Chromosome Res (2011) 19:669–682
DOI 10.1007/s10577-011-9226-3

Discovery and molecular mapping of a new gene conferring resistance to stem rust, *Sr53*, derived from *Aegilops geniculata* and characterization of spontaneous translocation stocks with reduced alien chromatin

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Received: 14 March 2011 / Revised: 27 May 2011 / Accepted: 16 June 2011 / Published online: 5 July 2011

Abstract This study reports the discovery and molecular mapping of a resistance gene effective against stem rust races RKQQC and TTKSK (Ug99) derived from *Aegilops geniculata* (2*n*=4*x*=28, $U^gU^gM^gM^g$). Two populations from the crosses TA5599 (T5DL-$5M^gL{\cdot}5M^gS$)/TA3809 (*ph1b* mutant in Chinese Spring background) and TA5599/Lakin were developed and used for genetic mapping to identify markers linked to the resistance gene. Further molecular and cytogenetic characterization resulted in the identification of nine spontaneous recombinants with shortened *Ae. geniculata* segments. Three of the wheat–*Ae. geniculata* recombinants (U6154-124, U6154-128, and U6200-113) are interstitial translocations (T5DS·5DL-$5M^gL$-5DL), with 20–30% proximal segments of $5M^gL$ translocated to 5DL; the other six are recombinants (T5DL-$5M^gL{\cdot}5M^gS$) have shortened segments of $5M^gL$ with fraction lengths (FL) of 0.32–0.45 compared with FL 0.55 for the $5M^gL$ segment in the original translocation donor, TA5599. Recombinants U6200-64, U6200-117, and U6154-124 carry the stem rust resistance gene *Sr53* with the same infection type as TA5599, the resistance gene donor. All recombinants were confirmed to be genetically compensating on the basis of genomic in situ hybridization and molecular marker analysis with chromosome 5D- and $5M^g$-specific SSR/STS-PCR markers. These recombinants between wheat and *Ae. geniculata* will provide another source for wheat stem rust resistance breeding and for physical mapping of the resistance locus and crossover hot spots between wheat chromosome 5D and chromosome $5M^gL$ of *Ae. geniculata*.

Keywords wheat · *Aegilops geniculata* · stem rust · Ug99 · recombinants

Abbreviations

STS	Sequence-tagged sites
PCR	Polymerase chain reaction
EST	Expressed sequence tag
NEB	New England biolab restriction endonuclease buffer
SSR	Simple sequence repeat
LOD	Logarithm of odds
GISH	Genomic in situ hybridization

Responsible Editor: Pat Heslop-Harrison.

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SPOT this is not an abbreviation but the name of a camera
IT Infection type
FL Fraction length

Introduction

Stem rust of wheat, caused by *Puccinia graminis* f. sp. *tritici*, is one of the most significant threats to global wheat production (Singh et al. 2008a). The emergence of the Ug99 group of stem rust races (TTKSK, TTKSF, TTKST, TTTSK) has reaffirmed the need to deploy diverse and effective resistance sources to safeguard wheat production (Pretorius et al. 2000; Wanyera et al. 2006; Jin and Singh 2006; Jin et al. 2008a, b, 2009a; Singh et al. 2006, 2008a, b). Races in the Ug99 race complex are virulent to resistance genes deployed in most wheat varieties currently under cultivation throughout the world, and more than half of the 46 catalogued wheat stem rust-resistant genes are ineffective (Singh et al. 2006, 2008a; McIntosh et al. 2008).

