RESEARCH ARTICLE

Mapping of stripe rust resistance gene in an *Aegilops caudata* introgression line in wheat and its genetic association with leaf rust resistance

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Abstract

A pair of stripe rust and leaf rust resistance genes was introgressed from *Aegilops caudata*, a nonprogenitor diploid species with the CC genome, to cultivated wheat. Inheritance and genetic mapping of stripe rust resistance gene in backcross-recombinant inbred line (BC-RIL) population derived from the cross of a wheat–*Ae. caudata* introgression line (IL) T291-2(pau16060) with wheat cv. PBW343 is reported here. Segregation of BC-RILs for stripe rust resistance depicted a single major gene conditioning adult plant resistance (APR) with stripe rust reaction varying from TR-20MS in resistant RILs signifying the presence of some minor genes as well. Genetic association with leaf rust resistance revealed that two genes are located at a recombination distance of 13%. IL T291-2 had earlier been reported to carry introgressions on wheat chromosomes 2D, 3D, 4D, 5D, 6D and 7D. Genetic mapping indicated the introgression of stripe rust resistance gene on wheat chromosome 5DS in the region carrying leaf rust resistance gene *LrAc*, but as an independent introgression. Simple sequence repeat (SSR) and sequence-tagged site (STS) markers designed from the survey sequence data of 5DS enriched the target region harbouring stripe and leaf rust resistance genes. Stripe rust resistance locus, temporarily designated as *YrAc*, mapped at the distal most end of 5DS linked with a group of four colocated SSRs and two resistance gene analogue (RGA)-STS markers at a distance of 5.3 cM. *LrAc* mapped at a distance of 9.0 cM from the *YrAc* and at 2.8 cM from RGA-STS marker *Ta5DS_2737450*, *YrAc* and *LrAc* appear to be the candidate genes for marker-assisted enrichment of the wheat gene pool for rust resistance.

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Introduction

Stripe rust (yellow rust) caused by *Puccinia striiformis* Westend f. sp. *tritici* (Pst) is a serious disease of wheat in cooler climate (2–15°C). Stripe rust reduces yield and quality of grain as seeds produced from the crop damaged by the stripe rust have low vigour and exhibit poor emergence after germination. The frequent use of limited parental genotypes in the modern wheat breeding practices and monoculture of few improved wheat varieties have resulted in narrow genetic base in the cultivated genepool. Limited variability in the host and emergence of new pathotypes render the varietal spectrum inadequate to combat the infection in almost all the wheat growing regions of the world. It also necessitates the availability of a battery of new genes in adapted backgrounds for breeding cultivars with new effective R genes.

Based on specificity, rust resistance can be classified as race-specific or race-nonspecific; and based on plant growth stage, it can be classified as seedling (also known as all-stage) resistance or adult plant resistance (APR) (Chen 2005, 2013). Seedling resistance is usually race-specific, but often provides complete control against avirulent races. Adult plant (AP) or postseedling resistance is often nonrace-specific. It is known to be ineffective during seedling stage, but effectiveness increases with plant age. Among the designated stripe rust resistance genes *Yr11*, *Yr12*, *Yr13*, *Yr14*, *Yr16*, *Yr18*, *Yr29*, *Yr30*, *Yr34*, *Yr36*, *Yr39*, *Yr46*, *Yr48* and *Yr52* confer APR, whereas the others confer all-stage resistance (Xu *et al.* 2013). Some slow rusting genes for stripe rust have also been catalogued and over 140 quantitative trait loci (QTL) for resistance to stripe rust in wheat have been published (Rosewarne *et al.* 2013), but it is likely that many of these QTLs are identical.

