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Molecular mapping of linked leaf rust resistance and non-glaucousness gene introgressed from *Aegilops tauschii* Coss. in hexaploid wheat *Triticum aestivum* L.

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Abstract

Aegilops tauschii, the D genome donor of wheat, is an invaluable source of genetic variability, which can be utilized for broadening the wheat gene pool. Linked leaf rust resistance and non-glaucousness genes transferred from *Ae. tauschii* to cultivated wheat variety WH542 were mapped in the present study. Genetic analysis in an F_2 population from a BC_3 plant derived from the cross *Triticum durum* cv. PBW114/*Ae. tauschii* acc. pau14195//4**T. aestivum* cv. WH542 revealed monogenic dominant inheritance for both the traits. The leaf rust resistance and the non-glaucousness gene were tentatively named *LrT* and *IwT*, respectively. Leaf rust resistance gene exhibited all stage resistance. SSR markers *Xbarc124, Xgdm5, Xgdm35, Xcfd51* and EST-derived markers *Xcau96* and *Xte6* on chromosome 2DS were linked with both genes. Chromosomal assignments of the genes were confirmed by testing linked SSR markers on Chinese Spring nulli-tetrasomics lines. SSR markers *Xcau96* (1.6 cM) and *Xbarc124* (0.6 cM) flanked *LrT* and *Xgdm35* (4.1 cM) and *Xte6* (2.5 cM) flanked non-glaucousness gene. *LrT* and *IwT* showed a recombination distance of 3.4 cM. Hence, *IwT* can be used as an easy to score morphological marker of *LrT* during its transfer to other glaucous backgrounds.

Keywords: *Aegilops tauschii*, bridging species, genetic mapping, leaf rust resistance, non-glaucousness, wide hybridization

Introduction

Cereal rusts are among the major biotic stresses that pose a substantial threat to wheat production worldwide. Leaf rust caused by *Puccinia triticina* is one of the most important and devastating foliar diseases of wheat. It causes significant yield losses all over the world due to more frequent and widespread occurrence (Bolton *et al.,* 2008). To date, 76 leaf rust resistance genes have been identified and catalogued in wheat (McIntosh *et al.*, 2013; Bansal *et al.*, 2017). Continuous emergence of new virulent races and monoculture of few improved cultivars result in increased vulnerability of wheat to this disease. Therefore, it is imperative to stay ahead of the pathogen and search for new resistance genes, not only in the cultivated gene-pool, but also in the wild relatives of wheat (Friebe *et al.*, 1996; Valkoun, 2001).

Cuticular wax deposits are known to reduce the loss of water due to transpiration and hence increasing drought tolerance in plants (Jenks and Ashworth, 1999; Riederer and Schreiber 2001). Glaucousness is a visual trait, which is related to greyish or whitish appearance on the leaves, sheaths, glumes and stems in wheat. This appearance is due to epicuticular wax exudates produced by the plant

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parts resulting in a waxy bloom on the surface of plant parts. Genetically, glaucousness is controlled by a single dominant gene (*W1*) mapped to wheat chromosome 2BS. Another single dominant gene, *Iw2* located on chromosome 2DS, acts as an epistatic inhibitor to glaucousness resulting in non-glaucous (NG) appearance of plant organs. *W1* has a duplicated gene *W2* and *Iw2* has duplicated gene *Iw1*, located on chromosome 2DS and 2BS, respectively (McIntosh *et al*., 2013), demonstrating that the genes for wax production and suppression are non-allelic (Tsunewaki and Ebona, 1999). *Iw3*, another glaucousness inhibitor gene has been located on chromosome 1BL (Dubcovsky *et al*., 1997).

Evaluation of wild *Triticum* and *Aegilops* species at the Punjab Agricultural University, Ludhiana, India has led to the identification of several *Aegilops* species including *Ae. tauschii* as very good sources of resistance to many diseases including leaf rust and stripe rust (Chhuneja *et al*., 2010). Present study was conducted to understand the genetic basis of leaf rust resistance and associated glaucousness trait introgressed into hexaploid wheat from *Ae. tauschii* and to determine the chromosomal location of genes for both traits.

