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ORIGINAL ARTICLE

Fine mapping of the stem rust resistance gene *SrTA10187*

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Abstract
***Key message SrTA10187* was fine-mapped to a 1.1 cM interval, candidate genes were identified in the region of interest, and molecular markers were developed for marker-assisted selection and *Sr* gene pyramiding.**
Abstract Stem rust (*Puccinia graminis* f. sp. *tritici*, *Pgt*) races belonging to the Ug99 (TTKSK) race group pose a serious threat to global wheat (*Triticum aestivum* L.) production. To improve *Pgt* host resistance, the Ug99-effective resistance gene *SrTA10187* previously identified in *Aegilops tauschii* Coss. was introgressed into wheat, and mapped to the short arm of wheat chromosome 6D. In this study, high-resolution mapping of *SrTA10187* was done using a population of 1,060 plants. *Pgt* resistance was screened using race QFCSC. PCR-based SNP and STS markers were developed from genotyping-by-sequencing tags and SNP sequences available in online databases. *SrTA10187* segregated as expected in a 3:1 ratio of resistant to susceptible individuals in three out of six BC_3F_2 families, and was fine-mapped to a 1.1 cM region on wheat chromosome 6DS. Marker context sequence was aligned to the reference *Ae. tauschii* genome to identify the physical region encompassing *SrTA10187*. Due to the size of the corresponding region, candidate disease resistance genes could not be identified with confidence. Comparisons with the *Ae. tauschii* genetic map developed by Luo et al. (PNAS 110(19):7940–7945, 2013) enabled identification of a discrete genetic locus and a BAC minimum tiling path of the region spanning *SrTA10187*. Annotation of pooled BAC library sequences led to the identification of candidate genes in the region of interest—including a single NB-ARC-LRR gene. The shorter genetic interval and flanking KASP™ and STS markers developed in this study will facilitate marker-assisted selection, gene pyramiding, and positional cloning of *SrTA10187*.

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Introduction

Stem rust, caused by the basidiomycete *Puccinia graminis* f. sp. *tritici* (*Pgt*), is one of the most serious diseases affecting global wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) production. During the early-to-mid twentieth century, *Pgt* caused large-scale wheat yield losses in the United States. In response, efforts were made to breed *Pgt*-resistant wheat varieties, and to eliminate the non-native alternative host, barberry (*Berberis vulgaris* L.), from the United States (Singh et al. 2015). In 1998, an isolate of *Pgt* from Uganda (Ug99) was found to be virulent to the widely deployed resistance gene *Sr31* (Pretorius et al. 2000) and pathotyped to the standard race nomenclature designation

TTKSK (Singh et al. 2011). In subsequent years, variants from the Ug99 race lineage developed additional virulence to *Sr24* (TTKST) and *Sr36* (TTTSK), placing an even greater number of wheat varieties at risk (Jin et al. 2008, 2009). Currently, thirteen races belonging to the Ug99 race group have been identified in a region extending from South Africa to Iran (Fetch et al. 2016). The majority of wheat varieties planted globally are susceptible to the Ug99 race group (Singh et al. 2015).

Committed efforts to identify novel sources of host resistance to *Pgt* in wheat and its wild wheat relatives have resulted in 27 numerically designated *Sr* genes available for stem rust resistance breeding efforts (Yu et al. 2014). Wild relatives of wheat are a valuable source of stem rust resistance, however, restricted recombination between wheat chromosomes and introgressions from anura genomes often require extensive chromosome engineering to generate genotypes with minimal linkage drag (Mago et al. 2009; Qi et al. 2011; Liu et al. 2011). The wheat D-genome donor species, *Aegilops tauschii* Coss., has homology to the D genome of wheat and has been a valuable source of disease resistance genes (Gill and Raupp 1987; Cox 1997; Cox et al. 1991; Olson et al. 2013b). *Aegilops tauschii* has contributed the Ug99 resistance genes *Sr33*, *Sr45*, *Sr46*, *SrTA1662*, *SrTA10171*, and *SrTA10187* (Olson et al. 2013a, b; Periyannan et al. 2013, 2014; Yu et al. 2015b). Gene introgression from *Ae. tauschii* can be achieved by crossing tetraploid wheat (*Triticum turgidum* L., $2n = 4x = 28$, AABB) with diploid *Ae. tauschii* ($2n = 2x = 14$, DD) to produce synthetic hexaploid wheat (McFadden and Sears 1944), or by direct hybridization between hexaploid wheat and *Ae. tauschii* as described by Gill and Raupp (1987). Using either method, genes can be transferred to wheat through normal meiotic recombination.

The *Ae. tauschii* accession TA10187 exhibited seedling resistance to multiple *Pgt* races (Rouse et al. 2011) and the resistance was transferred to hexaploid wheat (Olson et al. 2013b). A single gene, provisionally named *SrTA10187*, was mapped to the distal region of chromosome 6DS. Multiple stem rust resistance genes are located in this region including *Sr5* (Sears et al. 1957; McIntosh et al. 1995), *Sr42* (Ghazvini et al. 2012; Gao et al. 2015), *SrCad* (Hiebert et al. 2011; Kassa et al. 2016), and *SrTmp* (Hiebert et al. 2015; Lopez-Vera et al. 2014).

