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ORIGINAL PAPER

Introgression of stem rust resistance genes *SrTA10187* and *SrTA10171* from *Aegilops tauschii* to wheat

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Abstract *Aegilops tauschii*, the diploid progenitor of the wheat D genome, is a readily accessible germplasm pool for wheat breeding as genes can be transferred to elite wheat cultivars through direct hybridization followed by backcrossing. Gene transfer and genetic mapping can be integrated by developing mapping populations during backcrossing. Using direct crossing, two genes for resistance to the African stem rust fungus race TTKSK (Ug99), were transferred from the *Ae. tauschii* accessions TA10187 and TA10171 to an elite hard winter wheat line, KS05HW14. BC_2 mapping populations were created concurrently with developing advanced backcross lines carrying rust resistance. Bulked segregant analysis on the BC_2 populations identified marker loci on 6DS and 7DS linked to stem rust resistance genes transferred from TA10187 and TA10171, respectively. Linkage maps were developed for both genes and closely linked markers reported in this study will be useful for selection and pyramiding with other Ug99-effective stem rust resistance genes. The *Ae. tauschii*-derived resistance genes were temporarily designated *SrTA10187* and *SrTA10171* and will serve as valuable resources for stem rust resistance breeding.

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Introduction

Stem rust caused by the basidiomycete fungus *Puccinia graminis* Pers. f. sp. *tritici* Eriks & Henn. (*Pgt*) has great destructive potential and can result in complete yield loss on susceptible varieties. In North America, the impact of stem rust on wheat production has been limited in recent decades due to efforts to eradicate the alternate host, common barberry (*Berberis vulgaris* L.), and through development of resistant cultivars. Globally, the use of resistance genes in wheat cultivars has played a major role in controlling wheat stem rust. However, an isolate of *Pgt*, originally discovered in Uganda and commonly referred to as 'Ug99' (Pretorius et al. 2000), is virulent to the widely deployed *Sr31* and many other stem rust resistance genes present in cultivated wheat (Singh et al. 2011). Based on the North American nomenclature (Roelfs and Martens 1987; Jin et al. 2008), this race is designated TTKSK. Ug99 and

derived races have the potential to devastate global wheat production (Singh et al. 2011).

There are a limited number of resistance genes effective against race TTKSK that are native to the hexaploid wheat germplasm pool. To increase the number of stem rust resistance genes available to wheat breeding programs it is necessary to access the genetic diversity in the wild relatives of wheat. Cultivated wheat, *Triticum aestivum* L., is an allohexaploid (AABBDD, $2n = 6x = 42$) species derived from hybridization events between related Triticeae species (Sears 1948). Therefore, diploid and tetraploid species, *T. turgidum* (AABB, $2n = 4x = 28$) and *Aegilops tauschii* (DD, $2n = 2x = 14$) can be considered part of the primary gene pool of wheat. Other species with genomes homologous to the A, B and D genomes of *T. aestivum* are considered to be part of the secondary gene pool of hexaploid wheat and are relatively accessible sources of novel genes and allelic diversity. Species with related non-homologous genomes represent the tertiary gene pools of wheat. Stem rust resistance genes transferred from the tertiary gene pools of wheat are present among elite US wheat cultivars and breeding lines including *Sr24* from *Agropyron elongatum*, *Sr31* and *Sr1RS^{Amigo}* from *Secale cereale*, *Sr36* from *T. timopheevii* (Olson et al. 2010a), and *Sr38* from *Ae. ventricosa* (McIntosh et al. 1995). Stem rust resistance genes effective against race TTKSK originally present on large chromosome translocations have been made available as smaller introgressions including *Sr22* from *T. boeoticum* (Olson et al. 2010b), *Sr26* from *A. elongatum* (Dundas et al. 2007), *Sr39* from *Ae. speltoides* (Niu et al. 2011), *Sr44* from *A. intermedium* (Liu et al. 2013), *Sr47* from *Ae. speltoides* (Klindworth et al. 2012), and *Sr50* (formerly *SrR*) from *S. cereale* (Anugrahwati et al. 2008). In response to the threat of race TTKSK, new stem rust resistance genes *Sr51*, *Sr52* and *Sr53* transferred from the tertiary gene pool were identified in chromosome addition lines and Robertsonian translocations from *Ae. searsii*, *Dasypryum villosum*, and *Ae. geniculata*, respectively (Liu et al. 2011a, b; Qi et al. 2011).