Breeding resistant cultivars is the most practical approach to protect wheat from stem rust. Deployment of combinations of effective genes "stacked" or "pyramided" in combination with adult plant resistance should improve the durability of resistance in commercial cultivars by reducing the probability of corresponding simultaneous mutation events in the pathogen. Gene pyramiding is facilitated by the ability to use molecular markers closely or completely linked to resistance genes. Molecular markers have been developed for numerous stem rust resistance genes, including: *Sr2* (Spielmeyer et al. 2003; Hayden et al. 2004; Mago et al. 2011), *Sr6* (Tsilo et al. 2009), *Sr9a* (Tsilo et al. 2007), *Sr13* (Admassu et al. 2011; Simons et al. 2011), *Sr22* (Khan et al. 2005; Olson et al. 2010; Periyannan et al. 2011), *Sr24* (Mago et al. 2005), *Sr25* (Liu et al. 2010), *Sr26* (Mago et al. 2005; Liu et al. 2010), *Sr30* (Hiebert et al. 2010a), *Sr31* (Das et al. 2006; Weng et al. 2007), *Sr32* (Bariana et al. 2001), *Sr33* (Sambasivam et al. 2008), *Sr35* (Zhang et al. 2010), *Sr36* (Bariana et al. 2001; Tsilo et al. 2008), *Sr39* (Gold et al. 1999; Mago et al. 2009; Niu et al. 2011), *Sr40* (Wu et al. 2009), *Sr45* (Sambasivam et al. 2008), *Sr50* (synonym *SrR*, Anugrahwati et al. 2008), *Sr51* (Liu et al. 2011), *Sr52* (Qi et al. 2011), *SrCad* (Hiebert et al. 2010b), and *SrWeb* (Hiebert et al. 2010a). Recent progress on molecular marker development and improved donor sources should accelerate the pyramiding and deployment of cultivars with more durable resistance to stem rust.

New sources of Ug99 resistance from alien species of wheat have been recently reported (Jin et al. 2009b; Xu et al. 2009; Liu et al. 2011; Qi et al. 2011), and a number of resistance genes from *Aegilops speltoides* Tausch, *Dasypyrum villosum* (L.) Candargy, and *Aegilops searsii* Feldman & Kislev ex Hammer are being transferred into common wheat (Faris et al. 2008; Pumphrey et al. 2009). The successful use of alien genes is mostly determined by the ability of the introduced alien chromosome segments to substitute for homoeologous chromosome segments of wheat. Translocations with small alien fragments have less likelihood of a linkage drag, which can depress essential agronomic and end-use quality traits. The development of wheat-alien compensating translocations with minimal alien chromatin by manipulating homoeologous recombination can enhance the commercial exploitation of wild relatives in wheat improvement (Sears 1977; Friebe et al. 1996; Qi et al. 2007).

Aegilops geniculata Roth (syn. *Aegilops ovata* Roth) or ovate goatgrass is an annual allotetraploid species ($2n=4x=28$, $U^gU^gM^gM^g$) that arose from hybridization between *Aegilops umbellulata* Zhuk. ($2n=2x=14$, UU) and *Aegilops comosa* Sm. ($2n=2x=14$, MM; Kihara 1954; Kimber and Abu-Bakar 1981; Kimber and Yen 1988; Kimber et al. 1988). *Ae. geniculata* is native to the Balkans, Mediterranean region, Russia, and Near East (Kimber and Feldman 1987) and is an important source of resistance to diseases and pests (Gill et al. 1985; Zaharieva et al. 2001a; Schneider et al. 2008); salt tolerance (Siddiqui and Yosufzai 1988); drought and heat adaption (Zaharieva et al. 2001b); and high grain protein content, high iron and zinc content, and early maturity (Rawat et al. 2009). A complete set of wheat–*Ae. geniculata* disomic addition lines and several ditelosomic addition and disomic substitution lines were developed by Friebe et al. (1999). A number of agronomically useful traits such as the leaf rust resistance gene *Lr57*, stripe rust resistance gene *Yr40*, and powdery mildew resistance gene *Pm29* have been incorporated into cultivated wheat by the development of wheat–*Ae. geniculata* translocation lines (Dhaliwal et al. 2002; Zeller et al. 2002; Kuraparthy et al. 2007).

We report the identification of the stem rust resistance gene *Sr53* derived from the long arm of chromosome $5M^g$ of *Ae. geniculata*, which is effective against Ug99 (race TTKSK). This gene was introgressed into wheat in the form of a T5DL-$5M^gL{\cdot}M^gS$ translocation (Aghaee-Sarbarzeh et al. 2002; Kuraparthy et al. 2007). In addition to identifying this resistance gene, we further produced and characterized compensating recombinants with shortened $5M^gL$ segments derived from *Ae. geniculata*. Closely linked molecular makers were developed, which will facilitate its use in wheat improvement.