The objectives of this study were to: (1) fine map the *SrTA10187* locus on chromosome 6DS, (2) identify candidate resistance genes, and (3) develop single nucleotide polymorphism (SNP) markers for *SrTA10187*. Mapping the resistance locus to a shorter genetic region will improve breeding accuracy for *SrTA10187*, and identification of candidate resistance genes will expedite future map-based cloning efforts. Marker resources developed in this study will facilitate introgression and gene pyramiding of *SrTA10187* into elite wheat varieties using marker-assisted selection.

Methods and materials

Plant materials

The high-resolution mapping population used in this study comprised 1060 individuals from six BC_3F_2 families: U6897-1, U6897-2, U6897-3, U6897-4, U6897-5, and U6897-6. The population was developed by selfing six BC_3F_1 plants made from a cross of a single stem rust-resistant BC_2F_1 plant (Olson et al. 2013b) to the recurrent parent KS05HW14. All BC_3F_2 families segregated for previously mapped SSR loci (*Xcfd49* and *Xbarc173*) linked to *SrTA10187* (Olson et al. 2013b).

Stem rust resistance phenotyping

Seedling stem rust phenotyping was done using a Michigan-collected isolate of *Pgt* race QFCSC. Seedlings were inoculated at the two-leaf stage with *Pgt* urediniospores suspended in Soltrol 170 isoparaffin oil (Chevron Philips Chemical Company LP, The Woodlands, TX) using an airbrush. Plants were then placed into a dew chamber held at 20 °C and 100 % relative humidity for 16 h. At 14 days post-inoculation, the first leaf of each plant was scored for *Pgt* disease resistance using the 0-4 Stakman scale (Stakman et al. 1962). A χ^2 goodness-of-fit test was performed with *Pgt* infection types of 2-classified as resistant and infection types of 3 and higher classified as susceptible. Progeny tests were done on $BC_3F_{2:3}$ individuals that showed recombination between *Xcfd49* and *Xbarc173*.

DNA isolation

Genomic DNA was isolated from 1060 BC_3F_2 plants. Approximately 40 mg of leaf tissue from each plant was collected into separate 1.1 mL tubes containing stainless steel 5/32 in ball bearings (Grainger, Lake Forest, IL) in a 96-well plate format and ground using a Retsch MM 400 mill (Retsch, Newtown, PA). DNA was isolated using the Mag-Bind® Plant DNA Plus 96 Kit (M1128, Omega Bio-Tek, Norcross, GA) on a King Fisher Flex (Thermo Scientific, Waltham, MA) instrument and quantified using the Quant-iT™ PicoGreen® dsDNA Kit (Life Technologies Corp., Grand Island, NY) in a 384-well format on a CFX384 C1000 Real-Time thermal cycler (BioRad, Hercules, CA). Normalization to 30 ng μL^{-1} for SSR and STS markers and 10 ng μL^{-1} for KASP™ markers was done using a GBC Fit-X1 instrument (New England BioGroup, Atkinson, NH).

Marker development and genotyping

SNP markers were developed from GBS tags generated using a two-enzyme GBS protocol (Poland et al. 2012) from 94 BC_2F_1 plants segregating for *SrTA10187* and parental lines. TASSEL 3.0 (maizegenetic.net) and the UNEAK pipeline were used to call SNPs (Lu et al. 2013). Bulked segregant analysis was done using polymorphic GBS tags to identify SNPs associated with resistant individuals (Pujol et al. 2015; Trick et al. 2012).

Fifteen KASP™ (competitive allele-specific PCR, LGC, Teddington, Middlesex, UK) markers were used to fine map *SrTA10187*. KASP™ markers were developed from GBS SNPs and SNP sequences found in the online databases CerealsDB (Wilkinson et al. 2012, http://www.cerealsdb.uk.net/) and Sequencing the *Aegilops tauschii* Genome (Luo et al. 2013, http://aegilops.wheat.ucdavis.edu/) (Semagn et al. 2014). Source SNP identification codes are listed in Table 1. Initially, KASP™ markers were tested on parental genotypes to ensure that genotypic classes could be differentiated. Marker expression (codominant or dominant) and parental allele specificities are included in Table 1. The KASP™ markers *6DS0027*, *AT6D5273*, and *AT6D5280* were scored as dominant markers because heterozygous fluorescence clusters could not be differentiated. All other SNP markers were scored as codominant. Primer names include allele 1 (A1), allele 2 (A2), and common (C) primer designations. KASP™ marker loci were amplified in 5 µL reactions containing 10 ng of gDNA, 0.07 µL of primer mix (containing 12 µM of each allele-specific forward primer and 30 µM reverse primer), and 2.5 µL of KASP™ master mix. KASP™ thermal cycling was carried out according to the manufacturer's protocol: 94 °C for 15 min; 10 step-down cycles of 94 °C for 20 s, and 61–55 °C for 60 s (decreasing by 0.6 °C each cycle); and 26 cycles of 94 °C for 20 s, and 55 °C for 60 s. The BioRad CFX384 was used for thermal cycling and KASP™ marker fluorescence detection. BioRad CFX manager software was used for allelic discrimination.