Relative to the tertiary gene pool, the wheat D genome progenitor species, *Ae. tauschii* is a very tractable source of genes for resistance to wheat rust pathogens (Cox 1998). Four *Ae. tauschii*-derived stem rust resistance genes effective against race TTKSK are currently available including *Sr33*, *Sr45* (Kerber and Dyck 1979), *Sr46* (Rouse et al. 2011) and *SrTA1662* (Olson et al. 2013). Screening of *Ae. tauschii* accessions with stem rust race TTKSK identified 98 *Ae. tauschii* accessions with resistance that should be readily transferrable to *T. aestivum* (Rouse et al. 2011).

Direct hybridization of diploid *Ae. tauschii* accessions with hexaploid wheat (Gill and Raupp 1987) provides a means to transfer D genome regions carrying target genes of interest without disrupting adaptive allelic combinations in the A and B genomes while at the same time providing D genome allelic diversity for agronomically important traits (Cox et al. 1995a, b). An alternative route to transfer genes from *Ae. tauschii* is a two-step process of producing synthetic wheat via tetraploid × *Ae. tauschii* hybridization and colchicine doubling, followed by backcrossing with elite hexaploid wheat lines. Homologous recombination between *Ae. tauschii* and *T. aestivum* D genome chromosomes can readily break undesirable linkages between target genes and alleles associated with linkage drag which might not be possible with introgressions from other species. The integrated direct hybridization approach combining gene transfer, genomic localization and introgression (Olson et al. 2013) is an efficient method of expediting transfer of genes from *Ae. tauschii* into wheat breeding germplasm. In this study, direct hybridization with an elite hard winter wheat and development of backcross mapping populations was used to introgress and genetically map new sources resistance to stem rust race TTKSK from *Ae. tauschii* accessions TA10171 and TA10187. This work underscores the feasibility of transferring genes from *Ae. tauschii* and highlights the utility of D genome genetic resources in meeting future challenges to wheat production.

Materials and methods

Plant materials and direct hybridization

The diploid *Ae. tauschii* accessions TA10171 and TA10187 are from Turkmenistan and resistant to all *Pgt* races tested including TTKSK (Ug99), TTKST (Ug99 + *Sr24* virulence), TTTTF, RKQQC, TPMKC and QTHJC (Rouse et al. 2011). The *T. aestivum* recurrent parent is hard white winter wheat line KS05HW14 produced by Dr. Joe Martin, Kansas State University, Hays, KS. KS05HW14 has good agronomic performance but is seedling susceptible to most *Pgt* races including TTKSK, TTKST (Ug99 + *Sr24* virulence), TTTTF, TPMKC, RKQQC and QTHJC.

Emasculated florets of KS05WH14 were directly pollinated by TA10171 and TA10187 using the approach method (Rosenquist 1927). Between 14 and 17 days after pollination, caryopses containing fertilized embryos were removed from spikes for embryo rescue. Caryopses were surface-sterilized for 20 min in a 20 % bleach solution containing 0.001 % Tween 20® and rinsed three times in 40 mL ddH_2O for 20 min under sterile conditions in a laminar flow hood. Embryos were removed from caryopses and transferred to embryo culture media containing a mixture of 4.1 g/L Murashige and Skoog salts (Murashige and Skoog 1962) and Gamborg's B5 Vitamins (Gamborg et al. 1968) (Sigma Aldrich, M0404) with 3 % sucrose, 2 mg/L kinetin, 2 g/L phytagel (Sigma Aldrich, P8169) at pH 5.7.

