

# Simultaneous transfer, introgression, and genomic localization of genes for resistance to stem rust race TTKSK (Ug99) from *Aegilops tauschii* to wheat

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**Abstract** Wheat production is currently threatened by widely virulent races of the wheat stem rust fungus, *Puccinia graminis* f. sp. *tritici*, that are part of the TTKSK (also known as ‘Ug99’) race group. The diploid D genome donor species *Aegilops tauschii* ( $2n = 2x = 14$ , DD) is a readily accessible source of resistance to TTKSK and its derivatives that can be transferred to hexaploid wheat, *Triticum aestivum* ( $2n = 6x = 42$ , AABBDD). To expedite transfer of TTKSK resistance from *Ae. tauschii*, a direct hybridization approach was undertaken that integrates gene transfer, mapping, and introgression into one process. Direct crossing of *Ae. tauschii* accessions with an elite wheat breeding line combines the steps of gene

transfer and introgression while development of mapping populations during gene transfer enables the identification of closely linked markers. Direct crosses were made using TTKSK-resistant *Ae. tauschii* accessions TA1662 and PI 603225 as males and a stem rust-susceptible *T. aestivum* breeding line, KS05HW14, as a female. Embryo rescue enabled recovery of  $F_1$  (ABDD) plants that were backcrossed as females to the hexaploid recurrent parent. Stem rust-resistant  $BC_1F_1$  plants from each *Ae. tauschii* donor source were used as males to generate  $BC_2F_1$  mapping populations. Bulked segregant analysis of  $BC_2F_1$  genotypes was performed using 70 SSR loci distributed across the D genome. Using this approach, stem rust resistance genes from both accessions were located on chromosome arm 1DS and mapped using SSR and EST-STS markers. An allelism test indicated the stem rust resistance gene transferred from PI 603225 is *Sr33*. Race specificity suggests the stem rust resistance gene transferred from TA1662 is unique and this gene has been temporarily designated *SrTA1662*. Stem rust resistance genes derived from TA1662 and PI 603225 have been made available with selectable molecular markers in genetic backgrounds suitable for stem rust resistance breeding.

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## Introduction

A highly virulent race of the wheat stem rust fungus, *Puccinia graminis* f. sp. *tritici*, was discovered in east Africa over a decade ago. This race is distinguished by novel virulence to stem rust resistance gene *Sr31* and was initially referred to as ‘Ug99’, although it was later given the standard race nomenclature designation, TTKSK (Singh et al. 2011). Race TTKSK and its derivatives are considered a serious threat to wheat and barley production

and considerable effort is being devoted to breeding for resistance.

Resistance to stem rust race TTKSK in adapted wheat germplasm is limited and most currently effective resistance genes originated from wild relatives in the secondary or tertiary gene pools of wheat (Singh et al. 2011). Alien translocations carrying TTKSK resistance are already present among some elite U.S. wheat cultivars and breeding lines including *Sr1RS<sup>Amigo</sup>* from *Secale cereale*, *Sr24* from *Thinopyrum elongatum* and *Sr36* from *T. timopheevii* (Olson et al. 2010a). However, virulence to these genes exists within the TTKSK race group (Jin et al. 2008, 2009).

Additional alien sources of resistance against TTKSK include *Sr22* from *T. boeoticum* (Olson et al. 2010b), *Sr26* from *Agropyron elongatum* (Dundas et al. 2007), *Sr35* from *T. monococcum* (Zhang et al. 2010), *Sr39* from *Aegilops speltoides* (Niu et al. 2011), *Sr44* from *A. intermedium* (Bernd Friebe, personal communication), *Sr47* from *Ae. speltoides* (Klindworth et al. 2012), *Sr50* (formerly *SrR*) from *S. cereale* (Anugrahwati et al. 2008), *Sr51* from *Aegilops searsii*, (Liu et al. 2011a), *Sr52* from *Dasypyrum villosum* (Qi et al. 2011), and *Sr53* from *Ae. geniculata* (Liu et al. 2011b). Introgressing resistance genes from wild species is difficult because they typically require production of compensating Robertsonian translocations. These translocations are inherited as non-recombining chromosome segments carrying genes conferring undesirable agronomic phenotypes in addition to the target genes. Induced homoeologous recombination can be used to reduce alien segments derived from the secondary or tertiary gene pools but residual linkage drag may remain and require further rounds of segment reduction (Qi et al. 2007).

In contrast, *Ae. tauschii* belongs to the primary gene pool of common wheat and has several advantages for introgressing resistance. Cultivated bread wheat, *Triticum aestivum* L. ( $2n = 6x = 42$ , AABBDD), is an allohexaploid species that arose approximately 8000 years ago from the hybridization of cultivated tetraploid ( $2n = 4x = 28$ , AABB) wheat with the diploid D genome donor species, *Ae. tauschii* Coss. ( $2n = 2x = 14$ , DD) (Kihara 1944; McFadden and Sears 1946). McFadden and Sears (1946) produced the first synthetic allohexaploid by doubling the chromosome number of  $F_1$  hybrids between tetraploid wheat and *Ae. tauschii*. Kihara and colleagues made large collections of *Ae. tauschii*, documented extensive genetic diversity, including resistance to leaf and yellow rust, and produced a number of synthetic hexaploid wheat lines capturing new sources of rust resistance (Kihara et al. 1957; Tanaka 1961). Production of synthetic hexaploids is now a routine procedure requiring only embryo rescue rather than specialized cytological techniques. The D genome chromosomes of *Ae. tauschii*

undergo normal meiotic pairing with D genome chromosomes of *T. aestivum* so linkage drag can be reduced by normal homologous recombination.