One sequenced-tagged site (STS) marker (*6DS0050*) was also used for *SrTA10187* fine mapping. Primers were designed to amplify a 100 bp DNA segment of the gene designated *MSU_6DS_001*. The *6DS0050* primer sequence is shown in Table 1. The *6DS0050* marker locus was amplified in 20 µL reactions containing 120 ng of gDNA, 0.5 µL of each primer (10 µM), 2 µL of 10 × reaction buffer, 0.5 µL of dNTP (10 mM), and 0.2 µL of Taq polymerase (Empirical bioscience, Grand Rapids, MI, USA). Thermal cycling was done using the following protocol: 95 °C for 4 min; 34 cycles of 95 °C for 45 s, 57 °C for 45 s, and 72 °C for 30 s. A total of 20 µL of PCR product from each reaction was added to 4 µL of 6x loading dye and visualized on 1.5 % agarose gel containing ethidium bromide. A 100 bp DNA Ladder was used as a standard (New England Biolabs, Ipswich, MA, USA).

Linkage map construction and comparison to existing *Ae. tauschii* physical and genetic maps

A linkage map of the 6DS region harboring *SrTA10187* was constructed in JoinMap® 4 (Kyazma®, Wageningen, Netherlands) using the maximum likelihood algorithm. MapChart 2.3 was used for genetic map formatting (Voorrips 2002). Command-line BLAST was used to align each KASP™ marker context sequence to a unique location in the *Ae. tauschii* reference genome (Jia et al. 2013). Ten markers aligned to *Ae. tauschii* scaffolds with a known genomic position, whereas the remaining five markers aligned to unanchored scaffolds. The genetic map positions of markers in common with those published by Luo et al. (2013) were compared directly.

Scaffold sequence identification and annotation

The BAC-based physical map produced by Luo et al. (2013) was used to identify *Ae. tauschii* scaffold sequence in the region surrounding *SrTA10187*. Common SNP marker loci were identified between the *Ae. tauschii* genetic map (Luo et al. 2013) and *SrTA10187*. Physical positions of common markers as well as 6DS0050, 6DS0039, and BS0021983 on 6DS scaffolds were validated using BLAST. Scaffolds AT6D5270, AT6D5271, AT6D5272 and scaffolds of BAC library 6222 spanned the locus containing *SrTA10187* and were ordered based on the *SrTA10187* genetic map. The BAC 6222 scaffolds 6222.1 and 6222.2 could be ordered based on marker order. However, without marker coverage, many scaffolds of BAC library 6222 could not be ordered.

Scaffolds surrounding the *SrTA10187* locus were annotated using the MAKER pipeline. REPEATMASKER used a previously published wheat repeat library to mask the scaffold sequences (Campbell et al. 2014; Wicker et al. 2002; Smit et al. 2013). Several transcript files were used as evidence to aid the gene prediction programs: wheat full-length cDNAs (http://trifldb.psc.riken.jp/download/ver.3.0/TaRFL4905.fas.gz), wheat predicted coding sequences (http://trifldb.psc.riken.jp/download/nuc/Triticum_aestivum.full.fas.cds.fas.gz), and *Ae. tauschii* predicted coding sequences (ftp://climb.genomics.cn/pub/10.5524/100001_101000/100054/D/Annotation/wheatD_final_43150.gff.cds) (Jia et al. 2013; Mochida et al. 2009). UniProtKB/SwissProt plant proteins were also used as evidence for the gene model predictions (Apweiler et al. 2014). MAKER was run a single time allowing gene predictions to be made by both SNAP (using an existing rice HMM) and Augustus (using the wheat HMM from Augustus version 3.1) (Stanke et al. 2003; Korf et al. 2004). The predicted proteins were

Table 1 KASP™ and STS markers used for fine mapping of *SrTA10187* on wheat chromosome 6DS