Upon the development of shoot and root tissues, germinating embryos were transferred to 50 mL culture tubes containing a mixture of 4.1 g/L Murashige and Skoog salts and Gamborg's B5 Vitamins with 4 % maltose, 1.9 g/L MES buffer, 0.1 g/L ascorbic acid and 2 g/L phytagel at pH 5.7. After development of several roots greater than 10 cm, seedlings were placed in vernalization for 4 weeks at 4 °C. Vernalized seedlings were transferred directly to 12 cm pots containing Metro Mix 200 growth medium (Hummert, Earth City, MO) saturated with tap water. To prevent loss of turgor pressure, seedlings were immediately covered with clear plastic domes maintaining a 100 % humidity environment for 3–5 days. The domes were removed when guttation water was observed on the secondary leaves. F_1 plants were grown in the greenhouse for backcrossing under a regime of 10 h of supplemental light at 21 ± 4 °C for 6 weeks and then transitioned to 16 h of supplemental light at 23 ± 3 °C. At maturity, female spikes of the hybrids were pollinated by KS05HW14 to generate BC_1F_1 seed.

BC_1F_1 plants were evaluated for resistance to *Pgt* race QTHJC. Individual QTHJC-resistant BC_1F_1 plants derived from TA10187 and TA10171 were selected as males for backcrossing to generate BC_2F_1 mapping populations. The BC_2F_1 mapping populations consisted of 105 individuals (KS05HW14\3\KS05HW14\TA10187\\KS05HW14) and 107 individuals (KS05HW14\3\KS05HW14\TA10171\\KS05HW14) derived from TA10187 and TA10171, respectively. BC_2F_1 populations were evaluated for stem rust resistance as described below. Following the stem rust tests, BC_2F_1 plants were vernalized for 6 weeks at 4 °C, and then placed in a growth chamber with 10 h light at 20 °C and 14 h dark at 15 °C for 3 weeks and then transitioned to 16 h light at 23 °C and 8 h dark 20 °C until maturity. BC_2F_2 seed from individual plants was used for progeny testing of stem rust resistance as described below.

Stem rust resistance phenotyping

Reactions to *Pgt* races QTHJC (isolate 75ND717C) and race RKQQC (isolate 99KS76A-1) were assessed in facilities at Kansas State University. Urediniospores were removed from liquid nitrogen storage and heat-shocked in a 42 °C water bath for 5 min. Spores were suspended in Soltrol 170 isoparaffin oil (Chevron Phillips Chemical Company LP, The Woodlands, TX) and sprayed onto two to three-leaf-stage seedlings. Inoculated plants were incubated in a dew chamber at 20 ± 1 °C and 100 % relative humidity for 16 h and then placed in a growth chamber at 20 ± 1 °C with a 16 h light/8 h dark cycle. Infection types were scored 14 days after inoculation as described by Stakman et al. (1962). Seedlings with low infection types of ; to ;1+ to QTHJC and RKQQC were considered resistant and those with higher infection types of 3–4 were considered susceptible. Inoculations with *Pgt* race TTKSK (isolate 04KEN156/04) were done in a biocontainment safety level 3 facility at the University of Minnesota in conjunction with the USDA-ARS Cereal Disease Laboratory, St. Paul, MN, following previously described protocols (Jin et al. 2007). Seedlings yielding a low infection type of ;1–2+ to race TTKSK were considered resistant and seedlings yielding a high infection type of 3–4 were considered susceptible.

BC_1F_1 plants derived from direct crossing with TA10187 and TA10171 were tested with *Pgt* race QTHJC and resistant plants selected for backcrossing. BC_2F_1 mapping populations were evaluated using race RKQQC. To confirm the phenotypes of BC_2F_1 individuals in the mapping populations, 10 BC_2F_2 individuals from each BC_2F_1 plant were evaluated for reaction to race RKQQC.

To confirm that the new *Sr* genes are effective against race TTKSK, 24 individuals from each of 10 race RKQQC-resistant and -susceptible BC_2F_2 families were tested with race TTKSK. The donor parents TA10187 and TA10171 as well as the recurrent parent KS05HW14 were included as resistant and susceptible checks.

DNA isolation and bulked segregant analysis (BSA)

Genomic DNA from the BC_2F_1 mapping populations and TA10187, TA10171 and KS05HW14 parents was isolated using BioSprint 96 DNA Plant Kits (Cat. No. 941558) and a BioSprint 96 robot following the manufacturer's instructions (Qiagen, Valencia, CA).