As an alternative to creating synthetic hexaploids, genes can be transferred from *Ae. tauschii* to wheat through direct crossing. A direct hybrid between hexaploid wheat and *Ae. tauschii* was reported by The (1973) but backcrossing of  $F_1$  hybrids to wheat was not attempted. Gill and Raupp (1987) first demonstrated the transfer of genes from *Ae. tauschii* through direct hybridization, followed by embryo rescue and back-crossing. Direct hybridization of diploid *Ae. tauschii* accessions with adapted hexaploid wheat provides a means to transfer D genome regions carrying target alleles of interest without disrupting adaptive allelic combinations in the A and B genomes while also providing D genome allelic diversity for agronomically important traits (Gill and Raupp 1987; Cox et al. 1995a).

In crosses between diploids and hexaploids,  $F_1$  progenies display altered expression of paternal and maternal alleles leading to aberrant endosperm formation (Tiwari et al. 2010). Hybrid embryos with the ABDD genomic constitution require embryo rescue and generation of seedlings on artificial growth media. To restore fertility and recover target alleles, hybrids are first backcrossed as females to hexaploid wheat. Partially fertile  $BC_1F_1$  plants display aneuploidy (Gill and Raupp 1987) and are backcrossed again to hexaploid wheat as males generating a high frequency of euploid ( $n = 21$ ) gametes by certation. Target alleles can be recovered in  $BC_2$  plants with restored chromosome number and fertility.

To date, three genes have been transferred from *Ae. tauschii* that are effective against TTKSK. These include *Sr33*, *Sr45* (Kerber and Dyck 1979; Sambasivam et al. 2008) and *Sr46* (Rouse et al. 2011). Kerber and Dyck (1979) developed synthetic hexaploids carrying *Sr33* and *Sr45* by hybridization of *Ae. tauschii* with ‘Tetra-Canthatch’, the extracted tetraploid of ‘Canthatch’. The *Ae. tauschii* accessions carrying these genes originated from different subspecies of *Ae. tauschii*. The *Sr33* donor, *Ae. tauschii* ssp. *strangulata* accession RL5288 (syn. TA1600) originates from northern Iran, near Tehran and the *Sr45* source, *Ae. tauschii* ssp. *tauschii* RL5289 (syn. TA1599) originates from northwestern Iran near Gilan (Jon Raupp, pers. comm.). *Sr33* appears to be widespread throughout the *Ae. tauschii* center of diversity in countries bordering the South Caspian Sea. Innes and Kerber (1994) identified *Sr33* in *Ae. tauschii* accessions ranging from Armenia to Iran and Turkmenistan.

Recently, Rouse et al. (2011) identified 98 *Ae. tauschii* accessions with resistance to race TTKSK. Infection type patterns using six races of *Pgt* suggested that resistance in some accessions could not be explained by *Sr33*, *Sr45*, or

*Sr46* alone or in combination. *Ae. tauschii*, therefore, is potentially a source of new genes that can be transferred to cultivated wheat. Initial transfers and genetic mapping of useful genes from *Ae. tauschii* was completed in a two-step approach (Kerber and Dyck 1969, 1979; Gill and Raupp, 1987; Cox et al. 1995a, b; Murphy et al. 1998, 1999). In the first step, hybridization and gene transfer was completed followed by introgression into a genetic background suitable for breeding and production of true breeding lines homozygous for the target gene. In the second step, fixed lines were used for chromosome mapping of target genes using either monosomic analysis in earlier studies or molecular markers in later studies.

The D genome chromosomes of *Ae. tauschii* undergo normal meiotic pairing with D genome chromosomes of *T. aestivum* and genetic transfers between species is facilitated by homologous recombination. Unrestricted pairing and recombination between *Ae. tauschii* chromosomes and hexaploid wheat D genome chromosomes allows for genetic studies to be integrated with the process of gene transfer and introgression. This study reports the simultaneous transfer, genetic mapping, and introgression into elite wheat germplasm, of genes for resistance to stem rust race TTKSK from *Ae. tauschii* accessions TA1662 and PI 603225.