Marker ID	Marker expression	Primer name	Primer sequence[d]	Allele	Parent
BS00085937[a]	Codominant	BS00085937_A1	ATCGAGATGGCTATGCGACCC	G	KS05HW14
		BS00085937_A2	ATCGAGATGGCTATGCGACCG	C	TA10187
		BS00085937_C	ATATCGGCCATGCTGCCTTTGGAA		
6DS0034[b]	Codominant	6DS0034_A1	CGGATCTCCAAATAAAACAGTTAGCTATA	T	TA10187
		6DS0034_A2	GGATCTCCAAATAAAACAGTTAGCTATG	C	KS05HW14
		6DS0034_C	CCTCAACCTAACCGTCAAATCTTACA		
6DS0038[b]	Codominant	6DS0038_A1	CGTCTTGTTCTCGCTTGCTGT	A	TA10187
		6DS0038_A2	CGTCTTGTTCTCGCTTGCTGC	G	KS05HW14
		6DS0038_C	CATTGACGACCACCAGAGAGAAG		
6DS0023[b]	Codominant	6DS0023_A1	ACCCGTGACTTCCTTGGTCTTC	G	TA10187
		6DS0023_A2	ACCCGTGACTTCCTTGGTCTTG	C	KS05HW14
		6DS0023_C	GACGGCACGTTCCTGCAGAAAA		
AT6D5264[c]	Codominant	AT6D5264_A1	AAACAATTGGTATGCGTCTCTACC	G	KS05HW14
		AT6D5264_A2	AAACAATTGGTATGCGTCTCTACT	A	TA10187
		AT6D5264_C	ATATGAACCACAGCCGAAGG		
6DS0022[b]	Codominant	6DS0022_A1	CATGACCCCTCATTCATGCTG	C	TA10187
		6DS0022_A2	CATGACCCCTCATTCATGCTC	G	KS05HW14
		6DS0022_C	TGCTCTGAACTCGGAATCTCTTGAT		
6DS0050[c]	Dominant	6DS0050_F	AAAGGGAGTTGATATGGATCTGT	–	TA10187
		6DS0050_R	CAAGCTGGTTAAAATAGCTCTCA		
6DS0027[b]	Dominant	6DS0027_A1	AATGTCATCTCAGTAAAACAAATTGCC	G	TA10187
		6DS0027_A2	AATGTCATCTCAGTAAAACAAATTGCT	A	KS05HW14
		6DS0027_C	CATATTTTGGCCTTTCTGTCAGAGAGT		
6DS0039[b]	Codominant	6DS0039_A1	AAACTGCAGAGCATTTCATTTTTT	A	TA10187
		6DS0039_A2	AAACTGCAGAGCATTTCACTTTTG	C	KS05HW14
		6DS0039_C	GGTTACATCATAAACTACATGTGCATAG		
AT6D5273[c]	Dominant	AT6D5273_A1	ACTGTGAGTACATTGGGACTAGAATT	A	KS05HW14
		AT6D5273_A2	ACTGTGAGTACATTGGGACTAGAATC	G	TA10187
		AT6D5273_C	AAGTCTATCCCGGCTGGATGCA		
BS00021983[a]	Codominant	BS00021983_A1	AACAATCATATCAATCCCCATCGGC	G	KS05HW14
		BS00021983_A2	GAACAATCATATCAATCCCCATCGGA	T	TA10187
		BS00021983_C	CCAAACTTCCTTGTCTCGATGTAGCAT		
6DS0017[b]	Codominant	6DS0017_A1	GGAACATTACCATCATGGATT	A	TA10187
		6DS0017_A2	GGAACATTACCATCATGGATC	G	KS05HW14
		6DS0017_C	CCAGATATACTGTTTCCTGCTA		
BS00111704[a]	Codominant	BS00111704_A1	AGTGCATGTACCCAAACCAGTTGA	T	TA10187
		BS00111704_A2	GTGCATGTACCCAAACCAGTTGC	G	KS05HW14
		BS00111704_C	GCAGTGGGGCGCGGACGAA		
AT6D5276[c]	Codominant	AT6D5276_A1	TAAGATATGCAGAGGCGATGTTATTTCA	T	KS05HW14
		AT6D5276_A2	TAAGATATGCAGAGGCGATGTTATTTCG	C	TA10187
		AT6D5276_C	CAATGAAATAAGTATGTTTTGATCTGAAGC		
AT6D5280[c]	Dominant	AT6D5280_A1	TTCTCATTCCATTTGTGGCACCTGT	A	TA10187
		AT6D5280_A2	TTCTCATTCCATTTGTGGCACCTGC	G	KS05HW14
		AT6D5280_C	GCTAACTAGTTCTCCAATTTCTACTAAG		

Table 1 continued

Marker ID	Marker expression	Primer name	Primer sequence[d]	Allele	Parent
AT6D5282[c]	Codominant	AT6D5282_A1	TTTAATGGTAAAGTCAGCATGGTGCTA	T	TA10187
		AT6D5282_A2	TTTAATGGTAAAGTCAGCATGGTGCTC	G	KS05HW14
		AT6D5282_C	GCAAAACAATCTGACTGTTTTCCGAG		

[a] Source of the SNP context sequence: CerealsDB (http://www.cerealsdb.uk.net/)

[b] Source of the SNP context sequence: genotype-by-sequencing tags identified by bulk segregant analysis

[c] Source of the SNP context sequence: Sequencing the *Aegilops tauschii* Genome (http://aegilops.wheat.ucdavis.edu/)

[d] A1 and A2 primers are listed without FAM and HEX 5′ end sequences, in 5′ to 3′ orientation