Bulked segregant analysis was performed as described by Olson et al. (2013). Briefly, genomic DNA of eight RKQQC-resistant and eigh RKQQC-susceptible BC_2F_1 plants from each mapping population were pooled to generate resistant and susceptible bulks. A set of 70 D genome-specific SSR markers comprising five markers per chromosome arm were amplified from the resistant and susceptible bulks. In the BC_2F_1 mapping populations, SSR markers that were homozygous for the recurrent parent allele in the susceptible bulk and heterozygous in the resistant bulk indicated linkage to stem rust resistance.

Molecular marker analyses and genetic mapping

Following BSA, linked markers were analyzed on BC_2F_1 individuals of the mapping populations to develop genetic linkage maps. In the TA10187-derived BC_2F_1 mapping population, three SSR loci were evaluated on 6DS, including *Xbarc173*, *cfd49* and *psp3200*. In the TA10171-derived population, four SSR loci on 7DS including *Xcfd30*, *Xgdm88*, *Xwmc463*, and *Xwmc827* were evaluated. SSR loci *Xbarc70* and *Xgwm130*, located distally on 7DS, were evaluated to confirm a recombination breakpoint distal to *SrTA10171* in the BC_2F_1 mapping population. Marker loci

Table 1 Marker allele sizes, in base pairs, from the *SrTA10187* and *SrTA10171 Aegilops tauschii* donor accessions TA10187 and TA10171, and the hard winter wheat recurrent parent, KS05HW14

Gene	Locus	*Ae. tauschii*	KS05HW14
SrTA10187	*Xpsp3200*	182	165
	Xcfd49	196	219
	Xcfd135	148	124
	Xbarc173	275	237
SrTA10171	*Xwmc827*	132	171
	Xgdm88	118	116
	Xcfd30	177	174
	Xwmc463	156	152

were amplified following PCR conditions as described in Olson et al. (2013) (Table 1) and scored as co-dominant.

The *Lr34*-specific primer pairs L34DINT9F/L34MINUSR and L34SPF/L34DINT13R2 (Lagudah et al. 2009) were used to determine the *Lr34* status of *Ae. tauschii* accession TA10171, the recurrent parent KS05HW14 and stem rust-resistant and susceptible segregants. Reaction conditions for primer pairs L34DINT9F/L34MINUSR and L34SPF/L34DINT13R2 were as follows: 8.28 μL ddH_2O, 2.4 μL 10X reaction buffer, 0.9 μL 50 mM $MgCl_2$, 1.92 μL 2.5 mM dNTPs, 2.4 μL 1 pM forward primer, 2.0 μL 10 pM of reverse primer, 0.1 μL (0.5 U) Taq polymerase and 4 μL of template DNA (~30 ng/μL). Cycling conditions included an initial denaturation of 95 °C (1 min) followed by 35 cycles of 95 °C (1 min), 58 °C (60 s) and 72 °C (2 min), and a final extension at 72 °C (10 min).

Sizing of SSR marker PCR products was performed by capillary electrophoresis using a 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Analysis of SSR marker allele size was performed using GeneMarker 1.60 software (SoftGenetics, State College, PA).

Genetic maps were constructed using MAPMAKER v 3.0 (Lander et al. 1987). Marker orders were established using multipoint analysis and the Kosambi mapping function with a minimum LOD of 3.0 and validated using the ripple function. Segregation of marker loci and stem rust resistance was evaluated using a χ^2 goodness-of-fit test.