Materials and methods

Plant material

All plant material is maintained by the Wheat Genetic and Genomic Resources Center at Kansas State University (http://www.k-state.edu/wgrc/). A complete set of *Ae. geniculata* addition lines in a Chinese Spring background (*Ae. geniculata* donor accession TA2899; Friebe et al. 1999) was initially screened for stem rust response. TA7659 is a Chinese Spring disomic addition line with chromosome $5M^g$ from TA2899. The available ditelosomic addition line with the short arm of $5M^g$ (TA7670) was also screened for stem rust response; a long arm ditelosomic addition line is not available in this set. TA5599 (T5DL-$5M^gL{\cdot}M^gS$, synonym T550) is a wheat–*Ae. geniculata* translocation line derived from BC_2F_5 hybrids between a disomic substitution line DS$5M^g$(5D) and the Chinese Spring (CS) Ph^I stock (Chen et al. 1994), followed by backcrossing to the bread wheat cultivar WL711 (Aghaee-Sarbarzeh et al. 2002; Kuraparthy et al. 2007). TA5602 [T5DL·5DS-$5M^gS$ (0.95)] is a *Lr57*/*Yr40* donor characterized by Kuraparthy et al. (2007). TA5599, CS *ph1b ph1b* (TA3809), CS (TA3008), and Lakin (PI 617032) were used to select $5M^g$-specific molecular markers. The nullisomic–tetrasomic line CS N5DT5A (TA3066) and the ditelosomic lines CS Dt5DL (TA3127), TA3809, CS, Lakin, and WL711 were used as controls for the molecular characterization of wheat–*Ae. geniculata* recombinants.

Two mapping populations were developed to map molecular markers linked to the newly discovered resistance gene and for the development of wheat–*Ae. geniculata* recombinants. Population U6200 was derived from the cross TA5599/CS *ph1b ph1b* and consisted of 120 F_2 plants and F_3 families. Population U6154 was derived from the cross TA5599/Lakin and consisted of 150 F_2 plants and F_3 families. Lakin is a stem rust-susceptible hard winter wheat cultivar. Young leaves were collected from each individual line or F_2 plant, and genomic DNA was extracted using a BioSprint96 workstation following the protocol described in the BioSprint DNA Plant Handbook (Cat. no. 941558, Qiagen Inc., Valencia, CA, USA).

$5M^g$-specific PCR marker selection and molecular mapping of the resistance gene derived from *Ae. geniculata*

A total of 63 STS-PCR primers on the short arms and 63 STS-PCR primers on the long arms of wheat group 5 chromosomes were used to develop $5M^g$-specific PCR markers. STS-PCR primers specific for group 5 were designed on the basis of wheat expressed sequence tags (EST) mapped to wheat group 5 by the wheat EST Mapping Project (http://wheat.pw.usda.gov/NSF/project/mapping_data.html). STS-PCR amplification was performed according to Qi et al. (2007, 2008). PCR-amplified products were then divided into 10 μL aliquots and digested with six different four-base recognition restriction enzymes (*Alu*I, *Hae*III, *Mse*I, *Msp*I, *Rsa*I, and *Mbo*I) for 2 h at 37°C by adding 5 μL of an enzyme mixture composed of 3.25 μL ddH_2O, 1.5 μL NEB buffer 2 or 4, 0.15 μL 100X BSA, and 0.1 μL enzyme stock solution. PCR products were resolved on 1.5% agarose gels and visualized by ethidium bromide staining under UV light.

The SSR marker GWM292, located in the chromosome deletion bin C-5DL1-0.60 (Sourdille et al. 2004) and polymorphic for the long arm of chromosome $5M^g$ of *Ae. geniculata* (Aghaee-Sarbarzeh et al. 2002), together with four polymorphic STS-PCR markers developed in this study (Table 1), were used to genetically map the resistance gene. Genetic linkage between the molecular markers and the resistance gene was analyzed using JoinMap (version 3.0, Van Ooijen and Voorrips 2001) with a LOD (logarithm of odds) threshold at 3.0. The genetic distance in centimorgans (cM) was calculated according to Kosambi function (Kosambi 1944).