## Materials and methods

### Plant materials

The diploid *Ae. tauschii* ssp. *tauschii* accession TA1662 is from the Wheat Genetic and Genomic Resource Center collection and originates from Azerbaijan. TA1662 is resistant to *Pgt* races TTKSK (Ug99), TTTTF, TRTTF and QTHJC and is susceptible to *Pgt* races RKQQC and TPMKC (Rouse et al. 2011) (Table 1). The *Ae. tauschii* ssp. *tauschii* accession PI 603225 is part of the National Small Grains Collection and originates from Turkmenistan. PI 603225 is resistant to *Pgt* races QTHJC, RKQQC, TPMKC, TTKSK, TTTTF (Table 1). The *T. aestivum* recurrent parent, KS05HW14, is a high yielding hard white winter wheat line developed by Dr. Joe Martin at the Kansas State University wheat breeding program in Hays, KS with the pedigree KS98HW452(KS91H153/KS93HW255)/CO960293//KS920709B-5-2(T67/X84W063-9-45//K92). KS05HW14 is resistant to soilborne wheat mosaic virus, wheat streak mosaic virus, and moderately resistant to stripe rust, but susceptible to *Pgt* races QTHJC, RKQQC, TPMKC, and TTKSK (Table 1).

### Direct hybridization and population development

Emasculated florets of KS05WH14 were pollinated by TA1662 and PI 603225 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<sup>®</sup> and rinsed three times in 40 mL ddH<sub>2</sub>O. All handling of caryopses and embryos were done 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<sup>-1</sup>. Murashige and Skoog 1962 salts (Murashige and Skoog 1962) and Gamborg's B5 Vitamins (Gamborg et al. 1968) (Sigma-Aldrich, M0404) with 3 % sucrose, 2 mg L<sup>-1</sup> kinetin, 2 g L<sup>-1</sup> phytigel (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<sup>-1</sup> Murashige and Skoog salts and Gamborg's B5 Vitamins with 4 % maltose, 1.9 g L<sup>-1</sup> MES buffer, 0.1 g L<sup>-1</sup> ascorbic acid and 2 g L<sup>-1</sup> phytigel at pH 5.7. When seedlings developed several roots greater than 10 cm, they were placed in vernalization for 4 weeks at 4 °C. Vernalized seedlings were transferred directly to 5" pots containing Metro Mix 200 growth media (Hummert International, Earth City, MO) saturated with tap water. To prevent loss of turgor pressure, seedlings were immediately covered with a clear plastic dome maintaining a 100 % humidity environment for 3–5 days. Plastic domes were removed when guttation water was observed on the new leaves.

F<sub>1</sub> plants were grown in the greenhouse for 6 weeks at 21 ± 4 °C with 10 h of supplemental light and then transitioned to 23 ± 3 °C with 16 h of supplemental light until flowering. At anthesis, spikes of the F<sub>1</sub> hybrid plants were pollinated by KS05HW14 to generate BC<sub>1</sub>F<sub>1</sub> seed. A single BC<sub>1</sub>F<sub>1</sub> plant resistant to stem rust race QTHJC was used as a male to generate a BC<sub>2</sub>F<sub>1</sub> mapping population of 138 individuals designated U6714. After stem rust phenotyping, BC<sub>2</sub>F<sub>1</sub> plants were vernalized, grown out, and then selfed. Resulting BC<sub>2</sub>F<sub>2</sub> families from individual plants were used for progeny testing for stem rust resistance. Stem rust resistance from *Ae. tauschii* accession PI 603225 was transferred by a similar approach selecting a single resistant BC<sub>1</sub>F<sub>1</sub> plant resistant to stem rust race RKQQC. Analysis of a BC<sub>2</sub>F<sub>1</sub> population derived from the BC<sub>1</sub>F<sub>1</sub> plant was done to confirm segregation of a single dominant gene for stem rust resistance. The stem rust resistance gene from PI 603225 was genetically mapped using a BC<sub>1</sub>F<sub>2</sub> population of 153 individuals designated U6369, which was derived from the same BC<sub>1</sub>F<sub>1</sub> plant.

### Stem rust phenotyping

Reactions to *Pgt* race QTHJC isolate 75ND717C and race RKQQC isolate 99KS76A-1 were tested in facilities at

**Table 1** Infection types of *Aegilops tauschii* accessions and the *Triticum aestivum* recurrent parent, KS05HW14 to six races of *Puccinia graminis* f. sp. *tritici*

Line	Species	Gene	QTHJC	RKQQC	TPMKC	TRTTF	TTKSK	TTTTF
RL5288 (TA1600)	<i>Ae. tauschii</i>	<i>Sr33</i>	2 <sup>a</sup>	2–	2	– <sup>b</sup>	2–	2
RL5289 (TA1599)	<i>Ae. tauschii</i>	<i>Sr45</i>	4	4	4	–	1	4
AUS18913 <sup>c</sup>	<i>Ae. tauschii</i>	<i>Sr46</i>	3	3+	2–	2	1,2–	3+
PI 603225	<i>Ae. tauschii</i>	<i>Sr33</i>	2	2–	2	–	2–	22+
TA1662	<i>Ae. tauschii</i>	<i>SrTA1662</i>	2–	3–	3–	1	2–	2–
KS05HW14	<i>T. aestivum</i>	–	3+, 4	3+, 4	4	–	4	–

<sup>a</sup> Infection type based on Stakman 0–4 scale

<sup>b</sup> Data not available

<sup>c</sup> Data taken from Table 4 of Rouse et al. (2011)

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 seedlings at the two to three leaf stage. 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 yielding a low infection type of 2– to 2+ were considered resistant and seedlings yielding a high infection type of 3– to 4 were considered susceptible. Populations derived from TA1662 were assayed using race QTHJC and race RKQQC that are avirulent and virulent on TA1662, respectively. PI 603225-derived seedling stem rust resistance was assayed using the avirulent race RKQQC. Inoculation of *Pgt* race TTKSK isolate 04KEN156/04 was carried out at a biocontainment safety level 3 facility at the University of Minnesota in conjunction with the USDA-ARS Cereal Disease Laboratory, Saint Paul, MN following protocols described previously by Jin et al. (2007). Seedlings yielding a low infection type of 2– to 2+ to race TTKSK were considered resistant and seedlings yielding a high infection type of 3– to 4 were considered susceptible.