Results

Stem rust resistance from TA10187

Stem rust resistance was transferred from *Ae. tauschii* accession TA10187 to the hard winter wheat line KS05HW14 by direct crossing. Four F_1 hybrid plants were recovered by embryo rescue and 11 BC_1F_1 seeds were generated by backcrossing the male sterile F_1 plants. Four

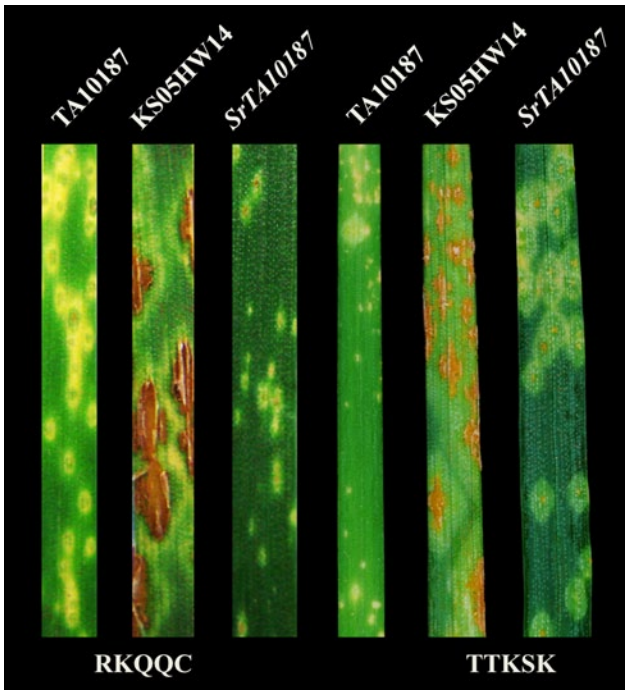

Fig. 1 Infection types produced by *Pgt* races RKQQC and TTKSK on *Aegilops tauschii* accession TA10187, recurrent parent hard winter wheat line KS05HW14 and backcross progenies with (*SrTA10187*) and without (*SrTA10187*) TA10187-derived stem rust resistance

BC_1F_1 seedlings were screened with *Pgt* race QTHJC and three seedlings yielded resistant ITs of ;1 to ;2-. A single race QTHJC-resistant BC_1F_1 plant was used as the male parent to generate a BC_2F_1 population for mapping. The stem rust resistance gene transferred from TA10187 is temporarily designated *SrTA10187*.

The *SrTA10187* BC_2F_1 mapping population segregated for reaction to race RKQQC with resistant plants yielding low infection types of ; to ;1+ similar to TA10187 with an infection type of ;1- and susceptible individuals yielding infection types of 3–4 similar to KS05HW14 with an infection type of 3+4 (Fig. 1). The population of 105 BC_2F_1 individuals segregated 39 resistant:66 susceptible. This ratio deviated from an expected 1:1 ratio for a single segregating locus ($\chi^2 = 4.94$, $p = 0.008$). However, the resistance appeared to be completely dominant with resistance fully expressed in heterozygous BC_2F_1 plants. Resistant and susceptible reactions to race RKQQC were confirmed in progeny tests of 10 BC_2F_2 progeny from each BC_2F_1 plant used in mapping *SrTA10187*.

Stem rust resistance selected with race RKQQC was confirmed to be effective against race TTKSK by progeny testing 20 BC_2F_2 families from the *SrTA10187* BC_2F_1 mapping population. When evaluated for reaction to race TTKSK, the *Ae. tauschii* donor, TA10187, yielded a low infection type of and KS05HW14 yielded a high infection

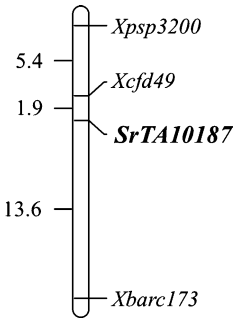

Fig. 2 Genetic map of *SrTA10187* on 6DS

type of 4. There was complete correspondence of resistance to *Pgt* races TTKSK and RKQQC in the BC_2F_2 families, confirming that resistance transferred from TA10187 selected with race QTHJC and mapped using race RKQQC, confers resistance to race TTKSK (Fig. 1).

Bulked segregant analysis using TA10187-derived BC_2F_1 individuals identified SSR locus *Xcfd135* as polymorphic between resistant and susceptible bulks and indicated 6DS was the chromosomal location of *SrTA10187*. TA10187 has a 148 bp allele at *Xcfd135* in contrast to the 124 bp allele from KS05HW14. At *Xcfd135*, the resistant bulk was heterozygous and the susceptible bulk was homozygous for the recurrent parent allele as expected for BSA in a backcross population and a dominant resistance phenotype. While *Xcfd135* was effective in distinguishing the bulks, this marker was not sufficiently consistent for evaluation of the entire BC_2F_1 mapping population and reliable genetic mapping.