Materials and methods

Development of the mapping population

Ae. tauschii pau14195 was crossed with tetraploid *Triticum turgidum* ssp. *durum* cv. PBW114 and F_1 was crossed with leaf rust susceptible hexaploid *T. aestivum* cv. WH542. The complex F_1s were further backcrossed with WH542 along with screening for rust resistance at the seedling and adult plant stages (APSs) in each backcross generations. Only the leaf and/or stripe rust resistant BC_1 and BC_2 plants were further backcrossed. Resistant BC_3F_1 plants were selfed to generate homozygous introgression lines as well as developing mapping populations. Mapping of a leaf rust resistance gene using BC_3F_2 and BC_3F_3 (PBW114/*Ae. tauschii* acc. pau14195//4*WH542) mapping populations derived from a single resistant BC_3F_1 plant is being described in the present investigation. Schematic representation of the development of mapping population is given in Figure S1. Populations of 305 BC_3F_2 plants and 293 BC_3F_3 families were tested against leaf rust at the seedling and APSs and 160 BC_3F_2 plants were selected for genotyping.

Evaluation for leaf rust resistance

Screening for leaf rust resistance was done at the seedling stage (SS) and the APS. Parental lines and six randomly selected homozygous resistant $BC_3F_{2:3}$ progenies were tested against 10 different leaf rust isolates at SS. Whole BC_3F_2 and BC_3F_3 populations were screened against isolate 77–5 at SS and mixture of races 77–1, 77–5, 104–2 and 77–2 at the APS. At the SS, 305 BC_3F_2 plants and 10–15 plants of each $BC_3F_{2:3}$ progeny along with parental lines WH542, *Ae. tauschii* pau14195 and PBW114 were screened following the method of Nayar *et al*. (1997). The landrace 'Agra Local' was used as susceptible check. Infection types (ITs) were recorded 14 days after inoculation on a 0–4 scale given by Stakman *et al*. (1962) with slight modifications as given in Roelfs *et al*. (1992).

After recording leaf rust data, BC_3F_2 seedlings were transplanted in the field in 1.0 m rows with row to row distance of 20 cm and plant to plant distance of 10 cm to record adult plant leaf rust response on the same F_2 plants. BC_3F_3 progenies of single F_2 plants, however, were simultaneously tested at SS and APS using 10–15 seeds for SS testing and 15–20 seeds for APS testing. In field evaluations, susceptible cultivar Agra Local and WL711 were used as spreader and planted after every 20 rows and all around the experimental plot. Artificial rust epidemic was created by spraying the mixture of uredinospores of leaf rust pathotypes 77–1, 77–5, 104–2, 77–2 every alternate day and keeping infected pots in between the rows. Irrigation was done at regular intervals to maintain high humidity in the field. Terminal disease severity was recorded according to the modified Cobb scale (Peterson *et al*., 1948).

Phenotypic characterization of glaucousness

The population also segregated for glaucousness (a waxy layer covering the leaves, stem and spike) as the *Ae. tauschii* pau14195 was NG, while recipient parent WH542 and bridging species PBW114 were glaucous (G). Glaucousness trait was scored at the APS when the spikes were fully visible. Glaucous plants were greyish green in appearance due to the presence of waxy coat on leaf, stem and ear, while NG plants were totally green. BC_3F_2 plants and $BC_3F_{2:3}$ progenies were scored for glaucousness (G) and non-glaucousness (NG).

Molecular analysis

Genomic DNA Extraction and bulk preparation

Genomic DNA of WH542, *Ae. tauschii* pau14195, PBW114 and BC_3F_2 plants was isolated from the young leaves using the CTAB method (Saghai-Maroof *et al*., 1994) with some modifications. Equimolar concentration of DNA of 10 phenotypically similar BC_3F_2 plants, i.e. leaf rust resistant-NG and leaf rust susceptible-glaucous plants were pooled separately to form 'Resistant-non-glaucous bulk' and 'Susceptible-glaucous bulk', respectively, for BSA (bulked segregant analysis).