Table 1 Primer sequences of *Ae. geniculata* $5M^g$-specific STS-PCR markers on wheat group 5 chromosomes and primer/enzyme combinations producing $5M^g$ polymorphism

Marker	Forward/reverse primer 5′–3′	Location (deletion bin)	Enzyme for polymorphism	EST accession/SSR
Xbe500291	5′ GCCTAACTCGGATGAGGATG 3′ 5′ TTTCTTCACTCGGTCCCTTG 3′	5AS1-0.4–0.75	*Mbo*I	BE500291
Xbe443021	5′ CATCGCCACTTCTGCTACAA 3′ 5′ CACTACGACTACGCGTGCAT 3′	C-5BL6-0.29	*Mbo*I	BE443021
Xbe442814	5′ CACCGACGAGTTGTACATGC 3′ 5′ ACCAGGACAATTTTGGCAAG 3′	C-5BL6-0.29	*Hae*III	BE442814
Xbe442600	5′ GAGGGAGATGGCCACAGATA 3′ 5′ GAATTTAGCTCCGCCTCCTT 3′	5BL-0.55–0.75	*Mse*I	BE442600
Xgwm292	5′ TCACCGTGGTCACCGAC 3′ 5′ CCACCGAGCCGATAATGTAC 3′	C-5DL1-0.60	–	GWM292

Evaluation of responses to stem rust

Stem rust response was assessed on TA5599, TA7659, TA7670, TA5602, TA3809, Lakin, each F_1, single F_2 plants, and the F_3 families (>12 plants per family) of the mapping populations with stem rust race RKQQC at Kansas State University, Manhattan, Kansas. Stem rust inoculation was as described by Wu et al. (2009). Infection types were scored at 12–14 days post-inoculation based on a 0–4 scale, where 0 corresponds to host immunity and 4 to complete host susceptibility (Roelfs and Martens 1988). Variation in pustule size within infection type classes was recorded by adding "+" or "–" signs if the infection types were larger or smaller than typical, respectively. When multiple infection types were observed on the same leaf, all were recorded. For example, infection type "33+" indicated that both infection types "3" and "3+" were observed. Plants with an infection type of 2+ or less were considered to be resistant to stem rust, whereas an infection type of 33+ or greater was scored as susceptible. Resistance to stem rust race TTKSK (Ug99) was evaluated on homozygous F_3 plants of the mapping populations and homozygous wheat–*Ae. geniculata* recombinants with shortened alien fragments at USDA-ARS Cereal Disease Laboratory, University of Minnesota, St. Paul, MN, USA, following procedures reported previously (Jin and Singh 2006).

Identification of wheat–*Ae. geniculata* recombinants with shortened alien chromatin

Root tips were collected from F_3 seeds derived from single resistant F_2 plants lacking at least one $5M^g$-specific marker to identify putative recombinants by genomic in situ hybridization (GISH). GISH was performed according to Zhang et al. (2001) with the following modifications: (1) genomic DNA for probe labeling was extracted from the M genome donor *Ae. comosa* using a DNeasy Plant Mini Kit following the manufacturer's instructions (Qiagen Inc.); (2) post-hybridization washes were in 2X SSC twice at room temperature for 5 min each, twice at 42°C for 10 then 5 min, and once at room temperature for 5 min. Fluorescent images were captured with a SPOT2.1 charge-coupled device camera (Diagnostic Instruments, Sterling Heights, MI, USA) using an epifluorescence Zeiss Axioplan 2 phase contrast and fluorescence. Images were processed with Adobe Photoshop CS3 (version 10.0.1, Adobe Systems Incorporated, San Jose, CA, USA).

Molecular characterization of wheat–*Ae. geniculata* recombinants

Homozygous recombinants confirmed by GISH were used for molecular characterization with 5D-specific SSR markers based on the SSR physical map of Sourdille et al. (2004). PCR was performed with 15 μL of the reaction mixture containing 1X PCR buffer (Bioline USA Inc., Taunton, MA, USA), 2 mM $MgCl_2$, 0.25 mM dNTPs, 5 pmol forward primer and reverse primer, respectively, 0.02 U/μL Taq DNA polymerase (Bioline USA Inc.), and 90 ng genomic DNA. PCR amplification was according to Wu et al. (2009). PCR products were resolved on 2.5% agarose gels and visualized by ethidium bromide staining under UV light.