To genetically map the resistance gene from TA10187, three SSR loci located distally on 6DS were evaluated on the entire BC_2F_1 mapping population. The three loci linked to *SrTA10187* on 6DS covered a genetic distance of 20.9 cM (Table 1; Fig. 2). The SSR locus, *Xcfd49* was 1.9 cM distal, and *Xbarc173* was 13.6 cM proximal to *SrTA10187*.

Stem rust resistance from TA10171

Stem rust resistance from *Ae. tauschii* accession TA10171 was also transferred to the hard winter wheat line KS05HW14 by direct crossing. One F_1 hybrid was recovered by direct crossing followed by embryo rescue. The F_1 plant was backcrossed as a female to KS05HW14 and 13 BC_1F_1 seeds were generated. Screening of six BC_1F_1 seedlings for stem rust resistance with race QTHJC identified two resistant plants with ITs ;1=. A single stem rust-resistant BC_1F_1 plant was backcrossed to KS05HW14 to produce a BC_2F_1 mapping population of 107 individuals. Stem rust resistance from TA10171 has been temporarily designated *SrTA10171*.

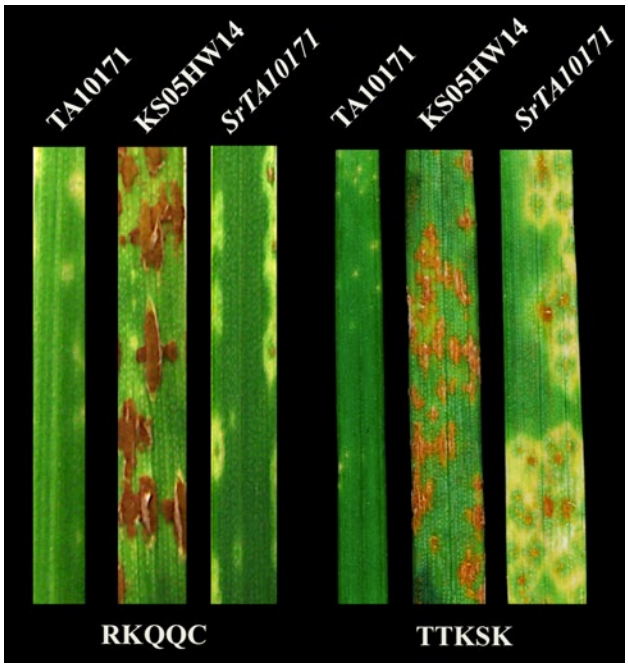

Fig. 3 Infection types produced by *Pgt* races RKQQC and TTKSK on *Aegilops tauschii* accession TA10171, recurrent parent hard winter wheat line KS05HW14 and backcross progenies with (*SrTA10171*) and without (*SrTA10171*) TA10171-derived stem rust resistance

The *SrTA10171* BC_2F_1 mapping population segregated for reaction to race RKQQC with a ratio of 42 resistant:65 susceptible. This again deviated from the expected 1:1 ratio ($\chi^2 = 4.94$, $p = 0.026$). However, as with *SrTA10187*, resistance was fully expressed in heterozygous BC_2F_1 plants. Resistant plants in this population had ITs ; to ;1 similar to the *Ae. tauschii* donor, TA10171, whereas the susceptible individuals were consistent with the recurrent parent KS05HW14 (IT of 3–4) (Fig. 3). Progeny testing of BC_2F_2 families with race RKQQC confirmed the stem rust phenotypes in the mapping population.

Stem rust resistance from TA10171 was also confirmed to be effective against race TTKSK. TA10171 and KS05HW14 gave the expected resistant and susceptible reactions, respectively, to TTKSK. The stem rust phenotype to race RKQQC in 20 BC_2F_2 families corresponded with resistance to race TTKSK (infection types of ;2- to 2 for resistant plants) demonstrating that *SrTA10171* mapped using race RKQQC conferred resistance to race TTKSK.