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SSR analysis

Sixty-eight SSR markers previously mapped on seven D genome chromosomes were selected at a distance of 8–10 cM based on the assumption that leaf rust resistance has been transferred from *Ae. tauschii*. Initial marker analysis was done at Punjab Agricultural University, Ludhiana, Punjab, though final linkage map generation was done at Plant Breeding Institute, The University of Sydney. PCR was performed in Applied Biosystems master cyclers in 20 µl reaction volumes containing 1× buffer, 60 ng of template DNA, 0.375 µM of each primer, 1.5 mM of $MgCl_2$, 0.15 mM of dNTP and 1 U of Taq DNA polymerase. PCR products were separated on 6% non-denaturing polyacrylamide gels and visualized under UV light in gel documentation system. SSR markers polymorphic between parents and bulks were genotyped on 160 BC_3F_2 plants. Chromosomal location of the markers was ascertained by genotyping on a set of nulli-tetrasomics Chinese spring lines.

Linkage and statistical analysis

Chi-squared (χ^2) test was used to check goodness of fit of the observed and expected ratios for leaf rust resistance and non-glaucousness in BC_3F_2 population and $BC_3F_{2:3}$ progenies. Linkage analysis was performed using MapDisto version 7.1 (Lorieux, 2012) using Kosambi mapping function (Kosambi, 1943) with a LOD threshold of 3.0.

Results

Characterization of leaf rust and glaucousness

The donor *Ae. tauschii* accession pau14195 showed high level of resistance against all 10 leaf rust isolates, *T. durum* cv. PBW114 showed susceptible reaction to six isolates and *T. aestivum* cv. WH542 showed susceptible infection type to four of the isolates (Table 1). At APS, *Ae. tauschii* pau14195 and PBW114 displayed resistant leaf rust responses (TR), while WH542 was susceptible with rust severity of 40S-60S. WH542 and PBW114 were glaucous, while *Ae. tauschii* pau14195 was NG (Figure 1).

In BC_3F_2, 215 plants were resistant, while 78 plants were susceptible ($\chi^2_{(3:1)} = 0.41$ non-significant at $P = 0.05$ and df = 1) (Table 2). Seedling and adult plant reaction of the parental lines and representative BC_3F_2 plants is shown in Figure 2. Plants which were resistant at SS were also resistant at APS and those susceptible at SS were susceptible at APS. For glaucousness trait the BC_3F_2 population segregated in a ratio of 216NG: 77 G ($\chi^2_{(3:1)} = 0.25$ non-significant at $P = 0.05$ and df = 1) indicating that the trait was controlled by single dominant gene. Two-way segregation indicated that out of the 215 leaf rust resistant plants, 209 were NG and six were glaucous. Out of the 78 leaf rust susceptible plants, 71 were glaucous and seven were NG (Table 2). The single gene control of both the traits was further confirmed in BC_3F_3 generation as F_3 families segregated for single gene for leaf rust resistance in 70HR: 143Seg: 75HS ($\chi^2_{(1:2:1)} = 0.18$, $P > 0.05$) at SS and APS and 80 Homozygous Non-Glaucous (HNG): 134Seg: 74 Homozygous Glaucous (HG) ($\chi^2_{(1:2:1)} = 1.65$, $P > 0.05$) for non-glaucousness (Table 3). Of the 70 homozygous resistant (against 77–5) BC_3F_3 families, six randomly selected families were tested against nine additional leaf rust races and found to be homozygous resistant against eight of these races (Table 1). ITs against race 12–5 could not be generated due to unavailability of race. These observations confirmed that resistance gene is from *Ae. tauschii* as PBW114 give susceptible reaction to races 11, 12–2, 16–1, 104–2 and 106. Predominance of the parental types in F_2 and F_3 populations indicated genetic linkage between genes controlling leaf rust resistance

Table 1. Infection types of *Ae. tauschii*, PBW114 and WH542 and six randomly selected BC_3F_3 families against 10 different Pt pathotypes

