Theor Appl Genet (2014) 127:947–955
DOI 10.1007/s00122-014-2270-6

ORIGINAL PAPER

Identification of a robust molecular marker for the detection of the stem rust resistance gene *Sr45* in common wheat

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Received: 26 September 2013 / Accepted: 12 January 2014 / Published online: 28 January 2014

Abstract
Key message **Fine mapping of the Ug99 effective stem rust resistance gene *Sr45* introgressed into common wheat from the D-genome goatgrass *Aegilops tauschii.***
Abstract Stem rust resistance gene *Sr45*, discovered in *Aegilops tauschii*, the progenitor of the D-genome of wheat, is effective against commercially important *Puccinia graminis* f. sp. *tritici* races prevalent in Australia, South Africa and the Ug99 race group. A synthetic hexaploid wheat (RL5406) generated by crossing *Ae. tauschii* accession RL5289 (carrying *Sr45* and the leaf rust resistance gene *Lr21*) with a tetraploid experimental line 'TetraCanthatch' was previously used as the source in the transfer of these rust resistance genes to other hexaploid cultivars. Previous genetic studies on hexaploid wheats mapped *Sr45* on the short arm of chromosome 1D with the following gene order: centromere–*Sr45*–*Sr33*–*Lr21*–telomere. To identify closely linked markers, we fine mapped the *Sr45* region in a large mapping population generated by crossing CS1D5406 (disomic substitution line with chromosome 1D of RL5406 substituted for Chinese Spring 1D) with Chinese Spring. Closely linked markers based on 1DS-specific microsatellites, expressed sequence tags and AFLP were useful in the delineation of the *Sr45* region. Sequences from an AFLP marker amplified a fragment that was linked with *Sr45* at a distance of 0.39 cM. The fragment was located in a bacterial artificial chromosome clone of contig (ctg)2981 of the *Ae. tauschii* accession AL8/78 physical map. A PCR marker derived from clone MI221O11 of ctg2981 amplified 1DS-specific sequence that harboured an 18-bp indel polymorphism that specifically tagged the *Sr45* carrying haplotype. This new *Sr45* marker can be combined with a previously reported marker for *Lr21*, which will facilitate selecting *Sr45* and *Lr21* in breeding populations.

Communicated by F. Ordon.

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Introduction

Aegilops tauschii Coss., the D-genome progenitor of *Triticum aestivum* L. (AABBDD), is a valuable resource for agronomically important traits such as tolerance to cold, salinity, drought, and pest and disease resistance (Gill et al. 1986; Friesen et al. 2008; Halloran et al. 2008). This diploid species is also the donor of stem rust resistance gene *Sr45* effective against diverse *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn (*Pgt*) races including the race group Ug99 (Singh et al. 2011). Because meiotic recombination between the *Ae. tauschii* (DD) chromosomes and *T. aestivum* D-genome chromosomes is nearly normal, these traits are easily transferable from *Ae. tauschii* into *T. aestivum* either through synthetic hexaploids generated from hybridisation between *T. turgidum* (AABB) and *Ae. tauschii* (Ogbonnaya et al. 2005) or through direct crossing with *T. aestivum* followed by backcrossing (Gill and Raupp 1987).

Ae. tauschii accession RL5289, the source of *Sr45*, was initially used as the source of leaf rust resistance gene *Lr21* (Kerber and Dyck 1969) that still remains effective against most *Puccinia triticina* Erikss. (*Pt*) races, except two north

American races TFBJQ and TFBGQ (Kolmer and Anderson 2011). Previous reports of susceptibility of RL5289 and the synthetic hexaploid RL5406 [generated by crossing RL5289 with the experimental tetraploid line 'TetraCanthatch'(Kerber 1964)] to Canadian *Pgt* races (Kerber and Dyck 1969) limited the use of this synthetic hexaploid for stem rust resistance studies until Marais et al. (1994) showed effectiveness of *Sr45* against South African *Pgt* races. In addition, RL5406 was also effective against commercially important Australian *Pgt* races and found to show race specificities similar to those of *Sr21,* a stem rust resistance gene introgressed into common wheat from *T. monococcum* (McIntosh 2009). Earlier genetic analysis using common wheat genotypes 87M66-2-1 (carrying *Sr45*) and Condor-*Sr33* (carrying *Sr33*—another stem rust resistance gene derived from *Ae. tauschii*) mapped *Sr45* on the short arm of chromosome 1D and linked in repulsion to *Sr33* at a distance of 10 map units; the predicted gene order was: centromere–*Sr45*–*Sr33*–*Lr21*–telomere (Kerber 1987; Marais et al. 1998).