In the *SrTA10171* BC_2F_1 mapping population, BSA identified SSR locus *Xwmc827* on the short arm of chromosome 7D as polymorphic between resistant and susceptible bulks. The donor parent TA10171 has a 132 bp allele at *Xwmc827* and the susceptible recurrent parent KS05HW14 has a 146 bp allele. The resistant bulk was heterozygous at this marker locus while the susceptible bulk was homozygous for the recurrent parent allele.

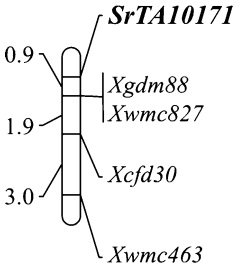

Fig. 4 Genetic map of *SrTA10171* on 7DS

Upon identification of 7D as the target chromosome, three additional SSR markers were used to genetically map *SrTA10171*. The four marker loci *Xcfd30, Xgdm88*, *Xwmc463* and *Xwmc827* were evaluated on the entire BC_2F_1 mapping population and covered a genetic distance of 5.8 cM (Fig. 4). Two co-segregating SSR loci, *Xgdm88* and *Xwmc827* mapped 0.9 cM proximal to *SrTA10171*. Two additional SSR loci, *Xbarc70* and *Xgwm130*, previously mapped in the distal region of 7DS, and located distally to *SrTA10171* based on consensus maps, were fixed for the KS05HW14 allele in the mapping population.

Lr34 status of parents and segregants in the *SrTA10171* mapping population was determined with the *Lr34*-specific primer pairs L34DINT9F/L34MINUSR and L34SPF/L34DINT13R2. Using L34DINT9F/L34MINUSR, the *Lr34* positive line Chinese Spring yielded no PCR product. In contrast, a ~550 bp PCR product was amplified from the *Ae. tauschii* accession TA10171, recurrent parent KS05HW14, two stem rust-resistant BC_2F_1 genotypes with *SrTA10171* and two stem rust-susceptible BC_2F_1 genotypes without *SrTA10171*. The primer pair L34SPF/L34DINT13R2, which indicates the presence of the *Lr34* functional allele, yielded a ~750 bp PCR product from Chinese Spring while the *Ae. tauschii* accession TA10171, recurrent parent KS05HW14, and BC_2F_1 genotypes with and without *SrTA10171* had no amplification. Taken together, these marker results for *Lr34* indicate that the *Ae. tauschii* accession TA10171 as well as the derived lines lack a functional *Lr34* allele.

Discussion

In this study, genes for resistance to stem rust race TTKSK were introgressed from *Ae. tauschii* accessions TA10187 and TA10171 and mapped on chromosome arms 6DS and 7DS, respectively. The integrated gene transfer and genetic mapping approach (Olson et al. 2013) delivered stem rust resistance genes from the diploid D genome species in a hexaploid wheat breeding background with good agronomic adaptation and closely linked genetic markers. The germplasm produced in this study will be useful for winter wheat breeding and the markers identified in this work can be used for marker-assisted selection (MAS) and gene pyramiding. The marker alleles identified in this study are expected to be unique to *Ae. tauschii*, and useful for MAS through backcrossing and forward breeding. As with any MAS application, though, it will be important to confirm resistance on marker selected individuals. The backcross-derived populations used for mapping *Sr* genes can also be used to develop resistant germplasm for breeding through phenotypic selection of resistant plants with good agronomic performance within the population. As *Pgt* race TTKSK is localized to Eastern and Southern Africa and the Middle East, identification and mapping of *SrTA10171* and *SrTA10187* was done with races QTHJC and RKQQC that are endemic to North America. All derived lines resistant to races QTHJC and RKQQC were confirmed to be resistant to TTKSK, thereby validating the effectiveness of mapping genes on 6DS and 7DS using races QTHJC and RKQQC as proxies.