The wild progenitor and nonprogenitor species of cultivated wheat are a valuable source of additional resistance

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genes (Jiang *et al.* 1994; Chhuneja *et al.* 2016). Species belonging to the genus *Aegilops* are important sources of genetic diversity for improving the variability of cultivated bread wheat. *Aegilops caudata* L. (= *Ae. markgrafii* = *T. dichasians*) is a diploid wild relative of wheat ($2n = 2x = 14$, genome CC). It carries resistance genes to powdery mildew, leaf and stripe rust, and also has high crude protein and lysine content (Valkoun *et al.* 1985). It is reported to be one of the most resistant representatives in the group of less closely-related species with homeologous genomes from which no rust resistance genes have been transferred to wheat (McIntosh *et al.* 2013). Riar *et al.* (2012) mapped a leaf rust resistance gene *LrAc* in the $F_{2:3}$ mapping population derived from one of the wheat–*Ae. caudata* accession pau3556 introgression lines on wheat chromosome 5DS. The present study was conducted to characterize and map a stripe rust resistance gene introgressed from same *Ae. caudata* acc. pau3556 into bread wheat and to study its genetic association with the leaf rust resistance gene *LrAc*.

Material and methods

Plant material and disease evaluation

A backcross-recombinant inbred line (BC-RIL) population derived from a cross of leaf rust and stripe rust resistant wheat–*Ae. caudata* introgression line, T291-2 (pau16060) with a susceptible wheat cv. PBW343, consisting of 354 RILs was used. The development of the introgression line and F_2 mapping population are described in Riar *et al.* (2012). The introgression line T291-2 was susceptible at the seedling stage to the two most prevalent Pst pathotypes 78S84 and 46S119 of Indian subcontinent, but showed a moderate resistance reaction of 20MR at the adult plant stage against a mixture of these Pst pathotypes. The RIL population was evaluated for stripe rust at the adult plant stage against a mixture of Pst isolates for two crop seasons 2013–2014 and 2014–2015. The population was planted at 1.5 m row with a row to row distance of 20 cm and recommended agronomic practices were followed for raising the crop (Anonymous 2013). Infector rows of the susceptible cultivars, WL711 and PBW343, were planted all around the experimental plot. Artificial rust epidemic was created by spraying the infector rows and experimental material with the mixture of uredinospores of Pst isolates 78S84 and 46S119. Stripe rust assessment was according to the modified Cobb's scale (Peterson *et al.* 1948). The RIL population was screened at the seedling stage against leaf rust pathotype 77-5 as described by Riar *et al.* (2012).

Molecular marker analysis

Genomic DNA was extracted using the CTAB method of Saghai-Maroof *et al.* (1984). Resistant and susceptible bulks comprising equal amounts of DNA from 10 resistant (phenotypic reaction of TR-5MR) and 10 susceptible (phenotypic reaction of 60S-80S) RILs were used for bulked segregant analysis (Michelmore *et al.* 1991). Riar *et al.* (2012) had developed an introgression profile of the donor introgression line T291-2 using whole genome SSR scan and reported *Ae. caudata* specific introgression on chromosomes 2D, 3D, 4D, 6D, 5D and 7D. All the introgressed markers including those linked to *LrAc* on 5DS were analysed in bulked segregant analysis. In addition, markers reported to be mapped on short arm of homeologous group 5A and 5B chromosomes were also genotyped on the bulks to find marker(s) cosegregating/linked with the target gene.

Polymerase chain reactions (PCR) were performed in Eppendorf and Applied Biosystems master cyclers. For SSR analysis, a 20 μL of PCR reaction mixture contained 40–50 ng genomic DNA, 4 μL of 5× PCR buffer, 1.2 μL of 25 mM $MgCl_2$, 3 μL of 1 mM dNTP mix, 1.5 μL each of 5 μM forward and reverse primers, 0.2 μL of 1U *Taq* polymerase. The PCR products were resolved on 6% polyacrylamide gels. For STS markers, a 20 μL of reaction mixture contained 80–100 ng genomic DNA, 4 μL of 5× PCR buffer, 1.5 μL of 25 mM $MgCl_2$, 6 μL of 1 mM dNTP mix, 1.5 μL each of 5 μM forward and reverse primers and 0.2 μL of 1U *Taq* polymerase. PCR conditions for SSR and RGA-STS primers were same as reported by Chhuneja *et al.* (2015).