Ug99 continues to pose threat to global wheat crop due to its virulence to majority of commonly used stem rust resistance genes including *Sr31*. There is a continued need for the deployment of widely effective combinations of genes in new cultivars to avoid production losses in the event of Ug99 spreading to major wheat growing zones. Combining multiple resistance genes can be more efficient if molecular markers tightly linked to target genes are available. To facilitate pyramiding of *Sr45*, here we exploit the available genomic resources [colinearity of wheat genome with other *Triticum* species and rice, barley, *Brachypodium* and *Sorghum* grasses (Guyot et al. 2004; Keller et al. 2005; Kota et al. 2006; Lagudah et al. 2006; Reddy et al. 2008; Zhang et al. 2010) and the physical map of *Ae. tauschii* accession AL8/78 (Luo et al. 2013)] to construct a linkage map and to develop markers linked to this stem rust resistance gene.

Materials and methods

Plant material

For initial screening of SSR markers, recombinant inbred line (RIL) populations derived from CS1D5406 (Chinese Spring 1D substitution carrying *Sr45*) × Chinese Spring (CS) and DT1D5406 (ditelosomic 1D substitution carrying *Sr45*) × CS comprising 46 and 97 lines, respectively, were used (Jones et al. 1990). The parental disomic substitution line CS1D5406 was crossed with CS to generate a high-resolution mapping population of 1,150 F_2 seeds. Backcross derivatives of hexaploid wheat with variable length of introgressed *Ae. tauschii* chromosome 1D segments carrying *Sr45* and *Lr21* were used as validation material (Table 1). Since *Sr45* and *Sr21* shared pathotypic specificity, backcross derivatives carrying *Sr21* in different genetic backgrounds were also used for the validation of the *Sr45* linked markers. CS1D5406 and *Ae. tauschii* accession AUS18911 (carrying *Lr21* and *Sr45*) were used as controls.

Rust screening

Parental lines, individuals of the two RIL populations (CS1D5406/CS and DT1D5406/CS) and F_3 families of the recombinants identified in the high-resolution mapping family were screened in the seedling stage using the Australian *Pgt* race 34-1,2,3,4,5,6,7,11 (PBI culture no. 171) according to Bariana and McIntosh (1993). *Sr21* and *Sr45* backcross derivatives and their recurrent parents were screened against *Pgt* races 11-1,2,3,4,5,6,7 (169) and 34-1,2,3,4,5,6,7 (103). As the *Sr45* donor accession RL5289 carries *Lr21* on the same chromosome (1D), the hexaploid derivatives were also screened for leaf rust resistance using *Pt* race 104-1,2,3,(6),(7),11,13 (547). Stakman Scale was used in scoring infection types (Stakman et al. 1962). Seedlings showing low infection scores (; to ;1 on their first leaf) typical to the standards carrying *Sr45* (CS1D5406) or *Sr21* (Einkorn C.I.2433) or *Lr21* (CS1D5406) genes were classified as resistant lines while the lines with other score types were grouped to be non-carrier of the targeted genes.

Screening with chromosome 1D-specific SSR markers and wheat ESTs

RILs were screened with 80 SSR markers specific to chromosome 1D (Somers et al. 2004) using the method described by Hayden et al. (2008). Genetic linkage analysis was performed with MAP MANAGER Version QTXb20 (Manly et al. 2001) using the Kosambi map function (Kosambi 1944). SSR markers that flanked *Sr45* in the two RIL populations (CS1D5406/CS and DT1D5406/CS) were used to identify recombinants among the 1,150 F_2 seeds of the high-resolution mapping family (CS1D5406/CS). Genomic DNA was isolated using the half-seed DNA extraction technique described in Kota et al. (2006). The genotype of the selected F_2 recombinants was identified using the rust test on the corresponding F_3 families. Wheat ESTs mapped to the short arm of chromosome 1D (Akhunov et al. 2010) were screened for polymorphism according to Lagudah et al. (2006). Closely linked wESTs were then used to reduce the number of recombinants for the high-resolution map and to search for orthologous regions in rice and *Brachypodium* (http://www.phytozome.net).