Results

Discovery of a new stem rust resistance gene

Screening the available set of *Ae. geniculata* addition lines in a Chinese Spring background (Friebe et al. 1999) revealed that the disomic addition line with 5M^g (TA7659) was resistant to race RKQQC. The ditelosomic addition line with the short arm of 5M^g (TA7670) was susceptible; a long arm ditelosomic addition line is not available. These results prompted testing of the existing 5M^g translocation stocks for stem rust resistance (Kuraparthy et al. 2007). The *Ae. geniculata* accession, TA10437, used to develop the *Lr57/Yr40* material described by Kuraparthy et al. (2007) is different from the donor TA2899 accession used for the available Chinese Spring stocks. However, TA5599 (T5M^{g}S·5M^{g}L-5DL) was resistant to RKQQC with an infection type (IT) 22+ (Table 5), while TA5602 [T5DL·5DS-5M^{g}S (0.95)] and the recurrent parent WL711 were susceptible (data not shown). The low infection types were similar between the TA7659 (IT 2) and TA5599 (2-2) when tested against TTKSK (Table 5), indicating that both *Ae. geniculata* donor accessions carry a resistance gene or genes on the long arm of 5M^g.

Development of 5M^g-specific STS-PCR markers for *Ae. geniculata*

One 5M^g short arm-specific STS-PCR marker BE500291/*Mbo*I (primer/enzyme combination) and three 5M^g long arm-specific makers (BE443021/*Mbo*I, BE442814/*Hae*III, and BE442600/*Mse*I) were developed after testing 126 STS-PCR primers (63 for each 5M^g arm) with six four-base recognition restriction enzymes (*Alu*I, *Hae*III, *Mse*I, *Msp*I, *Rsa*I, and *Mbo*I). The STS-PCR marker BE500291/*Mbo*I is a 5M^g short arm-specific marker, and its corresponding EST is located in the deletion bin 5AS1-0.4–0.75. The marker BE443021/*Mbo*I and BE442814/*Hae*III are proximal 5M^g long arm markers located in the deletion bin C-5BL6-0.29, and the marker BE442600/*Mse*I is a 5M^{g}L long arm marker located in the bin 5BL-0.55–0.75 (Table 1 and Fig. 1).

Molecular mapping of resistance derived from *Ae. geniculata*

A total of 120 F_2 plants and F_3 families of population U6200 and 150 F_2 plants and F_3 families of population U6154 were scored for their response to stem rust race RKQQC. Segregation of resistance in population U6200 was consistent with a 1:2:1 ratio, where 28 F_2 plants were homozygous resistant, 59 were heterozygous for resistance, and 33 were homozygous susceptible (Table 2). Population U6154 also showed a 1:2:1 segregation with 31 homozygous resistant plants, 68 heterozygous resistant plants, and 50 homozygous susceptible plants (Table 2).

The five SSR and STS-PCR markers tested in each population were closely linked to the resistance gene, designated as *Sr53*, at genetic distances ranging from

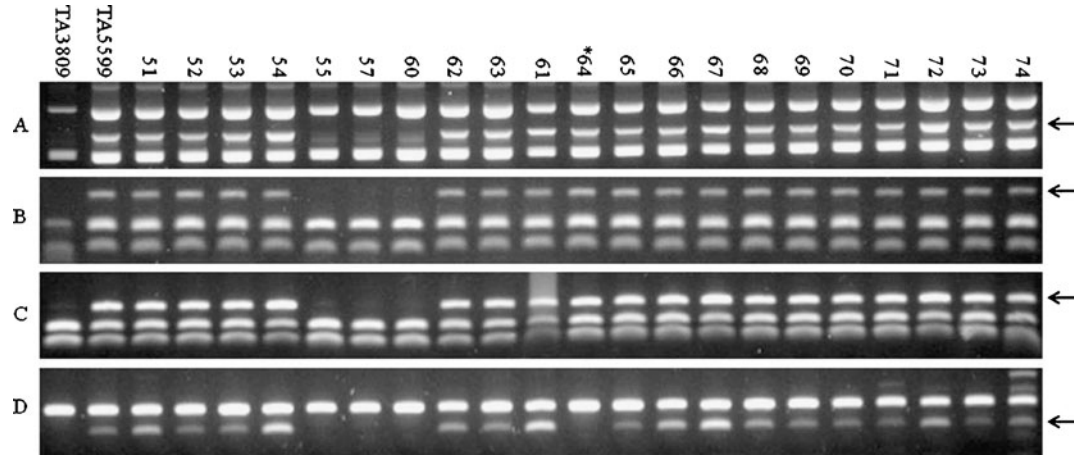


Fig. 1 STS-PCR patterns of TA3809 (*ph1b ph1b* mutant of Chinese Spring), TA5599 (T5DL-5M^{g}L·5M^{g}S), and single F_2 plants of population U6200 (TA5599/TA3809). Primer/enzyme combination is: BE500291/Mbo*I* (**a**); BE443021/Mbo*I* (**b**); BE442814/Hae*III* (**c**); E442600/Mse*I* (**d**). *Arrows* indicate the 5M^g-specific fragments. *Plant 64 is a recombinant missing marker BE442600/Mse*I*