To evaluate the effectiveness of the stem rust resistance gene from PI 603225 against TTKSK and related stem rust races in the field, PI 603225-derived lines were evaluated in hill plots in the US Winter Wheat Nursery at the KARI research station in Njoro, Kenya. In the 2011 nursery, three BC<sub>1</sub>F<sub>2;4</sub> lines homozygous for the gene from PI 603225: U6369R1-078, U6369R1-148, and U6369R1-200, were evaluated.

#### DNA isolation and PCR conditions

Genomic DNA was isolated using BioSprint 96 DNA Plant Kits (Cat. No. 941558) following the manufacturer's instructions (Qiagen, Valencia, CA). Reaction conditions

for simple sequence repeat (SSR) markers were as follows: 4.14 µL ddH<sub>2</sub>O, 1.2 µL 10× reaction buffer, 0.45 µL 50 mM MgCl<sub>2</sub>, 0.96 µL 2.5 mM dNTPs, 1.20 µL 1 pM of forward primer with a 19 bp M-13 tail (5'-ACGACGTTGT AAAACGAC), 1.00 µL 10 pM of reverse primer, 1.0 µL 10 pM of M-13 primer labeled with 6-FAM, VIC, PET, or NED, 0.1 µL (0.5 U) Taq polymerase and 2 µL of template DNA (30 ng/µL). Cycling conditions for all markers included an initial denaturation of 95 °C followed by 35 cycles of 95 °C (60 s), primer annealing temperature (48–62 °C; 60 s), and 72 °C (2 min), and a final extension at 72 °C (10 min). Primer sequences, specific annealing temperatures, and chromosome locations for SSR markers used in the bulked segregant analysis (BSA) and genetic mapping were obtained from GrainGenes2.0 (<http://wheat.pw.usda.gov/GG2/index.shtml>).

#### Molecular marker analyses and genetic mapping

Bulked segregant analysis was used to identify the chromosomal location of the resistance genes in TA1662 and PI 603225. Genomic DNA of eight resistant and eight susceptible BC<sub>2</sub>F<sub>1</sub> plants were pooled to generate resistant and susceptible bulks. A set of 70 D genome-specific SSR markers comprising five markers per chromosome arm was amplified from the resistant and susceptible bulks.

To map TA1662-derived resistance on 1DS, six SSR loci including *Xcfa2158*, *Xcfd15*, *Xgdm33*, *Xwmc147*, *Xwmc432* and *Xwmc222* and one EST-STS locus, *XBE489313*, were evaluated on the BC<sub>2</sub>F<sub>1</sub> population derived from TA1662 (U6714) following the PCR conditions described above. All markers were scored as co-dominant.

To map PI 603225-derived resistance on 1DS, six SSR loci on 1DS were evaluated on the BC<sub>1</sub>F<sub>2</sub> mapping population, U6369. Two loci, *Xbarc152* and *Xwmc147*, were scored as dominant for the KS05HW14 parent allele. The *Xcfa2158*, *Xwmc222*, *Xwmc336*, and *Xwmc432* loci were scored as co-dominant. Two EST-STS loci were also

evaluated, *XBE443103* and *XBE591682*, scored as dominant for the PI 603225 allele.

Sizing of PCR products was performed by capillary electrophoresis using a 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA). Analysis of allele sizes was performed using GeneMarker 1.60 software (SoftGenetics, State College, PA). Genetic linkage analysis was performed using MAPMAKER v 3.0 (Lander et al. 1987). Marker orders were established using multipoint analysis and the Kosambi centimorgan function with a minimum LOD of 3.0 and using the ripple function. Segregation of marker loci and stem rust resistance was evaluated using a  $\chi^2$  goodness-of-fit test.

An *Sr33* allelism test was conducted using diploid *Ae. tauschii* accessions TA1600 (RL5288)(*Sr33*) and PI 603225. TA1600 is resistant to *Pgt* races QTHJC, RKQQC, TPMKC and TTKSK (Rouse et al. 2011) (Table 1). TA1600 was crossed as a female to PI 603225. Progeny testing for stem rust resistance was performed with race RKQQC on 2,422 F<sub>2</sub> seedlings.