	Pt pathotypes									
Genotype	11	12–2	12–5	16–1	77–5	77–7	77–8	77–10	104–2	106
WH542	0;	0;	3+	0;	3+	2+	0;	3+	3	0;
PBW114	3	3	3+	3+	;	0;	0;	0;	3+	3+
Ae. tauchii pau14195	0;	0;	0;	0;	;	0;	0;	0;	0;	0;
BC_3F_3-P13-32	0;	;	–	0;	;	;1	;1	0;	0;	;
BC_3F_3-P13-35	0;	0;	–	0;	0;	12	;1	0;	0;	0;
BC_3F_3-P13-53	;	0;	–	0;	0;	0;	0;	0;	;–	0;
BC_3F_3-P13-61	0;	0;	–	;1	;	;1	2	;1	0;	0;
BC_3F_3-P13-84	0;	0;	–	0;	0;	;1	2-	0;	0;	0;
BC_3F_3-P13-96	0;	0;	–	0;	0;	0;	0;	0;	0;	0;

0; = no visible uredinia, ; = hypersensitive flecks, 2 = medium uredinia with necrosis, 3+ = large sporulating uredinia without chlorosis.

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Fig. 1. Spikes of parental lines (from left to right) PBW114, WH542, *Aegilops tauschii* and BC_3F_2 plants showing segregation for glaucousness trait.

and non-glaucousness. These genes are being temporarily designated as *LrT* and *IwT*, respectively.

Molecular mapping of LrT and IwT

A total of 56 SSR markers and two EST derived markers were polymorphic between parental genotypes *Ae. tauschii* pau14195 and WH542 and 15 of these polymorphic markers amplified *Ae. tauschii* specific introgressions in the bulks. Of the 15 introgressed markers, six markers *Xbarc124, Xgdm5, Xgdm35, Xcfd51, Xcau96* and *Xte6* from chromosome 2D (Liu *et al.*, 2007; Röder *et al.*, 1998; Somers *et al.*, 2004) amplified diagnostic polymorphic DNA fragments between the two contrasting bulks. None of the SSR markers selected from chromosome 2A and 2B showed polymorphism between the bulks. These six diagnostic markers were then genotyped on 160 BC_3F_2 plants.

Genotypic and phenotypic data were combined for mapping leaf rust resistance and non-glaucousness locus of *Ae. tauschii*. *LrT* was linked to *IwT* at a recombination distance of 3.4 cM. *LrT* was flanked by *Xbarc124* and *Xcau96* at 0.6 and 1.6 cM, respectively, and *IwT* was flanked by *Xgdm35* and *Xte6* at 4.1 and 2.5 cM, respectively with gene marker order as *Xgdm5-Xgdm35-IwT-Xte6-Xbarc124-LrT-Xcau96-Xcfd51* generating a partial linkage map of 18.4 cM (Figure 3).

Comparative analysis of markers mapped on present map with already published maps (Singh *et al.*, 2004; Somers *et al.*, 2004; Sourdille *et al.*, 2004; Liu *et al.*, 2007; Sun *et al.*, 2009) showed similarity in positions of markers though some rearrangements were also found. This indicated *LrT* is introgressed on chromosome 2DS. Linked SSR markers *Xgdm5, Xgdm35, Xbarc124* and *Xcfd51*

Table 2. Segregation of leaf rust resistance and glaucousness in the BC_3F_2 population derived from the cross BC_3F_1 PBW114/*Ae. tauschii* acc. pau14195//4*WH542

	Glaucousness			
Leaf rust reaction	Non-glaucous[a]	Glaucous	Total	$\chi^2_{(3:1)}$ leaf rust resistance
R (IT;)	209 (*LrT_IwT--*)	6 (*LrT_iwTiwT*)	215	0.41
S (IT 3+)	7 (*lrTlrTIwT---*)	71 (*lrTlrTiwTiwT*)	78	
Total	216	77	293	
$\chi^2_{(3:1)}$ glaucousness	0.25			

[a]Expected genotypes are given in parenthesis.