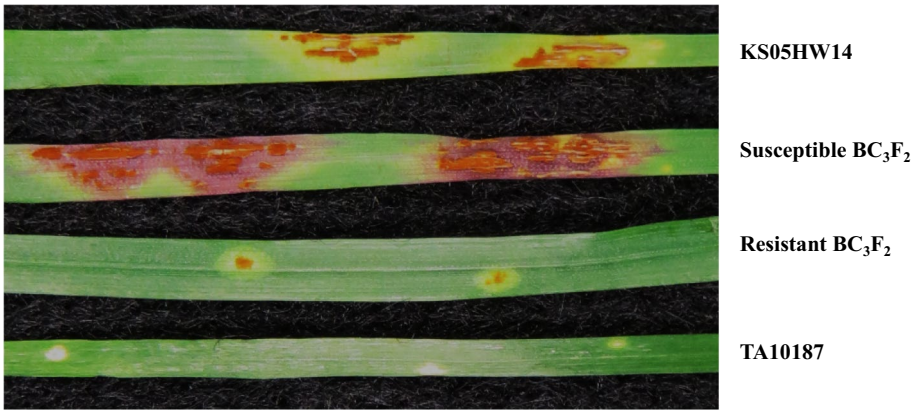

Fig. 1 *Pgt* race QFCSC leaf infection types on recurrent *T. aestivum* parent KS05HW14, susceptible BC_3F_2, resistant BC_3F_2, and *Ae. tauschii* accession TA10187

analyzed with Hmmscan to identify matching protein family (Pfam) domains (Finn et al. 2014; Eddy 2011). Gene models with support from transcript evidence, protein evidence, and/or matching Pfam domain(s) were retained as high-quality gene predictions. The context sequence used to design SNP markers 6DS0039, AT6D5273, and BS00021983 and primers used for 6DS0050 were aligned (using NCBI BLASTN version 2.2.26) and manually added to the scaffold annotations (Altschul et al. 1990). The coding sequences of two NB-ARC-LRR genes (*MSU_6DS_001* and *MSU_6DS_037*) identified in the region of interest corresponded to the previously annotated genes *F775_13570* and *F775_21138*, respectively (Jia et al. 2013). *F775_13570* and *F775_21138* coding sequences (obtained from http://plants.ensembl.org/Aegilops_tauschii) were aligned to scaffolds AT6D5270 and 6222.3 using Exonerate, and the corresponding MAKER gene predictions were revised (Jia et al. 2013; Slater and Birney 2005; Kersey et al. 2009).

The wheat chromosome 6DS GFF annotation file, transcript sequence file, protein sequence file, and Pfam annotation results are available for download from the Dryad Digital Repository (DOIs will be provided upon provisional acceptance). A genome browser displaying the annotation of these *Ae. tauschii* scaffolds is available for public access at http://childslab.plantbiology.msu.edu/jbrowse7/?data=data7%2Fjson%2Fwheat_AW4.

Results

High-resolution mapping of SrTA10187

In the high-resolution mapping population of 1060 BC_3F_2 plants, 683 plants had a resistant infection type of 2- and 377 had a susceptible infection type of 3 to *Pgt* race QFCSC (Fig. 1). Parental genotypes KS05HW14 and TA10187 had the expected susceptible and resistant infection types, respectively. Three BC_3F_2 families (U6897-2, U6897-4, and U6897-6) demonstrated the expected 3:1 segregation for QFCSC resistance, while the remaining three families (U6897-1, U6897-3, U6897-5) deviated from the expected ratio (Table 2).

Each individual of the high-resolution mapping population was genotyped using SSR markers *Xbarc173* and *Xcfd49*. A total of 69 individuals with recombination between SSR loci were identified and progeny tested for stem rust resistance. A genetic map was constructed spanning 6.8 cM in the distal region of wheat chromosome 6DS using 15 KASP™ SNP markers and one STS marker (Fig. 2b). Marker loci flanking *SrTA10187* include *6DS0027* located 0.9 cM distally, and *6DS0039* and *AT6D5273* located 0.2 cM proximally. The STS marker designated *6DS0050* maps 1 cM distal to *SrTA10187* and produces a 100 bp PCR product from *Ae. tauschii* accessions TA10187 (the *SrTA10187* donor) and AL8/78 (the *Ae.*

Table 2 Segregation of *Pgt* resistance in a high-resolution mapping population comprising six BC_3F_2 families

Family	Number of plants			χ^2 [b]	*P* value
	Resistant[a]	Susceptible	Total		
U6897-1	217	186	403	96.18	<0.001[c]
U6897-2	294	89	383	0.63	0.426
U6897-3	78	49	127	12.50	<0.001[c]
U6897-4	14	10	24	3.56	0.059
U6897-5	24	21	45	11.27	<0.001[c]
U6897-6	56	22	78	0.43	0.513

[a] Plants were classified resistant based on an infection type of 2- (Stakman et al. 1962)

[b] The expected segregation ratio of resistant to susceptible plants was 3:1

[c] Reject H_0 that plants are segregating as expected ($P > 0.05$)

tauschii reference genome accession) that is absent from KS05HW14 (Fig. 3).