Identification of AFLP fragment and *Ae. tauschii* BAC contigs linked with *Sr45*

Closely linked markers were also identified using AFLP analysis (Mago et al. 2002) with 408 primer combinations (derived from 17 *Pst*I and 24 *Mse*I selective amplification

Table 1 Evaluation of *Sr21* and *Sr45* backcross derivatives and their recurrent parents against Australian stem rust (*Pgt*) and leaf rust (*Pt*) races and the molecular markers linked with *Lr21*, *Sr45* and *sr33* haplotype present in AUS18911

Pedigrees	Rust response[a]			Marker score[b]			
	Rust1	Rust2	Rust3	M1	M2	*cssu45*	Classification group
Sr45/3*77W549	0;	;	;	+	+	+	I
Sr45/3*77W549	0;	;	;	+	+	+	I
77W549	0;	2+	2	−	−	−	
Sr45/2*Cocamba	;1	;	;;1−	+	+	+	I
Sr45/2*Cocamba	;1	;	;	+	+	+	I
Sr21/2*Cocamba	33+	33+	11+	−	−	−	
Cocamba	a	a	a	−	−	−	
Sr45/3*ZL27	;	;	;	+	+	+	I
ZL27	a	a	a	−	−	−	
Sr45/4*WW728	;1+	;1−	;1	±	+	+	I or II
WW728	3+	33+	23−	−	−	−	
Sr45/Kulin	;1+	;;1−	;	±	+	+	I or II
Sr45/Kulin	2	;;1−	;1−	−	+	+	II
Sr45/Kulin	23−	;;1−	;1−	−	+	+	II
Sr21/Kulin	2	23−	11−	−	−	−	
Kulin	33+	2+	23−	−	−	−	
Sr45/4*Lowan	23=	;1−	;1−	−	+	+	II
Lowan	23−	33+	33+	−	−	−	
Sr45/4*Kiata	3	;1−	1	−	+	+	II
Kiata	3	33+	23−	−	−	−	
Sr45/3*76W551	3+	;1	;;1−	−	+	+	II
Sr45/3*76W551	3+	;	;1−	−	+	+	II
76W551	a	a	a	−	−	−	
Sr45/4*XL30	3=	;1	11−	−	+	+	II
XL30	3+	2	22+	−	−	−	
Sr45/3*Lark	33+	;;1−	;1−	−	+	+	II
Lark	33+	33+	23−	−	−	−	
Sr45/3*K441	;;1,12	;, 2p2+	;;1=	−	−	+	III
K441	a	a	a	−	−	−	
Sr45/3*K2001	33+	1,2+	;1	−	−	+	III
K2001	3+	3+	3+	−	−	−	
Sr21/4*CO1568	3	2+	1	−	−	−	
CO1568	3	33+	23−	−	−	−	
Sr21/4*WT6/12	33+	33+	1c	−	−	−	
WT6/12	33+	33+	33+	−	−	−	
Sr21/4*Cranbrook	2	22−	1	−	−	−	
Cranbrook	2	22−	2−	−	−	−	
Thornbill	;;1−	;	;	+	+	+	I
CS1D5406	;1	;	;	+	+	+	I
Chinese Spring	3+	3+	3+	−	−	−	
AUS 18911	0;	;	;	+	+	+	I
Thatcher+*Lr21*				+	+	+	I
Thatcher				−	−	−	
Einkorn C.I.2433	;	3C	;	−	−	−	

[a] Rust1-*Pt* race 104-1,2,3,(6),(7),11,13, Rust2- *Pgt* race 11-1,2,3,4,5,6,7 and Rust 3-*Pgt* race 34-1,2,3,4,5,6,7. a—data missing