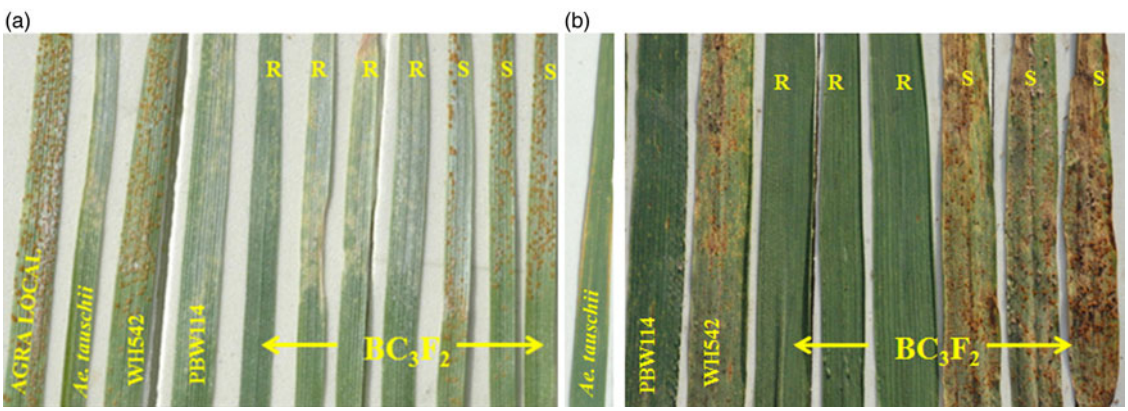

Fig. 2. Leaf rust reaction of the parental lines and BC_3F_2 population at seedling stage (A) and adult plant stage (B) with Agra local as susceptible check. R, Resistant; S, Susceptible.

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Table 3. The χ^2 analysis of leaf rust resistance and non-glaucousness segregation in BC_3F_3 families

	No. of families			
Genotype	*LrT LrT*	*LrT Lrt*	*LrtLrt*	Total
IwT IwT	67	13	–	80
IwT Iwt	2	124	8	134
Iwt Iwt	1	6	67	74
Total	70	143	75	288

$\chi^2_{(1:2:1)}$ (*LrT* versus *Lrt*) = 0.18, $P > 0.05$, $\chi^2_{(1:2:1)}$ (*IwT* versus *Iwt*) = 1.65, $P > 0.05$, $\chi^2_{(1:2:1:2:4:2:1:2:1)}$ (*LrT* versus *IwT*) = 431.9, $P < 0.0001$.

were verified for their chromosomal location using Chinese Spring nullisomic-tetrasomic lines for homoeologous group two chromosome (N2AT2D, N2BT2A, N2BT2D, N2DT2A and N2DT2B). SSR marker *Xgdm35* found to be present on chromosome 2D only with no allele amplifying on chromosome 2A and 2B. Markers *Xcfd51, Xbarc124* and *Xgdm5* amplified alleles from all the three homeologous chromosomes, i.e. 2A, 2B and 2D (Figure S2). Segregating alleles mapped in the population, were D-genome specific supporting the evidence that the genes in question are located on wheat chromosome 2D. Moreover, *Xcfd51* and *Xbarc124* have been physically mapped in the distal region of short arm of chromosome 2D (deletion bin 0.47–1.00) (http://wheat.pw.usda.gov/ggpages/SSRclub/GeneticPhysical/groupe2v2.xls) (Sourdille *et al.*, 2004). These results confirmed the location of *LrT* and *IwT* on chromosome 2DS.