Anchoring the SrTA10187 genetic map to Ae. tauschii reference genomes

Initially, the high-resolution *T. aestivum* genetic map developed in this study was compared to the *Ae. tauschii* reference genome physical map developed by Jia et al. (2013) (Fig. 2A, B). Ten KASP™ marker sequences aligned uniquely to *Ae. tauschii* scaffolds in the four most distal recombination bins of 6DS spanning 4 Mb of sequence. The remaining five KASP™ markers aligned uniquely to unanchored D-genome scaffolds (data not shown). Although *Ae. tauschii* scaffolds are ordered arbitrarily within recombination bins, only two inconsistencies were found between the physical and genetic maps at marker loci *AT6D5273* and either *BS00021983* or *BS00111704*. Due to the size of the physical map interval and reference genome recombination bins as well as discrepancies in marker order, the Jia et al. (2013) reference genome was unsuitable for candidate gene identification.

The genetic map developed in this study was subsequently compared to the *Ae. tauschii* genetic map developed by Luo et al. (2013) (Fig. 2B, D). Five KASP™ markers were developed from SNPs identified and mapped by Luo et al. (2013) including *AT6D5264*, *AT6D5273*, *AT6D5276*, *AT6D5280,* and *AT6D5282*. These KASP™ markers were mapped in the *SrTA10187* populations to enable direct comparisons of genetic and physical positions. Conserved marker order across the region enabled the identification of a distinct interval on 6DS harboring *SrTA10187*.

To identify the genomic sequence spanning the *SrTA10187* interval, genetic markers were aligned to *Ae. tauschii* scaffolds assembled by Luo et al. (2013) (Fig. 2C). The STS marker designed to amplify the *MSU_6DS_001* exon sequence aligned to scaffold AT6D5270 and is located distal to the resistance locus (Fig. 2C). KASP™ markers *6DS0039*, *AT6D5273*, and *BS00021983* aligned to scaffold sequences from BAC pool 6222 and are located proximal to *SrTA10187*. Scaffolds 6222.1 and 6222.2 were ordered based on the genetic map. Unfortunately, 745 Kb of sequence from BAC pool 6222 could not be anchored to a genetic map position.

Candidate gene identification

Gene predictions were made in 1.5 Mb of sequence from scaffolds AT6D5270, AT6D5271, AT6D5272, and BAC library 6222 spanning the *SrTA10187* interval. Scaffolds 6222.1 and 6222.2 were located proximal to *SrTA10187* and did not contain defense response genes. Pfam domains consistent with known NB-ARC-LRR defense response genes were identified in the unanchored 745 Kb of sequence from BAC pool 6222. In this region, one NB-ARC-LRR gene was identified and designated *MSU_6DS_037* (Fig. 2C). A gap is present in the BAC-based physical map and it remains unknown if other defense response genes are present in the identified genomic interval.

Discussion

In this study, the TTKSK-effective resistance gene *SrTA10187* derived from *Ae. tauschii* was assigned to a 1.1 cM genomic interval on chromosome 6DS. The availability of SNP sequences in public databases, combined with GBS-derived SNPs and bulked segregant analysis, facilitated the development of KASP™ markers to produce a refined map of the *SrTA10187* region. Saturation of the *SrTA10187* region on 6DS helped to resolve the genetic position of this important resistance gene. Assignment of marker sequence to the publicly available *Ae. tauschii* genome sequence enabled the identification of a discrete physical map interval harboring putative defense response genes.

To date, five *Pgt* resistance genes have been identified on wheat chromosome 6DS: *Sr5*, *Sr42*, *SrCad*, *SrTmp*, and *SrTA10187* (McIntosh et al. 1995; Gao et al. 2015; Kassa et al. 2016; Hiebert et al. 2015; Olson et al. 2013b). *Sr42*, *SrCad*, *SrTmp*, and *SrTA10187* are effective against TTKSK, but *Sr5* is not (Jin et al. 2007). Furthermore, *SrTmp* virulence has been identified in *Pgt* race TTKTK (Patpour et al. 2016).

Differences in race specificity exist between *Sr42* and *SrTA10187*. North American *Pgt* races QFCSC and QTHJC are avirulent on *SrTA10187* while these races are virulent