The TA10187-derived stem rust resistance gene *SrTA10187* maps distally on chromosome 6DS close to the SSR locus *Xcfd49* (1.9 cM distal). This SSR marker should be useful in MAS for *SrTA10187*. The closest proximal locus is *Xbarc173* at 13.6 cM. Marker development in the region proximal to *SrTA10187* and high-resolution mapping are in progress and should yield more closely linked markers for use in high throughput SNP genotyping assays.

Several *Sr* genes are located in the distal region of 6DS including *Sr5* (RA McIntosh unpublished), *Sr42* (Ghazvini et al. 2012) and *SrCad* (Hiebert et al. 2011). All of the isolates used in this study are virulent on *Sr5*, suggesting *SrTA10187* is not *Sr5*. Both *Sr42* and *SrCad* confer resistance to stem rust races TTKSK and RKQQC. However, several races are virulent on these genes including race QTHJC. *SrTA10187* confers resistance to race QTHJC, thus it is likely to be different from *Sr42* and *SrCad* or a superior allele. Based on the mapping results from this study, *SrTA10187* appears to be approximately 5 cM distal to the published location of *Sr42* and *SrCad* (Hiebert et al. 2011). However, it is possible that *SrTA10187* is actually allelic with *Sr42* and *SrCad*. Allelism tests, are needed to confirm whether *SrTA10187* is a unique locus.

SrTA10171 was mapped on chromosome arm 7DS and closely linked SSR loci were identified. Marker *Xwmc827* mapped 0.9 cM proximal to *SrTA10171* and could be useful for MAS. Markers distal to the map location of *SrTA10171* were fixed for the recurrent parent alleles and did not segregate in the BC_2F_1 mapping population, allowing only proximal markers to be mapped. This suggests a recombination breakpoint located very closely distal to *SrTA10171* in the meiotic event producing the female gamete that

formed the BC_1F_1 plant used to develop the BC_2F_1 mapping population.

The *SrTA10171* gene from *Ae. tauschii* accession TA10171 is located approximately 9 cM distal to the pleiotropic stem rust, leaf rust, stripe rust and powdery mildew resistance gene designated *Sr57/Lr34/Yr18/Pm38* on 7DS (Krattinger et al. 2009). *Sr57* is known to act as a modifier of major genes (Hiebert et al. 2011) and confer quantitative stem rust resistance (Kolmer et al. 2011) in addition to conferring quantitative resistance to leaf rust, stripe rust and powdery mildew (Spielmeyer et al. 2005). Resistance conferred by *Sr57* is expressed at the adult plant stage through a non-hypersensitive response, whereas *SrTA10171* resistance is expressed at the seedling stage through a hypersensitive reaction (Rubiales and Niks 1995) (Fig. 4). The *Ae. tauschii* accession TA10171 and derived lines were confirmed to carry non-*Lr34* alleles demonstrating that *SrTA10171* is not the same as *Sr57/Lr34/Yr18/Pm38*.

Both *SrTA10171* and *SrTA10187* could be pyramided with other Ug99-effective *Sr* genes located on 7DS and 6DS, respectively. On 7DS, *SrTA10171* could be coupled with *Sr57* (*Lr34*) (Krattinger et al. 2009). A recombinant haplotype carrying both genes would be highly desirable to combine high levels of seedling stem rust resistance from *SrTA10171* with the broad spectrum adult plant resistance locus *Sr57/Lr34/Yr18/Pm38*. Further, the combination of seedling resistance genes and adult plant resistance genes for stem rust resistance has been shown to confer higher levels of resistance than each gene independently (Hiebert et al. 2011). While yet to be confirmed, *SrTA10187* may be linked distal to *Sr42* (Ghazvini et al. 2012) and *SrCad* (Hiebert et al. 2011). Although *Sr42* and *SrCad* may occupy the same locus as speculated by Ghazvini et al. (2012), perhaps at least two genes could be phased in coupling to generate a two-gene pyramid in the distal region of 6DS, thereby enhancing the durability of each resistance gene while targeting marker-assisted selection to a single chromosome. Both *SrTA10171* and *SrTA10187* will serve to complement existing stem rust resistance genes in chromosome-specific gene pyramids providing greater utility in breeding and extended durability.

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