[b] M1, SCAR marker linked with *Lr21* (Fu et al. 2010); M2, PCR marker linked with *sr33* haplotype present in AUS18911 (unpublished data); +, indicates the marker allele linked with these genes while − indicates the alternate allele. Groups I, II and III are the three groups of backcross derivatives based on the length of *Ae. tauschii* (RL5289) segment introgression on 1DS of wheat

primers). Markers polymorphic between resistant (DNA mixture of 10 homozygous resistant) and susceptible (10 homozygous susceptible) bulks were further mapped on the critical recombinants of the high-resolution mapping family. Linked AFLP fragments were sequenced and used to identify orthologous regions in rice and *Brachypodium* (http://www.phytozome.net). Tightly linked AFLP fragments were also used to screen the BAC library made from the genomic DNA of the D-genome reference *Ae. tauschii* accession AL8/78 (Luo et al. 2003). BAC contigs related to clones harbouring AFLP fragment were identified using the *Ae. tauschii* physical map information (Luo et al. 2013).

Identification of PCR based markers linked with *Sr45* resistance

End-sequences and low-copy fragments from the clones picked at random position of the contigs were isolated using the method described by Lagudah et al. (2006). Using PCR, sequences related to the low-copy fragments were amplified from the two contrasting parents (CS1D5406 and CS), sequenced and compared to identify nucleotide polymorphism. Nucleotide differences present between the two isolated sequences were then targeted to develop PCR-based markers linked with *Sr45* resistance. Tightly linked markers were identified based on the screening of the critical F_2 recombinants of the high-resolution mapping family (CS1D5406/CS). PCR amplification was performed using the method described in Lagudah et al. (2009).

Validation of *Sr45*-linked PCR markers on backcross derivatives

PCR markers tightly linked with *Sr45* were further validated on a set of *Sr45*-carrying backcross derivatives (Table 1). As the short arm of chromosome 1D of *Ae. tauschii* acc. RL5289 harbouring *Sr45* is also the source for *Lr21*; the presence of this later gene in these derivatives was detected using the *Lr21* gene specific marker described in Fu et al. (2010). Since hexaploid lines carrying *Sr21* or *Sr45* gene produce identical resistance response against wider range of *Pgt* races, here backcross derivatives carrying *Sr21* were included as negative control.

Results

Rust phenotype of the mapping population and the backcross derivatives

The *Sr45* carrying parental lines (CS1D5406 and DDT1D5406) and the resistant RILs of the mapping populations produced low infection type (IT); 1−, when tested with *Pgt* race 34-1,2,3,4,5,6,7,11. In contrast, CS and the RILs without *Sr45* showed susceptible response of IT 3+ (Fig. 1). The F_3 families derived from the heterozygous genotypes of the selected F_2 recombinants of the high-resolution mapping population (CS1D5405/CS) had plants exhibiting both infection types (;1− and 3+). The CS1D5406/CS RILs exhibited monogenic segregation for stem rust response [20R (resistant):26S (susceptible), $\chi^2 = 0.783$, $p = 0.37$]. The segregation among DDT1D5406/CS RIL population showed significant deviation (62R:35S, $\chi^2 = 7.515$, $p = 0.0061$) from monogenic segregation at the *Sr45* locus and it was attributed to segregation distortion caused by selection against the telosome during the development of this population. *Sr45* and *Sr21* carrying backcross derivatives produced low stem rust responses (; to ;1), when tested with *Pgt*

Fig. 1 Rust response of **a** CS1D5406 and **b** Chinese Spring against the Australian *Pgt* race 34-1,2,3,4,5,6,7,11

race 34-1,2,3,4,5,6,7 and the recurrent parents showed relatively higher responses (2− to 3+). But with *Pgt* race 11-1,2,3,4,5,6,7, there was no change in the resistant phenotype expressed by derivatives carrying *Sr45*, while the lines with *Sr21* showed higher infections similar to their recurrent parents. Subsequently, in the test against *Pt* race 104-1,2,3,(6),(7),11,13, only the derivatives with large introgressed RL5289 segments carrying both *Sr45* and *Lr21* produced an infection type IT 0; to ;1.