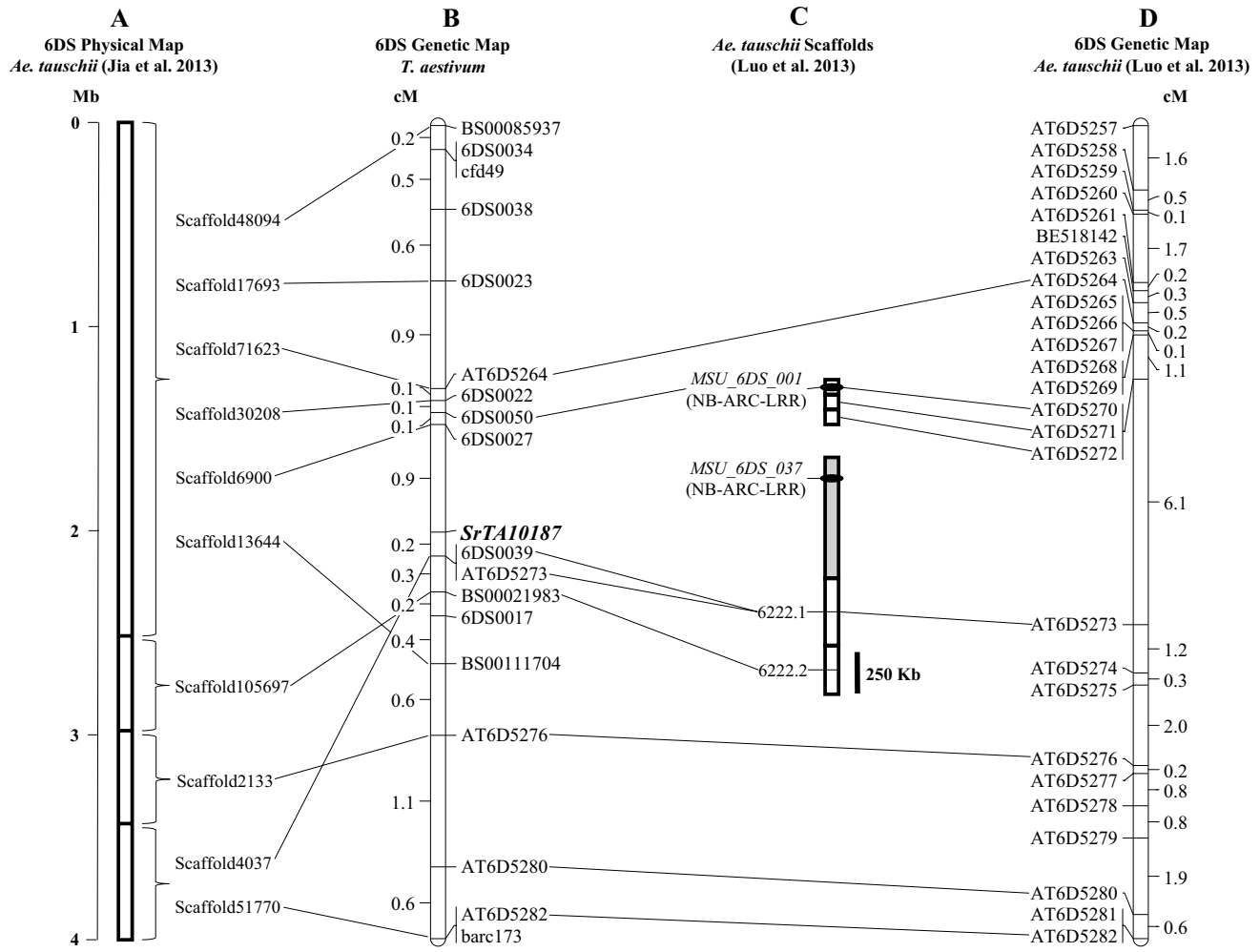

Fig. 2 Comparative genetic and physical maps of the 6DS chromosome region linked to the *Pgt* resistance gene *SrTA10187*. (*A*) *Ae. tauschii* reference genome 6DS physical map (Jia et al. 2013). Ten KASP™ marker sequences aligned uniquely to scaffolds in the four most distal recombination bins of 6DS. (*B*) A high-resolution *T. aestivum* genetic map encompassing *SrTA10187* was developed from 1060 BC_3F_2 individuals. (*C*) Graphical representation of annotated *Ae. tauschii* scaffolds. *Lines* connecting the scaffolds to each genetic map indicate shared sequence. *Shaded region* = unanchored scaffold sequence from BAC pool 6222. *Black marks* indicate genes with Pfam domains consistent with plant defense response. (*D*) *Ae. tauschii* 6DS genetic map developed by Luo et al. (2013)

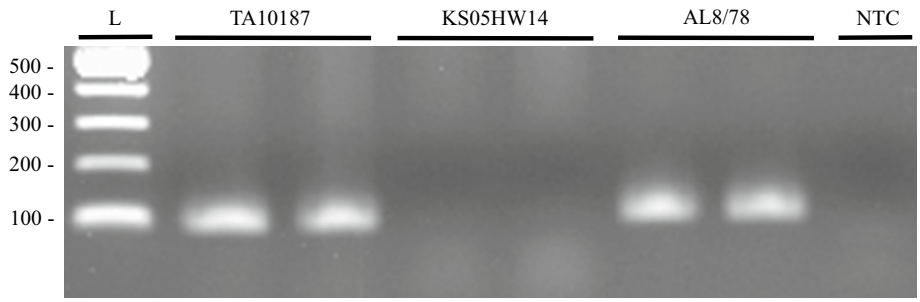

Fig. 3 The STS marker 6DS0050 amplifies a 100 bp product from *Ae. tauschii* accessions TA10187 and AL8/78, but not the *T. aestivum* parent KS05HW14. Primers were designed within an exon of *MSU_6DS_001*. *6DS0050* mapped 1 cM distal to *SrTA10187*. L = DNA ladder; NTC = no template control

on *Sr42* (Ghazvini et al. 2012; Olson et al. 2013b). Virulence or avirulence to *SrCad* by QFCSC or QTHJC is currently unknown. Future testing of *Sr42*, *SrCad*, *SrTmp*, and *SrTA10187* race specificity to TTKTK, QFCSC, and QTHJC will help elucidate the effectiveness of these genes on prevalent North American races as well as the Ug99 race

group. Furthermore, allelism tests between *SrTA10187* and other 6DS genes should be done using *Pgt* race TTKSK to determine the allelic relationship between these genes.