Development of SSR and STS markers from wheat survey sequence data

Survey sequence data of wheat chromosome 5DS was obtained from International Wheat Genome Sequence Consortium (IWGSC 2014). Transcript and protein data files of *Brachypodium distachyon* was also retrieved. Using a tool BLASTN, wheat 5DS survey sequence was BLAST against transcript data of *Brachypodium* to obtain gene containing contigs of wheat. The new SSR primers were designed from the genic contigs of short arm of 5DS using primer designing tool PerlPrimer from the sequences containing at least 10 mononucleotide or dinucleotide repeats and six tri, tetra, penta and hexa nucleotide repeats were selected.

For designing STS primers specific for nucleotide-binding site and leucine-rich repeats (NBS-LRR) genes on wheat chromosome 5DS, NBS-LRR protein sequences were fetched from *B. distachyon* protein file and BLAST searched against 5DS survey sequence. Wheat contigs containing NBS-LRR sequences were annotated to locate the positions of NBS-LRR encoding sequences using FGENESH (Solovyev *et al.* 2006). After getting protein and mRNA sequences of the genes, simple modular architecture research tool (SMART), an online tool for the identification and annotation of protein domains and the analysis of protein architectures (Letunic *et al.* 2012), was applied on protein sequence file with a default parameter of amino acid length of $\geq$30. The results were confirmed with the help of another online tool LRRfinder (http://www.lrrfinder.com/). RGA-STS primers were then designed from LRR regions of wheat sequence using PerlPrimer.

Genetic mapping

Mapping was conducted using MapDisto 1.7.5.1 (Lorieux 2012). A LOD score of 4.0 and recombination fraction 0.2 was used for preparing the linkage map. RILs with stripe rust reaction from 0 to 20MS were classified as resistant and those with more than 20MS as susceptible. Markers and genes were grouped and ordered using 'find groups' and 'order sequence' command, respectively. Order of markers was refined using 'ripple order' and 'check inversions' commands. The robustness of the marker order was evaluated using boostrap order with 1000 trials. The map was drawn using MAPCHART program ver. 2.1 developed by Voorrips (2002).

Results

Inheritance studies

Donor introgression line was susceptible at the seedling stage, but moderately resistant at the adult plant stage implying that it carries APR gene(s) for stripe rust. The stripe rust reaction in the T291-2/PBW343 BC-RILs varied from TR to 80S in both the crop seasons (figure 1a) with overall 184 resistant (0–20MS) and 161 susceptible (>20MS) RILs with a $\chi^{2\,(1:1)} = 1.53$ conforming to a single gene segregation ratio. The segregating RILs were not taken into consideration for calculating the χ^2. Figure 1b exhibits stripe rust reaction of the resistant and susceptible RILs at the adult plant stage. The stripe rust resistance gene from IL T291-2 was tentatively designated as *YrAc*. For leaf rust resistance, 182/349 BC-RILs exhibited a hypersensitive resistant reaction with infection type ; to 1 and 159/349 were susceptible and eight BC-RILs were segregating showing goodness of fit to a single gene ratio which was consistent with the results reported by Riar *et al.* (2012) based on $F_{2:3}$ population.

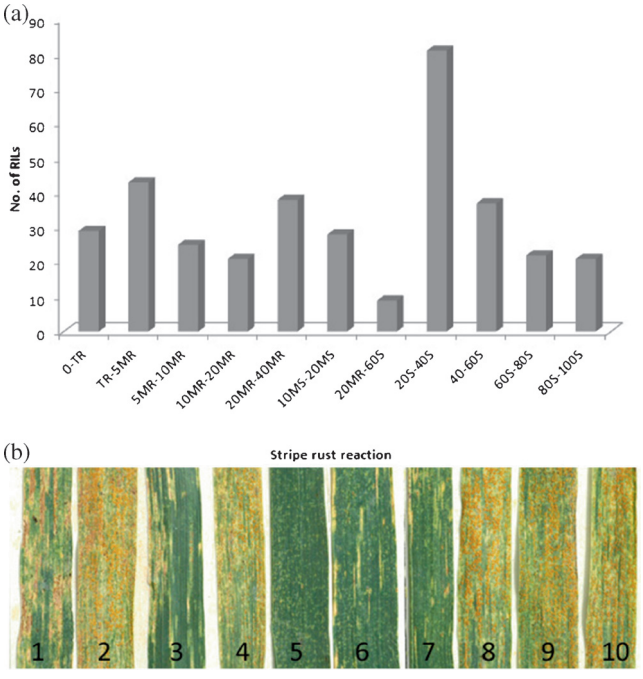

Figure 1. (a) Frequency distribution of T291-2/PBW343 RIL population for stripe rust reaction in the field conditions under artificial epiphytotic conditions. (b) Stripe rust reaction of the parental lines, resistant and susceptible RILs at the adult plant stage. 1. WH868; 2. WL711; 3. IL T291-2; 4. PBW343; 5–7. resistant RILs and 8–10. susceptible RILs.

