross breeding to combine multiple rust resistance in wheat. Plant Breed., **134**: 172-177.

McIntosh R. A., Dubcovsky J., Rogers W. J., Morris C., Appels R. and Xia X. C. 2020. Catalogue of gene symbols for wheat: 2015-16 Supplement. https:// shigen. nig.ac.jp/wheat/komugi/genes/ symbol ClassList.jsp

Murray M. G., and Thompson W. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res., **8**(19): 4321-4326.

Nayar S., Jain S. K., Prashar M., Bhardwaj S. C., Kumar S., and Menon M. K. 2003. Appearance of new pathotype of *Puccinia recondita tritici* virulent on *Lr9* in India. Indian Phytopathol., **56**(2): 196-198.

Raghunandan K., Tanwar J., Patil S. N., Chandra A. K., Tyagi S., Agarwal P., Mallick N., Murukan N., Kumari J., Sahu T.K., Jacob S. R., Kumar A., Yadav S., Nymagoud S., Vinod., Singh A. K., and Jha S. K. 2022. Identification of novel broad-spectrum leaf rust resistance sources from Khapli wheat landraces. Plants, **11**(15): 1965.

Rani K., Raghu B. R., Jha S. K., Agarwal P., Mallick N., Niranjana M., Sharma J.B., Singh A. K., Sharma N. K., Rajkumar S. and Vinod. 2020. A novel leaf rust resistance gene introgressed from *Aegilops markgrafii* maps on chromosome arm 2AS of wheat. Theor. Appl. Genet., **133**: 2685-2694

Sharma A., Srivastava P., Mavi G. S., Kaur S., Kaur J., Bala R., Singh T. P., Sohu V. S., Chhuneja P., Bains N. S. and Singh G. P. 2021. Resurrection of wheat cultivar PBW343 using marker-assisted gene pyramiding for rust resistance. Front. Plant Sci., **12**: 570408.

Singh R.P., Huerta-Espino J. and Roelfs A.P. 2002 The wheat rusts. In: Curtis BC, Rajaram S, Gómez Macpherson H (eds) Bread wheat: improvement and production, plant production and protection series no. 30, FAO, Rome: 227–249

Stakman E.C. and Steward D.M. 1962. Loegering, W.Q. Identification of Physiologic races of *Puccinia graminis* var. *tritici*; USDA: Washington, DC, USA.

Sun X., Bai G., Carver B.F. and Bowden R. 2010. Molecular mapping of wheat leaf rust resistance gene *Lr42*. Crop Sci., **50**(1): 59-66.

Sunilkumar V. P., Krishna H., Devate N. B., Manjunath K. K., Chauhan D., Singh S., Sinha N., Singh J.B., Prakasha T.L., Pal D., Sivasamy M., Jain N., Singh G.P. and Singh P. K. 2022. Marker assisted improvement for leaf rust and moisture deficit stress tolerance in wheat variety HD3086. Front. Plant Sci., **13**: 1035016.

Tomar S.M.S., Singh S.K., Sivasamy M. and Vinod. 2014. Wheat rusts in India: Resistance breeding and gene deployment-A review. Indian J. Genet.Plant Breed., **74**: 129-156.

Tyagi S., Jha S.K., Kumar A., Saripalli G., Bhurta R., Hurali D.T., Sathee L., Mallick N., Mir R.R. and Chinnusamy V. 2022. Genome-wide characterization and identification of cyclophilin genes associated with leaf rust resistance in bread wheat (*Triticum aestivum* L.). Front. Genet., **13**: 972474

USDA Circular series, World agriculture production, July, 2024. https://apps.fas.usda.gov/psdonline/circulars/production.pdf