Discussion

This study identified and characterized a leaf rust resistance gene that showed linkage with a morphological marker non-glaucousness introgressed from *Ae. tauschii* into cultivated wheat background. Genetic studies conducted on BC_3F_2 plants and $BC_3F_{2:3}$ progenies revealed monogenic dominant inheritance for leaf rust resistance and non-glaucousness. *T. durum* PBW114 used as bridging species in crosses of *Ae. tauschii* with hexaploid wheat WH542. Since PBW114 was also showed resistance to four leaf rust isolates (77–5, 77–7, 77–8 and 77–10) at SS and mixture of races at APS and but resistance gene identified and mapped in the present study was contributed by *Ae. tauschii* acc. pau14195. PBW114 is susceptible to six of the 10 leaf rust races tested but selected six BC_3F_3 families were homozygous resistant against the nine leaf rust isolates. *Ae. tauschii* accession pau14195 also showed

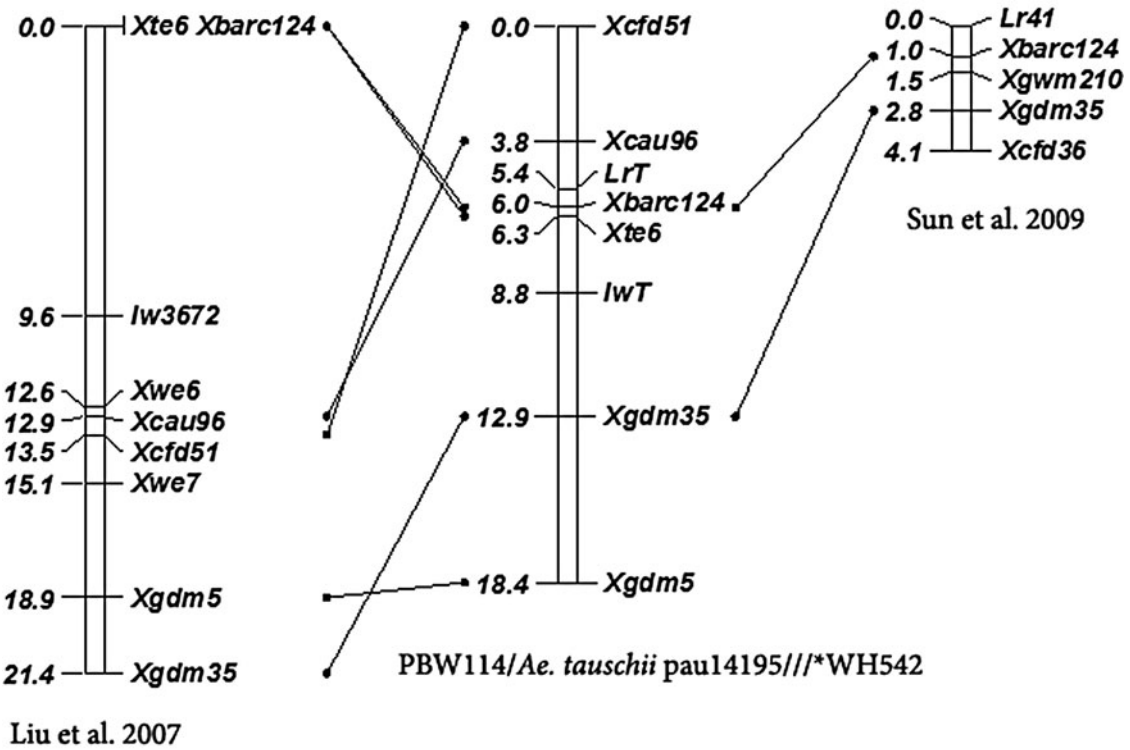

Fig. 3. Partial linkage map of chromosome 2D based on BC_3F_2 population showing leaf rust resistance gene (*LrT*) and non-glaucousness gene (*IwT*) transferred from *Aegilops tauschii* on the short arm of chromosome 2D. Comparison with the maps published by Liu *et al.* (2007) and Sun *et al.* (2009) is also depicted.