Table 2 Segregation of stem rust resistance gene *Sr53* and linked molecular marker loci in F_3 populations derived from the crosses TA5599/TA3809 (U6200) and TA5599/Lakin (U6154)

Population	Gene/Marker	F_2 genotype[a]			Total	χ^2	$P_{0.05}$	$p_{0.01}$
		A_1A_1	A_1A_2	A_2A_2				
U6200	*Sr53*	28	59	33	120	0.29	10.6	13.82
	Xbe442600	90		30	120	0.54	7.88	10.83
	Xbe443201	90		30	120	0.01	7.88	10.83
	Xbe442814	90		30	120	0.01	7.88	10.83
	Xbe500291	90		30	120	0.01	7.88	10.83
	Xgwm292	89		30	119	0.00	7.88	10.83
U6154	*Sr53*	31	68	50	149	5.40	10.6	13.82
	Xbe442600	100		50	150	5.12	7.88	10.83
	Xbe443201	99		51	149	6.01	7.88	10.83
	Xbe442814	99		51	149	6.01	7.88	10.83
	Xbe500291	99		51	149	6.01	7.88	10.83
	Xgwm292	97		51	148	6.57	7.88	10.83

[a] F_2 genotype was determined by phenotypes of F_3 families. A_1A_1: homozygous for resistant allele; A_1A_2: heterozygous; A_2A_2: homozygous for susceptible allele

1.2 to 4.7 cM (Fig. 2). The *Sr53* locus was mapped between maker *Xbe442600* and the other four markers in population U6200, whereas the gene was proximal to all the markers in U6154. The closest markers linked to the resistance gene *Sr53* were *Xbe442600* located in the bin 5BL1-0.55–0.75 and *Xbe443201* (C-5DL6-0.29).

Identification of wheat–*Ae. geniculata* recombinants by molecular markers

Nine putative recombinants based on the disassociation of linked markers were identified by screening single F_2 plants from populations U6154 and U6200 with four $5M^g$-specific STS-PCR markers. Four recombinants

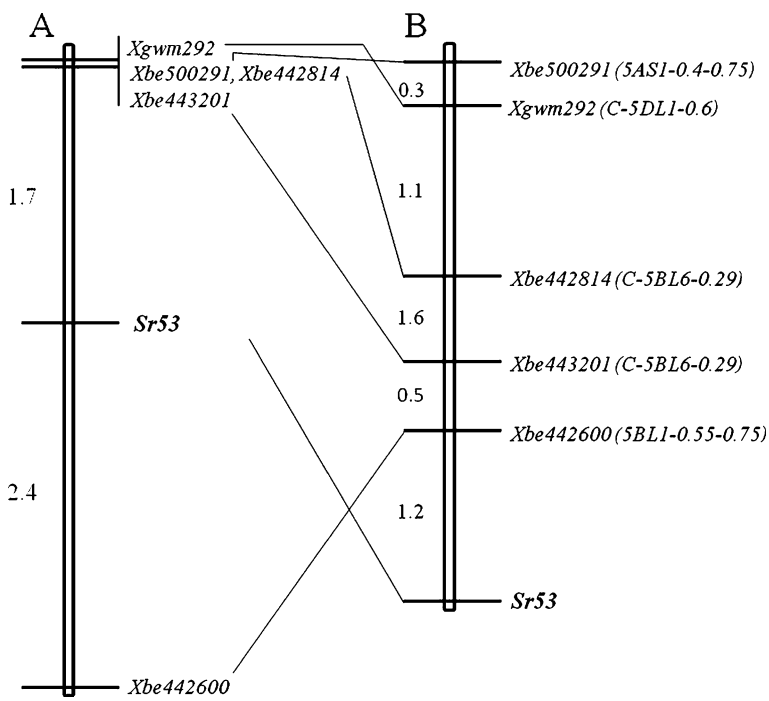


Fig. 2 Genetic linkage maps of *Sr53* derived from $5M^gL$ of *Ae. geniculata*. Map distances are in centimorgans. **a**. Map based on population U6200 (TA5599/TA3809). **b** Map based on population U6154 (TA5599/Lakin)