Chromosome 1D-specific SSRs and wESTs linked with *Sr45*

A total of 15 from 80 SSRs markers specific to chromosome 1D were polymorphic between the resistant and susceptible bulks and the parents. These 15 SSR markers were used to genotype the 2 RIL populations (CS1D5406/CS and DDT1D5406/CS). Due to a small size ($n = 46$) of the CS1D5406/CS RIL population, SSR markers linked to *Sr45* clustered, while in DDT1D5406/CS ($n = 97$), markers *gwm106* and *gwm337* flanked the gene. In 421 F_2 seeds in the CS1D5406/CS high-resolution mapping population *gwm106* and *gwm337* were recombined and were used to construct a linkage map of the *Sr45* region. The embryo sections of the 421 seeds were advanced to F_3 and based on the rust test they grouped into three genotypic classes (56R, 283 segregating and 82S).

Wheat ESTs *BE586140, BE405749, BE442682, BE446624, KsuE18, BE499711, BE499070, BE590575, BE500570, BF483372* and *BE444266* from the short arm of chromosome 1D, used as RFLP markers, were polymorphic between CS1D5406 and CS. They were mapped using 12 DDT1D5406/CS RILs showing recombination between *Sr45* and *gwm106* and *gwm337*. EST *BE446624* and marker *KsuE18* cosegregated with *Sr45*, while EST *BE444266* was mapped distally and cosegregated with *gwm106*. To resolve the linkage of *BE446624* and *KsuE18* with *Sr45*, the two markers were mapped using 103 susceptible F_2 plants from the CS1D5406/CS high-resolution mapping population. Two recombinants were obtained for the markers, mapping them proximal to *Sr45*. The EST *BE446624* mapped distal to *KsuE18* in Akhunov et al. (2010) and was therefore used for further mapping. In the high-resolution mapping population, 23 recombinants were identified between *BE446624* and *Sr45*. Markers *BE444266* and *BE446624* identified a syntenic region in chromosome 2 of *Brachypodium*. A total of 7 wheat ESTs (*BJ279892, CJ580198, BE418347, BJ319804, CJ542788, CJ573217* and *CJ534799*) having homology with *Brachypodium* genes present in this syntenic region were found from the wheat genome database (http://www.jcvi.org/euk-blast/index.cgi?project=tae1), but all were monomorphic between resistant and susceptible parents when screened as RFLP markers.

AFLP fragment and the *Ae. tauschii* BAC contig linked with *Sr45*

AFLP analysis using selective amplification primer pair *Pst*I+ATT and *Mse*I+GAA amplified a fragment associated with resistance. Screening of the critical recombinants selected between *BE444266* and *BE446624* mapped the AFLP marker (henceforth *AF45*) proximally to *Sr45* at a distance of

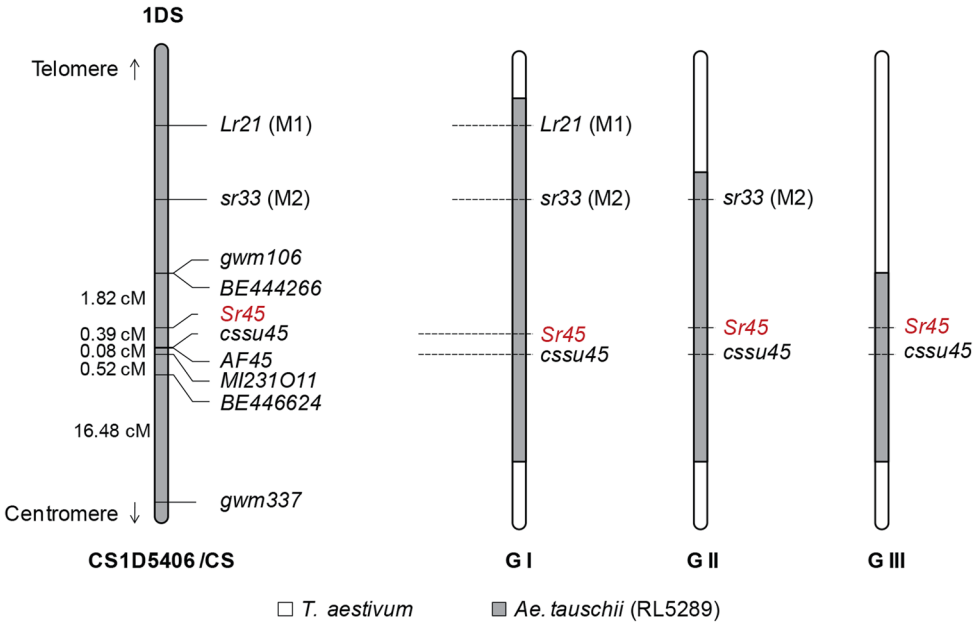

Fig. 2 Genetic linkage map of chromosome 1DS showing *Sr45* and linked markers on CS1D5406/CS high-resolution mapping population. *GI*, *GII* and *GIII* are the three groups of backcross derivatives based on the length of *Ae. tauschii* (RL5289) segment introgression on 1DS of wheat