## Results

### Stem rust resistance from TA1662

By direct hybridization, stem rust resistance was transferred from the *Ae. tauschii* accession TA1662 to hexaploid wheat. Nine hybrid F<sub>1</sub> plants were recovered by embryo rescue. The male-sterile F<sub>1</sub> plants were backcrossed as females to KS05HW14 and nine BC<sub>1</sub>F<sub>1</sub> seed were recovered. To identify resistance BC progeny, the BC<sub>1</sub>F<sub>1</sub> plants were screened with race QTHJC along with the two parents. The stem rust-susceptible recurrent parent, KS05HW14, yielded a high infection type of 3 + 4 and the donor parent, TA1662, yielded a low infection type of 2–. Two BC<sub>1</sub>F<sub>1</sub> seedlings screened with race QTHJC gave infection types of 2 and 3. The QTHJC-resistant BC<sub>1</sub>F<sub>1</sub> plant was used as the male donor parent to generate a BC<sub>2</sub>F<sub>1</sub> mapping population (U6714), comprised of 138 progeny.

Segregation of a single gene for resistance to *Pgt* race QTHJC was observed in the BC<sub>2</sub>F<sub>1</sub> mapping population with resistant plants yielding low infection types of 2– to 2 and susceptible individuals yielding high infection types of 3 to 4. The observed segregation of 64 resistant: 74 susceptible in the BC<sub>2</sub>F<sub>1</sub> population was consistent with a 1:1 segregation ratio ( $\chi^2 = 0.72$ ,  $p = 0.39$ ) for a single dominant gene for stem rust resistance. All resistant and susceptible reactions to QTHJC in the mapping population were confirmed by progeny testing of 24 BC<sub>2</sub>F<sub>2</sub> progeny from each BC<sub>2</sub>F<sub>1</sub> plant. The TA1662-derived stem rust resistance gene is temporarily designated as *SrTA1662*.

Stem rust resistance conferred by *SrTA1662* is race specific (Table 1). Race QTHJC was used as an avirulent race to map *SrTA1662*. This indicates *SrTA1662* race specificity is different from *Sr45*, to which race QTHJC is virulent (Rouse et al. 2011). Ten individuals from all 138 BC<sub>2</sub>F<sub>2</sub> families in U6714 were inoculated with race RKQQC, which is virulent to *SrTA1662*. All individuals in all BC<sub>2</sub>F<sub>2</sub> families, including race QTHJC-resistant families, yielded high infection types of 3– to 4 to race RKQQC (Table 2), thus confirming virulence of race RKQQC to *SrTA1662*. RKQQC is avirulent to *Sr33*. Race RKQQC virulence to *SrTA1662* indicates race specificity that is different from *Sr33* (Rouse et al. 2011).

*SrTA1662* was confirmed to be effective against race TTKSK. Accession TA1662, recurrent hexaploid parent KS05HW14, and 20 different BC<sub>2</sub>F<sub>2</sub> families (24 individuals for each family) from the mapping population were assayed with race TTKSK. TA1662 and KS05HW14 gave expected resistant and susceptible reactions to race TTKSK. Segregation in all BC<sub>2</sub>F<sub>2</sub> families for resistance to race QTHJC correlated with resistance to TTKSK (infection types of 2– to 2+ for resistant plants) demonstrating that *SrTA1662* mapped using race QTHJC is the same gene that confers resistance to race TTKSK (Table 2).

**Table 2** Infection types of TA1662, KS05HW14, and 20 BC<sub>2</sub>F<sub>2</sub> families to wheat stem rust races QTHJC, RKQQC and TTKSK

Line	QTHJC	TTKSK	RKQQC
TA1662	2– <sup>a</sup>	2–	3–
KS05HW14	3	3	3+
U6714-004	3	3	3
U6714-022	3,3–	3,3–	3
U6714-069	33–	33–	3–,3
U6714-074	3	3	3
U6714-100	3	3	3–,3
U6714-118	3	4	3–,3
U6714-125	3	3	3
U6714-133	3	3	3
U6714-153	3	3	3,3–
U6714-159	3	3	3
U6714-002	2–/3 <sup>b</sup>	2–/3	3
U6714-016	2–/3	2–/3	3,3–
U6714-042	2–/3	2–/3	3,3+
U6714-075	2–/2 + 3,3	2–/2 + 3,3	3,3–
U6714-109	2/3	2/3	3
U6714-110	2/3–	2/3–	3,3–
U6714-112	2–/3	2–/3	3–,3+
U6714-141	2,2–/3	2,2–/3	3
U6714-155	2/3	2/3	3
U6714-163	2–/3	2–/3	3

<sup>a</sup> Infection type based on Stakman 0–4 scale

<sup>b</sup> Segregation for resistant and susceptible phenotypes indicated by “/”

BSA identified 1DS as the chromosome location of *SrTA1662*. Markers *Xwmc222* and *Xgdm33* differentiated the resistant and susceptible bulks from the BC<sub>2</sub>F<sub>1</sub> population. The resistant bulk carried the 161 bp TA1662 allele and the 167 bp KS05HW14 allele at *Xwmc222* whereas the susceptible bulk was fixed for the 167 bp allele from KS05HW14. The 154 bp allele from TA1662 at *Xgdm33* was present in the resistant bulk while the susceptible bulk was fixed for the 178 bp KS05HW14 allele. Together, these marker data place *SrTA1662* on chromosome 1DS. The seven loci linked to *SrTA1662* on 1DS (Table 3) cover a genetic distance of 35.8 cM in the BC<sub>2</sub>F<sub>1</sub> mapping population, U6714 (Fig. 1). Two SSR loci, *Xwmc432* and *Xwmc222* flank *SrTA1662* at 4.4 cM distal and 4.4 cM proximal, respectively. Eight recombinant genotypes in the BC<sub>2</sub>F<sub>1</sub> mapping population, U6714, place *SrTA1662* in the interval between *Xwmc432* and *Xwmc222* (Table 4).