For deciphering the linkage between stripe rust resistance gene *YrAc* and leaf rust resistance gene *LrAc* (Riar *et al.* 2012), BC-RILs were classified into various groups based on the phenotypic reaction to leaf rust and stripe rust (table 1). A 13.3% recombination distance was observed between *LrAc* and *YrAc* taking into account only the homozygous RILs.

Molecular mapping of stripe rust resistance

The SSR markers exhibiting *Ae. caudata* specific introgression in T291-2 were analysed on the resistant and susceptible bulks (RB and SB) pooled from stripe-rust resistant and susceptible RILs. SSR markers from the introgressed segments on chromosome 5DS only gave diagnostic polymorphism between R and S bulks. The leaf rust resistance gene *LrAc* introgressed from *Ae. caudata* was mapped on short arm of wheat chromosome 5DS. Of a total of 75 SSR markers from homeologous group 5 amplified on the bulks, only six (*Xgwm205*, *Xcfa2104*, *Xgwm190*, *Xcfd81*, *Xbarc130* and *Xgwm234*) gave diagnostic polymorphism between R and S bulks. A pair of colocated leaf rust and stripe rust resistance genes *Lr57* and *Yr40* transferred from M genome of *Ae. geniculata* has also been mapped on 5DS (Kuraparthy *et al.* 2007). An STS marker *Lr57-Yr40_caps16* linked to *Lr57-Yr40*, showed polymorphism between R and S bulks for stripe rust also. Analysis of six informative SSR markers and *Lr57-Yr40_caps16* on the whole RIL population mapped *YrAc* at distal end of 5DS with *Xgwm190* as the nearest marker and *LrAc* at a distance of 10.0 cM with *Xgwm234* as the nearest marker.

To further enrich the 5DS region carrying *YrAc* and *LrAc*, 184 SSR markers designed from the survey sequences of 5DS were used for BSA and only 14 SSR markers were found polymorphic between the resistant and susceptible bulks. Three RGA-STS markers, two from 5DS contig *Ta5DS_2737450* and one from *Ta5DS_2759608*, were also found to be polymorphic on bulks. The primer sequences and

Table 1. Cosegregation of leaf rust and stripe rust resistance in IL T291-2/PBW343 RIL population.

		Stripe rust			
Rust reaction		R	H	S	Total
Leaf rust	R	158	4	20	182
	H	1	4	3	8
	S	25	1	133	159
Total		184	9	156	349

R, resistant; S, susceptible; H, heterozygous.

Table 2. The polymorphic SSR and RGA_STS markers named after contig IDs, SSR motif, forward and reverse primer sequences, annealing temperature and amplicon size.