0.39 cM (Fig. 2). The *AF45* was located by PCR to BAC clone HB079K08 in BAC contig ctg2981 (Luo et al. 2003, 2013).

PCR-based marker from the *Ae. tauschii* BAC sequence linked with *Sr45*

In addition to HB079K08, markers (from BAC clones HI229K10, MI262C21, MI231O11, HI243P06 and MI221O11) derived at the random positions of the contig were targeted for further mapping. Since the BAC end sequences of these clones were highly repetitive, low-copy fragments were therefore identified in sub-clones and used to generate PCR-based markers. A low-copy sequence derived from BAC clone MI221O11 was polymorphic with a 18-bp deletion and mapped at a genetic distance of 0.39 cM to *Sr45* (Fig. 3). Primer *cssu45* (Forward-5′CGAGTTTCAATACTTCGCCC3′+Reverse-5′GATTACTATGCAATAGGGCCC3′) designed to span the deletion (annealing temperature of 60 °C), amplified 220- and 238-bp products in the resistant and susceptible plants, respectively, and both products in the heterozygous plants (Fig. 4).

Validation of *Sr45* linked marker and the three genotypic classes

PCR marker *cssu45* detected the presence of *Sr45* in all backcross derivatives used in this study (Table 1). In contrast, the marker amplified the 238-bp fragment in all of the *Sr21* carrying lines. To estimate the size of the *Ae. tauschii* chromosome 1D introgressed from RL5289, these hexaploid derivatives were also screened with markers specific to *Lr21* (Fu et al. 2010) and to the *Sr33* locus which detected a haplotype (haplotype-V) homologous with *sr33* present in the *Ae. tauschii* accessions carrying

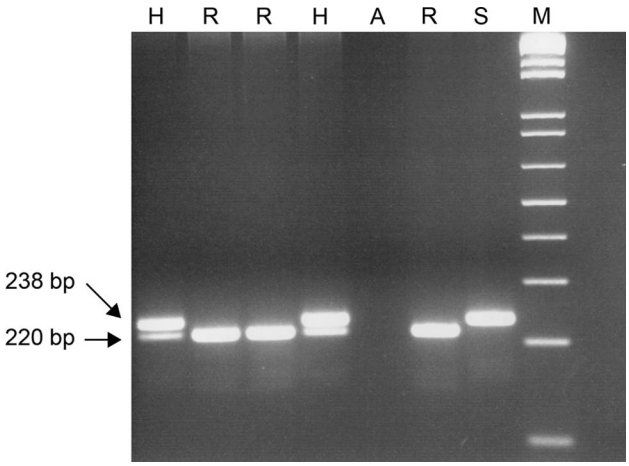

Fig. 4 PCR product amplified by *cssu45* marker on *R* (resistant), *H* (heterozygous), *S* (susceptible) and *A* (1D nullisomic) lines and *M* refers to 1 kb size ladder

Sr45 and *Lr21* (Periyannan et al. 2013). Based on these markers, backcross derivatives were categorised into three groups (Fig. 2). Group I included lines with large introgressed segments carrying *Lr21*, *sr33*-haplotype-V and *Sr45*. This haplotype includes CS1D5406 and Thatcher+*Lr21*. Group II carried *sr33*-haplotype-V and *Sr45*. Group III derivatives carried the shortest introgressions with *Sr45* alone and was found in *Sr45*/3*K441 and *Sr45*/3*K2001 lines.