#### Stem rust resistance from PI 603225

Stem rust resistance from *Ae. tauschii* accession PI 603225 was transferred and mapped using a similar approach. A BC<sub>1</sub>F<sub>2</sub> mapping population, designated as U6369, was used for mapping of PI 603225-derived resistance. The *Ae. tauschii* accession PI 603225 yields a low infection type of 2– to RKQQC while the stem rust-susceptible recurrent parent KS05HW14 yields a high infection type of 3+ to 4 to RKQQC. PI 603225-derived stem rust resistance followed segregation for a single dominant gene in BC<sub>2</sub>F<sub>1</sub> ( $\chi^2 = 2.08$ ,  $p = 0.15$ ) and BC<sub>1</sub>F<sub>2</sub> (U6369) ( $\chi^2 = 0.79$ ,  $p = 0.67$ ) populations with race RKQQC-resistant individuals yielding low infection types of 2– to 2 and susceptible individuals yielding high infection types of 3+ to 4.

**Table 3** Parental allele sizes in base pairs of SSR loci on 1DS linked to *SrTA1662* from TA1662 and *Sr33* from PI 603225

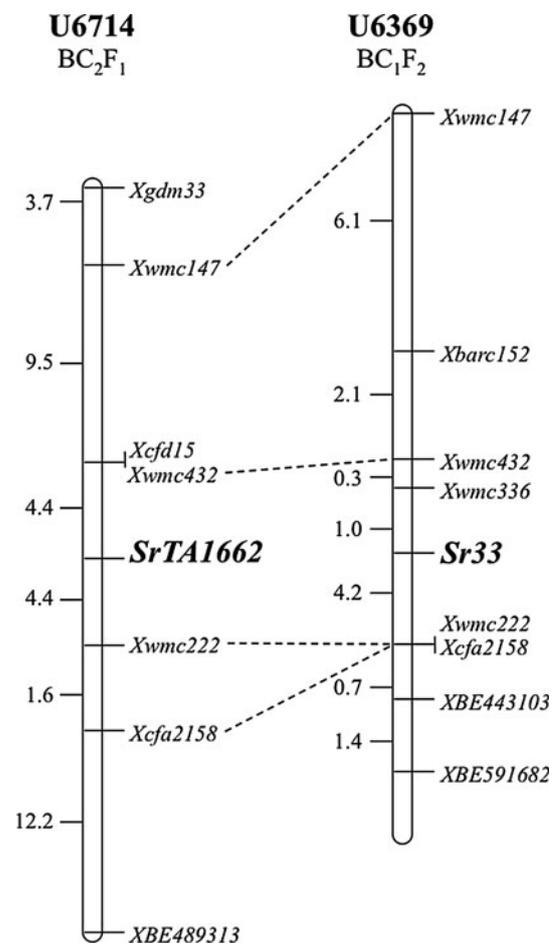
Locus	TA1662	PI 603225	KS05HW14
<i>Xgdm33</i>	154	na <sup>a</sup>	178
<i>Xwmc147</i>	143	– <sup>b</sup>	150
<i>Xbarc152</i>	na	–	134
<i>Xwmc432</i>	174	202	193
<i>Xcfd15</i>	196	na	159
<i>Xwmc336</i>	na	91	97
<i>Xwmc222</i>	161	160	167
<i>Xcfa2158</i>	214	214	204
<i>XBE591682</i>	na	921	930
<i>XBE489313</i>	339	na	342
<i>XBE443103</i>	na	781	783

<sup>a</sup> “na” indicates data are not available

<sup>b</sup> “–” indicates no allele is amplified from the *Aegilops tauschii* parent and the marker is dominant for the KS05HW14 allele

BSA determined 1DS as the chromosome location of PI 603225-derived stem rust resistance. The 160 bp PI 603225 allele of *Xwmc222* was present in the resistant bulk while the susceptible bulk was fixed for the 167 bp KS05HW14 allele. This placed the PI 603225-derived gene on chromosome arm 1DS. Further mapping of 1DS markers (Table 3) placed this gene in the same interval on 1DS as *SrTA1662* (Table 4; Fig. 1). Sixteen recombinants place PI 603225-derived stem rust resistance between SSR markers *Xwmc432* and *Xwmc222* (Table 5). All 2,422 F<sub>2</sub> seedlings from the allelism cross TA1600 (*Sr33*)/PI 603225 yielded low infection types of 2– to 2 to stem rust race RKQQC.