	Contig ID	Motif	Forward primer	Reverse primer	Annealing temp. (°C)	Amplicon size (bp)
SSR markers						
1	*Ta5DS_2774886**	$(AAG)_{21}$	TGACCCCTCTAGTGCTTGGT	GCTGTCCGTGACTATCCTCC	52	243
2	*Ta5DS_1270508*	$(ACA)_{14}$	TGGGAGGGAGAGTTTGTCTG	CCCCAACTTGTTTGGGACTA	50	192
3	*Ta5DS_2736874*	$(TTG)_{10}$	TCAGCTCTAGCCTACCCCAA	GGCTGACGACAAGAGGCTAC	52	208
4	*Ta5DS_2764692*	$(AAC)_8$	CCCCCCTCTGAACATCTTGAA	CTTGCTATCCATGTGCCAGA	50	255
5	*Ta5DS_2755465*	$(TTG)_7$	TTTTGCCGTATTGGAGAAGG	TTTGCACGTTACTCTGGCTG	49	102
6	*Ta5DS_2736800*	$(GGC)_6$	GCGTCGCTGAGGATAGAGAC	TCCTGTAAAACGCAGTCGTG	51	176
7	*Ta5DS_2744525*	$(CCG)_6$	CCCCTATTTCTCACTCCCGT	CGTAGTACCTTTGCCGAGGA	52	231
8	*Ta5DS_2744983*	$(CAA)_6$	TGTTGCTGTCTGTTCTTGCC	GGCGAGCCAATGTAAAACTC	52	191
9	*Ta5DS_2757949*	$(CGG)_6$	CTCTCGACCCAAATCCTCAC	AACCTTGTACGGACGTGGAG	53	140
10	*Ta5DS_2764999*	$(TTG)_6$	CTTCTTGAAAGTTTGCCCCA	TTCTTTAATTGGTGTGCCCC	49	221
11	*Ta5DS_515626*	$(AACA)_6$	CATGAAAGTCGAGCAGCAGA	GCGGTCTGTTTATTTACCCG	50	240
12	*Ta5DS_2683050*	$(ATGA)_6$	GCTCATCATCGATCCTCCAT	GAATAATCACGGACGGTTGG	50	133
13	*Ta5DS_2735414*	$(GAAG)_6$	AGGATCGGAACCGAGAAGAT	GCTCTGAGTTTTGGACTGGC	50	132
14	*Ta5DS_2736392*	$(CAAA)_8$	TGGTACAAGAGACCAGCAACA	TACTGCCGCATCGATTGTAG	50	180
RGA_STS						
15	*Ta5DS_2737450*	–	CGATTATTCTGTTAGTCTGGCAC	TGTAGGTTTGAATCCTGTGGT	48	637
16	*Ta5DS_2737450a*	–	CATGAGAAGATACAAAGTGTGGCAG	GAAGGAGACAGGTTCAGTTTCAG	52	508
17	*Ta5DS_2759608*	–	CACTTTGTGAGCCTGGTTGGA	CTTTCTCAACTGCGTCAACTC	50	562

*Primer names were given same as the contig names from which these were designed.

PCR conditions for the 14 markers mapped on 5DS are given in table 2.

A consensus data derived from the individual year's stripe rust and leaf rust data for 2013–2014 and 2014–2015 was used for mapping stripe rust resistance gene *YrAc* as a major gene. Stripe rust resistance gene *YrAc* mapped at the distal most end of 5DS linked with five colocated SSR markers, *Ta5DS_2736392*, *Ta5DS_2744983*, *Ta5DS_2744525*, *Ta5DS_2683050*, *Xgwm190* and two RGA-STS markers *Ta5DS_2737450a* and *Ta5DS_2759608* at a distance of 5.3 cM (figure 2). Leaf rust resistance gene *LrAc*, mapped at the other end of the 5DS segment with *Ta5DS_2737450* as the closest marker at a distance of 2.8 cM. *LrAc* and *YrAc* were thus found to be linked with a recombination distance of 9.0 cM. To determine orientation of the 5DS segment carrying *YrAc* and *LrAc*, the comparison was made with

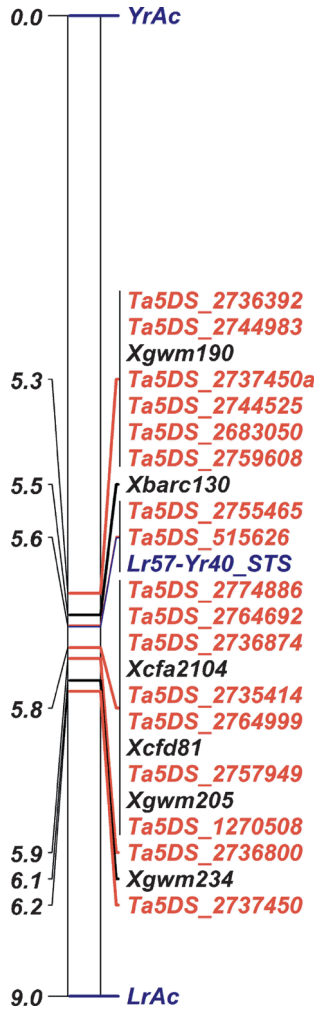

Figure 2. Partial linkage map of recombinant chromosome 5DS carrying an *Ae. caudata* introgression with stripe rust and leaf rust resistance genes *YrAc* and *LrAc*, respectively. Markers in black are the SSR markers from the map of Somers *et al.* (2004) and those in red are new SSR and STS markers designed from survey sequence data of 5DS. *YrAc* and *LrAc* mapped at a distance of 9.0 cM. Blue indicate the *LrAc* and *YrAc* genes and the STS markers linked to rust resistance genes *Lr57* and *Yr40*.