Discussion

Sr45 remains effective against Ug99 and the commonly detected *Pgt* races in Australia, South Africa, India and is

Fig. 3 Alignment of CS1D5405, CS, AL8/78, AUS18911 and CS1D5406 sequence showing the 18-bp deletion specific to *Sr45* carrying lines (AUS18911 and CS1D5406). Sequences in red represent the primer region and the dotted line corresponds to the deletion specific to *Sr45*-carrying lines

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CS1D5405   CGAGTTTCAATACTTCGCCCAGTTCATTCTATCGGGAACTCGGCAAACTTCTCTCTGTCA  60
CS         CGAGTTTCAATACTTCGCCCAGTTCATTCTATCGGGAACTCGGCAAACTTCTCTCTGTCA  60
AL8/78     CGAGTTTCAATACTTCGCCCAGTTCATTCTATCGGGAACTCGGCAAACTTCTCTCTGTCA  60
AUS18911   CGAGTTTCAATACTTCGCCCAGTTTATTCTATCGGGAACTCGGCAAACTTCTCTCTGTCA  60
CS1D5406   CGAGTTTCAATACTTCGCCCAGTTTATTCTATCGGGAACTCGGCAAACTTCTCTCTGTCA  60

CS1D5405   AGTTTGGTCCAAAATTGAATAGACAGTGATAAGCATCTCGGCGAGCTCTGGTTTTGCCAG  120
CS         AGTTTGGTCCAAAATTGAATAGACAGTGATAAGCATCTCGGCGAGCTCTGGTTTTGCCAG  120
AL8/78     AGTTTGGTCCAAAATTGAATAGACAGTGATAAGCATCTCGGCGAGCTCTGGTTTTGCCAG  120
AUS18911   AGTTTGGTCCAAAATTGAATAGACAGTGATAAGCATCTCGGCG----------------  103
CS1D5406   AGTTTGGTCCAAAATTGAATAGACAGTGATAAGCATCTCGGCG----------------  103

CS1D5405   GTTCCAATGAAAAACTACTCGGTGAACTAAATAAACTAGGTAGAATCTCAAGTTTGCAGA  180
CS         GTTCCAATGAAAAACTACTCGGTGAACTAAATAAACTAGGTAGAATCTCAAGTTTGCAGA  180
AL8/78     GTTCCAATGAAAAACTACTCGGTGAACTAAATAAACTAGGTAGAATCTCAAGTTCGCAGA  180
AUS18911   -TTCCAATGAAAAACTACTCGGTGAACTAAATAAACTAGGTAGAATCTCAAGTTCGCAGA  162
CS1D5406   -TTCCAATGAAAAACTACTCGGTGAACTAAATAAACTAGGTAGAATCTCAAGTTCGCAGA  162

CS1D5405   GTTCCCGGCGAAAACAACTTGGCAAGGCCTGACAGGTGGGCCCTATTGCATAGTAATC    238
CS         GTTCCCGGCGAAAACAACTTGGCAAGGCCTGACAGGTGGGCCCTATTGCATAGTAATC    238
AL8/78     GTTCCCGGCGAAAACAACTTGGCAAGGCCTGACAGGTGGGCCCTATTGCATAGTAATC    238
AUS18911   GTTCCCGGCGAAAACAACTTGGCAAGGCCTGACAGGTGGGCCCTATTGCATAGTAATC    220
CS1D5406   GTTCCCGGCGAAAACAACTTGGCAAGGCCTGACAGGTGGGCCCTATTGCATAGTAATC    220
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therefore a useful gene to combine with other stem rust resistance genes. Apart from rapid selection of individual genes, markers linked with resistance are valuable for pyramiding genes with similar race specificities. Linkage with SSRs was used as a first step in the attempt to identify markers tightly linked with *Sr45*. SSRs *gwm106* and *gwm337* were found to flank *Sr45* and enabled the selection of recombinants for high-resolution mapping of the *Sr45* region. Subsequently, 2 wESTs *BE444266* and *BE446624* from the chromosome group 1 EST map of Akhunov et al. (2010) drastically reduced the number of recombinants for further marker analysis.