The stem rust resistance gene derived from PI 603225 is effective against race TTKSK under field conditions. Three homozygous-resistant BC<sub>1</sub>F<sub>2.4</sub> families were evaluated in the field in Njoro, Kenya in 2011. U6369R1-078, U6369R1-148, U6369R1-200 yielded adult plant severities and infection responses of 20RMR, 25RMR and 25RMR, respectively, while the recurrent parent KS05HW14



**Fig. 1** Genetic linkage maps showing the position of *SrTA1662* and *Sr33* from PI 603225 on 1DS

**Table 4** Recombinant genotypes at co-dominant SSR loci from the BC<sub>2</sub>F<sub>1</sub> mapping population, U6714 (*n* = 138), placing *SrTA1662* in the interval between *Xwmc432* and *Xwmc222*

Line	<i>Xgdm33</i>	<i>Xwmc147</i>	<i>Xcfd15</i>	<i>Xwmc432</i>	<i>SrTA1662</i> <sup>a</sup>	<i>Xwmc222</i>	<i>Xcfa2158</i>	<i>XBE489313</i>
U6714-170	a <sup>b</sup>	a	a	a	<i>srsr</i>	h <sup>c</sup>	h	h
U6714-121	a	a	a	a	<i>Srsr</i>	h	h	h
U6714-156	a	a	a	a	<i>Srsr</i>	h	h	h
U6714-063	a	a	a	a	<i>Srsr</i>	h	h	h
U6714-003	h	h	h	h	<i>Srsr</i>	a	a	a
U6714-084	h	h	h	h	<i>Srsr</i>	a	a	– <sup>d</sup>
U6714-030	h	h	h	h	<i>srsr</i>	a	a	a
U6714-062	h	h	h	h	<i>srsr</i>	a	a	a

<sup>a</sup> *srsr* indicates a line lacks the *SrTA1662* gene and is homozygous for stem rust susceptibility and *Srsr* indicates a line is heterozygous for *SrTA1662*

<sup>b</sup> “a” indicates a line is homozygous for the KS05HW14 parent allele at the marker locus

<sup>c</sup> “h” indicates a line is heterozygous for both TA1662 and KS05HW14 alleles

<sup>d</sup> “–” missing data

yielded a severity and infection response of 50S. Susceptible check cultivar ‘Jagger’ was rated 80S.

## Discussion

This study demonstrates the rapid and efficient transfer and introgression of two genes from the D genomes of diploid *Ae. tauschii* accessions into an elite hexaploid wheat background while concurrently mapping the genes to identify linked markers in one integrated process. Direct hybridization of *Ae. tauschii* with an elite winter wheat breeding line allows preservation of adapted gene complexes in the A and B genomes. By backcrossing introgression lines first as females to overcome male sterility and then as males to take advantage of pollen certation, fertile hexaploid lines carrying *Ae. tauschii*-derived stem rust resistance are quickly recovered. Development of segregating populations in the process of transferring genes from *Ae. tauschii* to *T. aestivum* facilitates chromosome localization and genetic mapping of stem rust resistance genes. Phenotypic selection of lines for use in stem rust resistance breeding can be done within progenies derived from BC<sub>2</sub>F<sub>1</sub> mapping populations.

A dominant stem rust resistance gene was transferred from PI 603225 to an elite hexaploid wheat breeding line. Race specificity and infection types for this gene are different from *Sr45* and *Sr46*, but similar to those of *Sr33* (Table 1). Bulk segregant analysis placed PI 603225-derived stem rust resistance on chromosome 1DS distal to *Sr45* and proximal to the published location of *Sr33* on chromosome arm 1DS (Sambasivam et al. 2008). It was initially thought to be a novel resistance gene, but a revised SSR map for *Sr33* places the PI 603225-derived

gene at the same locus flanked by *Xwmc336* and *Xwmc222* (S. Periyannan and E. Lagudah, pers. comm.). An allelism test using diploid *Ae. tauschii* accessions confirmed that the stem rust resistance gene from PI 603225 is *Sr33*.

Another dominant stem rust resistance gene was successfully transferred from the WGGRC *Ae. tauschii* accession TA1662 to the same elite hexaploid wheat breeding line. *SrTA1662* was mapped on chromosome 1DS and was demonstrated to confer resistance to TTKSK and QTHJC. Race QTHJC is avirulent to *SrTA1662*, thus differentiating this gene from *Sr45* on 1DS. Both *Sr33* and *SrTA1662* map to a region on 1DS flanked by the SSR loci *Xwmc432* and *Xwmc222* suggesting they occupy either the same locus or closely linked loci (Fig. 1). Race RKQQC is virulent to *SrTA1662*, thus differentiating this gene from *Sr33*. Presence–absence polymorphism and structural variation has been shown to generate variable haplotypes for disease resistance genes and resistance gene analogs in *Ae. tauschii* (Brooks et al. 2006). Similar structural variation could exist among *Ae. tauschii* genotypes carrying *Sr33* and *SrTA1662*. We cannot exclude the possibility that *SrTA1662* is a new allele of *Sr33*. However, evidence that *SrTA1662* is different from *Sr33* comes from an assay done using a marker derived from the *Sr33* gene sequence (S. Periyannan and E. Lagudah, pers. comm.). The TA1662 donor and lines carrying *SrTA1662* resistance lack the diagnostic *Sr33* marker. However, the PI 603225 donor and lines homozygous for PI 603225-derived resistance are positive for the diagnostic *Sr33* allele, supporting the previous conclusion that PI 603225-derived resistance is *Sr33*. Further work including allelism testing is needed to determine whether *SrTA1662* is a new allele of *Sr33* or represents a new stem rust resistance locus closely linked to *Sr33*.