In recent studies, genetic markers closely linked to *Sr42* and *SrCad* were developed for marker-assisted selection (Gao et al. 2015; Kassa et al. 2016). *Sr42* and *SrCad* have been mapped to the same region of 6DS and differences in SNP marker haplotypes have been identified, however, the genetic relationship between *Sr42* and *SrCad* is still unclear (Kassa et al. 2016). To make comparisons between the genetic map developed in this study and those published by Gao et al. (2015) and Kassa et al. (2016), the KASP™ markers Excalibur_s114066_kwm918 and contig32737_kwm112 were tested on our mapping population. The marker Excalibur_s114066_kwm918 (=IWB31561) was used because it co-segregated with *Sr42* in a bi-parental population of 94 $F_{2:3}$ plants (Gao et al. 2015). Excalibur_s114066_kwm918 also co-segregated with *SrCad* in a RIL population of 384 lines, a DH population of 334 lines, and mapped 0.37 cM proximal to *SrCad* in a RIL population of 141 lines (Kassa et al. 2016). The marker contig32737_kwm112 was used because it was derived from a SNP identified in *Ae. tauschii*, and it co-segregated with *SrCad* in three bi-parental populations (Kassa et al. 2016). Both markers were monomorphic in our mapping population, and could not be used for linkage map construction. All individuals tested (including parental lines KS05HW14 and TA10187) carry the C allele for Excalibur_s114066_kwm918, and for contig32737_kwm112. This result demonstrates the importance of identifying unique SNP markers, and knowing the parental alleles to select for each gene.

Olson et al. (2013b) reported *SrTA10187* segregation distortion in a population of 105 BC_2F_1 plants. One consequence of using genes from a wild relative such as *Ae. tauschii* for the improvement of cultivated wheat is the potential for linkage drag. In the present study, three of the six BC_3F_2 families (U6897-2, U6897-4, and U6897-6) exhibited no segregation distortion, but three families (U6897-1, U6897-3, and U6897-5) deviated from the expected 3:1 segregation ratio (Table 2). Based on this observation, an additional backcross to the recurrent parent KS05HW14 may have broken linkage between *SrTA10187* and deleterious allele(s) causing linkage drag in BC_3-derived families U6897-2, U6897-4, and U6897-6. The segregation distortion observed in U6897-1, U6897-3, and U6897-5 likely increased recombination fractions and therefore genetic map length estimates, but it is unlikely that marker order was affected given the high marker coverage across the region.

While the publicly available *Ae. tauschii* reference genome was useful for identifying a physical region that corresponds to our genetic map, large recombination bins, unanchored scaffolds, and differences in marker order limited its effectiveness in identifying candidate genes. The physical region corresponding to our 6DS genetic map spans a relatively large distance of 4 Mb, comprised of only 4 recombination bins. Due to lower recombination in the *Ae. tauschii* reference genome mapping population, the order of many scaffolds located within recombination bins is unknown. The most distal 6DS recombination bin spans nearly 2 Mb of unordered sequence. Of the 15 KASP™ markers aligned to the *Ae. tauschii* reference, five markers aligned to unanchored scaffolds. The high-resolution genetic map developed by Luo et al. (2013) enabled the identification of a more discrete interval of sequences spanning the *SrTA10187* locus.

In this study, we have developed the codominant KASP™ marker *6DS0039,* located 0.2 cM proximal to *SrTA10187,* which will be useful for germplasm development, marker-assisted selection, and resistance gene pyramiding. Additionally, the STS marker *6DS0050* could be used as an alternative to the KASP™ markers developed in this study. Markers identified in this study will be useful for deployment of *SrTA10187* into wheat breeding programs (Yu et al. 2015a). The codominant KASP™ marker *6DS0039* enables pyramiding multiple *Pgt* resistance genes with complementary race specificities into the same wheat line that could not be efficiently combined using phenotypic data alone.

Author contribution statement A.T.W., E.L.O. designed research and objectives; A.T.W., L.K.B., E.I.B., T.L.L. performed research; A.T.W., S.K.S., E.L.O. analyzed data; K.L.C., J.A.P., S.K.S., E.L.O. contributed analytical support; A.T.W., E.L.O. wrote and edited the manuscript.

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Compliance with ethical standards

This research complies with the current laws of the United States of America.

Conflict of interest The authors of this study declare that there is no conflict of interest for this study.

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