In earlier comparative genomic studies, the short arms of wheat chromosome group 1 were found to have markers conserved with chromosome 5 and 1H of rice and barley, respectively (Feuillet and Keller 1999; Reddy et al. 2008). In this study, the 2 wESTs *BE444266* and *BE446624* were unable to predict syntenic regions in any of these grasses but identified a syntenic region in chromosome 2 (Bd2) of *Brachypodium*. Unfortunately, genes present within the region in the Bd2 pseudomolecule were not polymorphic between genotypes with and without *Sr45*. This contrasts with the successful use of synteny with *Brachypodium* to identify polymorphic markers linked to *Sr35* (Zhang et al. 2010) or to *Lr34* (Bossolini et al. 2007).

Given the limitations that can be encountered in using comparative genomic information from completely sequenced grasses (rice, *Brachypodium* and *Sorghum*), BAC clones from wheat diploid relatives have proven to be useful for characterising genes present in the corresponding genomes of hexaploid wheat (Keller et al. 2005). Using a subgenomic approach, the physical maps for rust resistance genes *Lr21* (Huang et al. 2003), *Lr10* (Feuillet et al. 2003), *Lr34* (Lagudah et al. 2006; Krattinger et al. 2009), *Sr33* (Periyannan et al. 2013) and *Sr35* (Zhang et al. 2010; Saintenac et al. 2013) were generated using sequence information from related diploid species. For *Sr45*, an AFLP fragment closely linked to gene was used to identify *Ae. tauschii* BAC clones and contigs representing the region. *Ae. tauschii* genomic sequences from the BAC ends of the isolated clones were highly repetitive and proved to be a challenge for physical mapping, as reported earlier by Ling et al. (2003). Low-copy fragments identified from the internal region of the BAC sequence were helpful in genetic mapping. BAC clones separated by large physical distances in the *Ae. tauschii* BAC contig co-segregated and were mapped to the same genetic position, suggesting low recombination in the region. The entire *Ae. tauschii* fragment cloned in the BAC MI221O11 did not contain any genes and nearly 95 % of the sequence was highly repetitive. Chromosome walking using additional BAC clones will be required to identify candidate genes responsible for resistance conditioned by *Sr45*.

PCR-based co-dominant marker *cssu45* identified from the *Ae. tauschii* genomic sequence distinguished the presence of *Sr45* in different genetic backgrounds and together with *Lr21* specific marker (Fu et al. 2010), breeders can now select material with both stem and leaf rust resistance. In this study, we also showed that Thatcher+*Lr21* (Thatcher*6/RL5406), the reference genotype for *Lr21,* used in several studies also carries *Sr45*. Marker *cssu45* is specific to *Sr45* haplotype, as it amplifies a different allele in derivatives with *Sr21*, a stem rust resistance gene derived from *T. monococcum* having similar rust specificities. In addition to Ug99 infection in hexaploids (Singh et al. 2011) and marker *cssu45*, an Australian *Pgt* race 11-1,2,3,4,5,6,7 differentiates *Sr45* and *Sr21* carrying wheat genotypes (Table 1). *Sr21*, however, is shown to be resistant to Ug99 in diploid wheats (Zhang et al. 2010).

In addition to *Sr45*, the short arm of wheat chromosome 1D carries other *Pgt* R genes introgressed from *Ae. tauschii* or rye: *Sr33* (Periyannan et al. 2013), *Sr50* (Anugrahwati et al. 2008) and *SrTA1662* (Olson et al. 2013). Like *Sr45,* these genes confer resistance against diverse *Pgt* races including Ug99 and its derivatives. Markers linked with *Sr33, Sr50* and *SrTA1662* and *Sr45* will serve as a useful selection tool to generate chromosome 1DS-specific stem rust resistance gene block to provide long-lasting resistance against all the known *Pgt* races.

Acknowledgments This work was funded in part by Borlaug Global Rust Initiative (BGRI) Durable Rust Resistance in Wheat (DRRW) Project (administered by Cornell University with a grant from the Bill & Melinda Gates Foundation and the UK Department for International Development); Australian Centre for International Agricultural Research (ACIAR), Grain Research and Development Corporation (GRDC), National Science Foundation Plant Genome Research DBI-0701916 (Principal Investigator, J. Dvorak). The first author acknowledges the University of Sydney for providing Endeavour International Postgraduate Research Scholarship. We are grateful to Sutha Chandramohan, Libby Viccars, Xiaodi Xia and Hanif Miah for excellent technical support.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments comply with the current laws of Australia.

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