**Table 5** Recombinant genotypes at co-dominant SSR loci from the BC<sub>1</sub>F<sub>2</sub> mapping population, U6369 ( $n = 153$ ), placing PI 603225-derived *Sr33* in the interval between *Xwmc432* and *Xwmc222*

Line	<i>Xwmc432</i>	<i>Xwmc336</i>	<i>Sr33</i> <sup>a</sup>	<i>Xwmc222</i>	<i>Xcfa2158</i>
U6369R1-103	a <sup>b</sup>	a	<i>srsr</i>	h <sup>c</sup>	h
U6369R1-150	a	a	<i>srsr</i>	h	h
U6369R1-053	a	a	<i>srsr</i>	h	h
U6369R1-183	a	a	<i>srsr</i>	h	h
U6369R1-048	h	a	<i>srsr</i>	h	h
U6369R1-049	h	h	<i>srsr</i>	a	a
U6369R1-082	h	h	<i>Srsr</i>	a	a
U6369R1-132	h	h	<i>Srsr</i>	a	a
U6369R1-180	h	h	<i>Srsr</i>	a	a
U6369R1-177	h	h	<i>Srsr</i>	b <sup>d</sup>	b
U6369R1-033	h	h	<i>SrSr</i>	b	b
U6369R1-126	h	h	<i>SrSr</i>	b	b
U6369R1-140	h	h	<i>SrSr</i>	b	b
U6369R1-182	b	b	<i>SrSr</i>	b	b
U6369R1-055	b	b	<i>SrSr</i>	h	h
U6369R1-167	b	b	<i>SrSr</i>	h	h

<sup>a</sup> Genotype of PI 603225-derived *Sr33*. An *srsr* indicates the line is homozygous stem rust susceptible; *Srsr* indicates the line is heterozygous stem rust-resistant and *SrSr* indicates the line is homozygous stem rust-resistant

<sup>b</sup> A black “a” in a white background indicates the line is homozygous for the KS05HW14 parent allele

<sup>c</sup> A black “h” in a gray background indicates the line is heterozygous for both PI 603225 and KS05HW14 alleles

<sup>d</sup> A white “b” in a black background indicates the line is homozygous for the PI 603225 parent allele

The addition of *Sr33* from PI 603225 and *SrTA1662* from TA1662 provides depth to the pool of stem rust resistance genes effective against the Ug99 lineage of stem rust races. The effectiveness of PI 603225-derived *Sr33* in an elite winter wheat background has been demonstrated under heavy disease pressure in Kenya against races TTKSK and TTKST, conferring adult plant severity and infection type from 20 to 25RMR. Many PI 603225-derived lines have shown acceptable agronomic traits in field tests and are ready for distribution to breeding programs. The effectiveness of *SrTA1662* to TTKSK has been demonstrated at the seedling stage and is currently being tested at the adult plant stage in the 2012 U.S. Winter Wheat Nursery at the KARI research station in Njoro, Kenya. Germplasm is currently being tested and selected for future distribution of *SrTA1662* resistance in adapted agronomic types.

Three stem rust resistance genes effective against TTKSK transferred from *Ae. tauschii* are present on 1DS including *Sr33*, *Sr45* (Sambasivam et al. 2008), and *SrTA1662*. At least two genes from *Ae. tauschii*, and potentially three if *Sr33* and *SrTA1662* are different loci, can be phased in coupling on 1DS. Combining resistance genes into coupling phase linkage blocks is desirable for two reasons: (1) combinations of effective genes are more likely to be durable than single genes; and (2) linkage increases the frequency of recovering the desired gene set in segregating populations. The rye-derived stem rust resistance gene, *Sr50* (formerly *SrR*) is also located on 1DS and recombinants with reduced rye chromatin are available

for stem rust resistance breeding efforts (Anugrahwati et al. 2008). Markers for *SrTA1662*, *Sr33*, *Sr45* and *Sr50* provide a means to generate gene pyramid linkage blocks on 1DS. With marker-assisted selection for stem rust resistance genes targeted to a single chromosome arm, genome-wide constraints associated with selection of multiple unlinked genes are relaxed, thereby reducing constraints placed on breeding population sizes by early generation enrichment of target alleles.

This study demonstrates the exceptional feasibility of transferring major gene disease resistance from *Ae. tauschii* directly to cultivated hexaploid wheat. Genes for resistance to the ‘Ug99’ stem rust pathogen were transferred from the diploid species to hexaploid wheat and mapped to chromosomal locations within three generations, highlighting the speed with which alleles can be recovered from *Ae. tauschii*. Homology between the *Ae. tauschii* D genome and the D genome of hexaploid wheat allows for genetic studies to be integrated with the introgression and germplasm development process yielding valuable closely linked markers for use in breeding. This study further demonstrates the *Ae. tauschii* D genome is a valuable genetic resource for meeting future challenges to wheat production.

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