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complete resistance against all the races. Difference in infection types of donor *Ae. tauschii* and derived BC_3F_3 families against different isolates as shown in Table 1 could be attributed to their complex background generated during three way crosses. Resistance gene from PBW114 might have been lost inadvertently while selecting for resistance plants from backcrosses. Based on molecular mapping and nullisomic-tetrasomic analysis *LrT* and *IwT* were located on the short arm of chromosome 2D. The linked markers/marker alleles to these two genes were also D-genome specific. One of the linked marker *Xgdm35* did not amplify any allele in lines nullisomic for chromosome 2D, confirming its position on chromosome 2D. Though other markers amplified alleles from all the three homeologous chromosomes but alleles segregating in mapping population were chromosome 2D specific as evident from phenotypic data of six selected BC_3F_3 families against nine leaf rust isolates. Linkage of *LrT* with NG phenotype acted as a morphological marker in the present study further supports the conclusion that leaf rust resistance and non-glaucousness is the result of introgression from *Ae. tauschii* as PBW114 and WH542 are NG and only *Ae. tauschii* has glaucous phenotype. Thus gene mapped in present study on chromosome 2DS is neither a contribution of A or B genome of durum wheat PBW114 used as bridging species.

A dominant inhibitor of glaucousness derived from *Ae. tauschii, Iw2* has been previously mapped on the distal region of chromosome 2DS (Tsunewaki and Ebona, 1999). Liu *et al.* (2007) mapped a dominant non-glaucousness gene *Iw3672* from a synthetic hexaploid wheat in the same region as *Iw2* that showed linkage with SSR markers *Xbarc124* and *Xte6* (0.9 cM) and *Xwe6* (1.4 cM) on chromosome 2DS and concluded that *Iw3672* and *Iw2* were the same. In the present study, *IwT* mapping at a similar location with *Xte6* as the closest marker, indicates that *IwT* and *Iw2* could be allelic or the same genes.

Chromosome 2DS of wheat has many other catalogued leaf rust resistance genes such as *Lr2, Lr22a* and *Lr39. Lr2* is close to the centromere (McIntosh, 1998) and is not effective against Indian races. *LrT* is different from *Lr2* as it has been mapped at the distal region of chromosome 2DS and is resistant to prevalent races in the Indian Subcontinent. There are two alleles of *Lr22* (*Lr22a* and *Lr22b*) and both of these confer adult plant resistance. In contrast, *LrT* exhibited leaf rust resistance at both seedling and APS. *Lr39* a seedling resistance gene has been mapped on 2DS and is closely linked to *Xbarc124* (Singh *et al.*, 2004; Sun *et al.*, 2009). *LrT* being mapped in the same region can be an allele of *Lr39*.

Marker order *Xcfd51-Xcau96-LrT-Xbarc124-Xte6-IwT-Xgdm35-Xgdm5* on 2DS, however, showed an inversion in the present map in comparison to the map of Liu *et al.* (2007) used for mapping of *Iw3672*, Sun *et al.* (2009) for *Lr41* (Figure 3) and consensus map of Somers *et al.* (2004). *LrT* was distal to *IwT* at a recombination distance of 3.4 cM. Marker *Xbarc124* mapped at 0.6 cM from *LrT*, can be used for marker assisted mobilization of this gene to other wheat backgrounds.

Because of evolution of virulent leaf rust races, single disease resistance genes become ineffective rapidly. Therefore, pyramiding of various all-stage resistance genes with APR genes can be a more effective breeding strategy to achieve durable resistance. As there is no reported virulence against *LrT* in India, it is a good candidate gene for use in breeding programmes. It can be pyramided with other leaf rust resistance genes to achieve durable control of leaf rust. We have identified closely linked flanking markers for *LrT* that can aid in transfer of this gene to other wheat backgrounds through marker assisted selection for wheat germplasm improvement. Owing to ease of selection, linkage with an easily scorable morphological marker can improve selection efficiency in the breeding programmes. Leaf tip necrosis has been utilized as phenotypic marker for selection of progenies carrying *Lr34/Yr18* and *Lr46/Yr29* (Singh, 1992; Rosewarne *et al.*, 2006; Shah *et al.*, 2011). Lines carrying *LrT* with and without non-glaucousness can be used in the breeding programmes to transfer this gene to elite wheat backgrounds using selection based on molecular and/or morphological markers.

Supplementary Material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262116000460

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Conflict of Interests

None.

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