the consensus map of Somers *et al.* (2004) which showed that *YrAc* is at the distal end of 5DS and *LrAc* is proximal. However, no markers distal to *YrAc* and *LrAc* could be identified.

Discussion

Two pairs of linked leaf rust and stripe rust all stage resistance genes (seedling resistance genes) *Lr57-Yr40* and *Lr76-Yr70*, introgressed from *Ae. geniculata* (Kuraparthy *et al.* 2007) and *Ae. umbellulata* (Bansal *et al.* 2016), respectively, were mapped at the distal end of 5DS. *YrAc* characterized and mapped during present investigation is supposedly new gene as *YrAc* showed susceptibility to Pst isolates 78S84 and 46S119 at the seedling stage, while *Yr40* and *Yr70* both depicted seedling resistance for these two Pst isolates. However, it is not feasible to determine novelty of *LrAc* regarding *Lr57* and *Lr76* as all three genes show complete resistance against the leaf rust races prevalent in India.

Ae. caudata introgression, being homeologous to its wheat counterparts, was not expected to recombine but *YrAc*, *LrAc* and the markers mapped on 5DS showed recombination albeit at much lower frequency. According to consensus map by Somers *et al.* (2004), the SSR markers *Xgwm190*, *Xbarc130*, *Xcfa2104*, *Xcfd81* and *Xgwm205* spanned 28 cM on 5DS. However, in this study, these markers showed a recombination distance of 5.76 cM indicating suppressed recombination between *Ae. caudata* introgression and its wheat counterpart. Recombination between the genes and/or markers on the introgressed segment and wheat chromosomes might be due to the presence of some genes on homeologous group 5, which either partially suppress *Ph1* gene (Sears 1976) or modify its action in some way. Although there is no direct evidence, but some other studies also reported spontaneous recombination leading to gene transfers from 5M chromosome of *Ae. geniculata* (Liu *et al.* 2011).

The *APR* genes have been known to provide moderate level of resistance, which is more desirable as it puts less selection pressure on the pathogen. The donor introgression line T291-2 showed stripe rust response of 20MR but some of the RILs depicted much higher level of resistance indicating that some minor QTL from the recipient parent or the bridging parents might also be segregating in the population and as a whole population showed a quantitative type of resistance. RILs showing transgressive segregation will be used to develop a subpopulation for the genetic dissection of the other resistance loci.

Identification of markers closely linked with disease resistance has progressed in the last decade through the development of high-throughput and cost-effective genotyping facilities. Advances in wheat genome sequencing and NGS technologies have led to immense data which can be used to develop markers for wheat (Varshney *et al.* 2014; Jordan *et al.* 2015). All these technologies individually or in combination can be used to fine map the genes of interest. In the present investigation, the target region was enriched using survey sequence data of 5DS chromosome of Chinese Spring (IWGSC 2014). SSR markers designed from the contigs carrying genic sequences and mapped on 5DS, indicate the location of potential genes. Mapping of three RGA_STS markers designed from NBS-LRR genes identified the putative disease-resistance gene loci present on 5DS. *APR* gene for stripe rust resistance *YrAc* identified in the present study is a putatively new gene as per the published evidence, no other stripe rust resistance gene has been transferred from the C genome of *Ae. caudata* to bread wheat.

SSR and STS markers linked to *LrAc* and *YrAc* genes are suitable for marker-assisted transfer of the *Ae. caudata* genes to elite wheat backgrounds. Selected RILs will be used as donor for marker-assisted mobilization of *LrAc* and *YrAc* to advance breeding lines.

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