Table 3 Amplification patterns of STS-PCR markers on putative wheat–*Ae. geniculata* recombinant lines

Entries	BE500291/*Mbo*I (5AS1-0.4–0.75)	Gwm292 (C-5DL1-0.60)	BE442814/*Hae*III (C-5BL6-0.29)	BE443021/*Mbo*I (C-5BL6-0.29)	BE442600/*Mse*I (5BL1-0.55–0.75)
U6154-2	+[a]	+	+	−	−
U61540-124	−[b]	−	−	+	+
U6154-128	−	−	−	−	+
U6154-139	+	+	+	+	−
U6200-42	+	+	+	+	−
U6200-64	+	+	+	+	−
U6200-95	+	+	+	+	−
U6200-113	−	−	−	−	+
U6200-117	+	+	+	+	−
TA5599	+	+	+	+	+
Chinese spring	−	−	−	−	−

[a] Marker is present

[b] Marker is absent

(out of 150, 2.7%) originated from the U6154 F_2 population, and the remaining five recombinants (out of 120, 4.2%) were from the U6200 F_2 population. Among the nine putative recombinants, U6154-124, U6154-128, and U6200-113 were negative for the $5M^g$ short arm marker BE500291/*Mbo*I and positive for at least one of the three $5M^g$ long arm-specific markers. These three putative recombinants were considered likely interstitial recombinants between 5DL and $5M^gL$, lacking the short arm of $5M^g$ (Table 3). The remaining six recombinants were positive for the $5M^gS$ short arm marker BE500291/*Mbo*I, but were missing at least one $5M^gL$ marker (BE443021/*Mbo*I, BE442814/*Hae*III, and/or BE442600/*Mse*I). These six putative recombinants most likely had shortened long arm segments of $5M^g$ (Table 3).

GISH analysis of the putative recombinants

Genomic in situ hybridization analysis confirmed that recombinants U6154-124, U6154-128, and U6200-113 are interstitial recombinants with a proximal segment of $5M^g$ recombined in the long arm of a wheat chromosome. The length of the $5M^g$ segment in U6154-124 is 32% of the long arm of the translocation chromosome, 19% for U6154-128, and 20% for U6200-113 (Table 4 and Fig. 3). The crossover breakpoint is located at FL (fraction length) 0.16 and 0.47 for U6154-124, at FL 0.25 and 0.44 for U6154-128, and at 0.33 and 0.53 for U6200-113 (Table 4 and Fig. 3). GISH analysis of the other six putative recombinants confirmed that they consisted of the short arm and centromere of $5M^g$, a proximal part of the long arm of $5M^g$ and the distal part derived from wheat (Fig. 3). Crossovers for the six interstitial recombinants ranged from FL 0.31 to FL 0.45 compared with FL 0.55 for TA5599 (Table 4).

SSR marker analysis of the recombinants

Analysis with 20 5D-specific SSR markers (6 for the short arm, 14 for the long arm) confirmed that the nine recombinants are genetically compensating. All

Table 4 Summary of crossover fraction lengths (FL) of $5M^gL$ in recombinant lines between wheat 5D and *Ae. geniculata* chromosomes

Translocation line	$5M^g$ FL (%)	Cross over 1	Cross over 2
TA5599	54.4±0.8	54.4±0.8	−
U6154-2	33.3±2.6	33.3±2.6	−
U6154-124	31.8±2.1	15.5±2.7	47.3+3.4
U6154-128	18.8±1.0	24.9±3.9	44.1±3.9
U6154-139	34.3±3.0	34.3±3.0	−
U6200-42	31.9±2.0	31.9±2.0	−
U6200-64	45.5±2.3	45.5±2.3	−
U6200-95	31.4±3.8	31.4±3.8	−
U6200-113	19.6±1.9	33.3±1.3	52.9±2.1
U6200-117	33.1±4.9	33.1±4.9	−

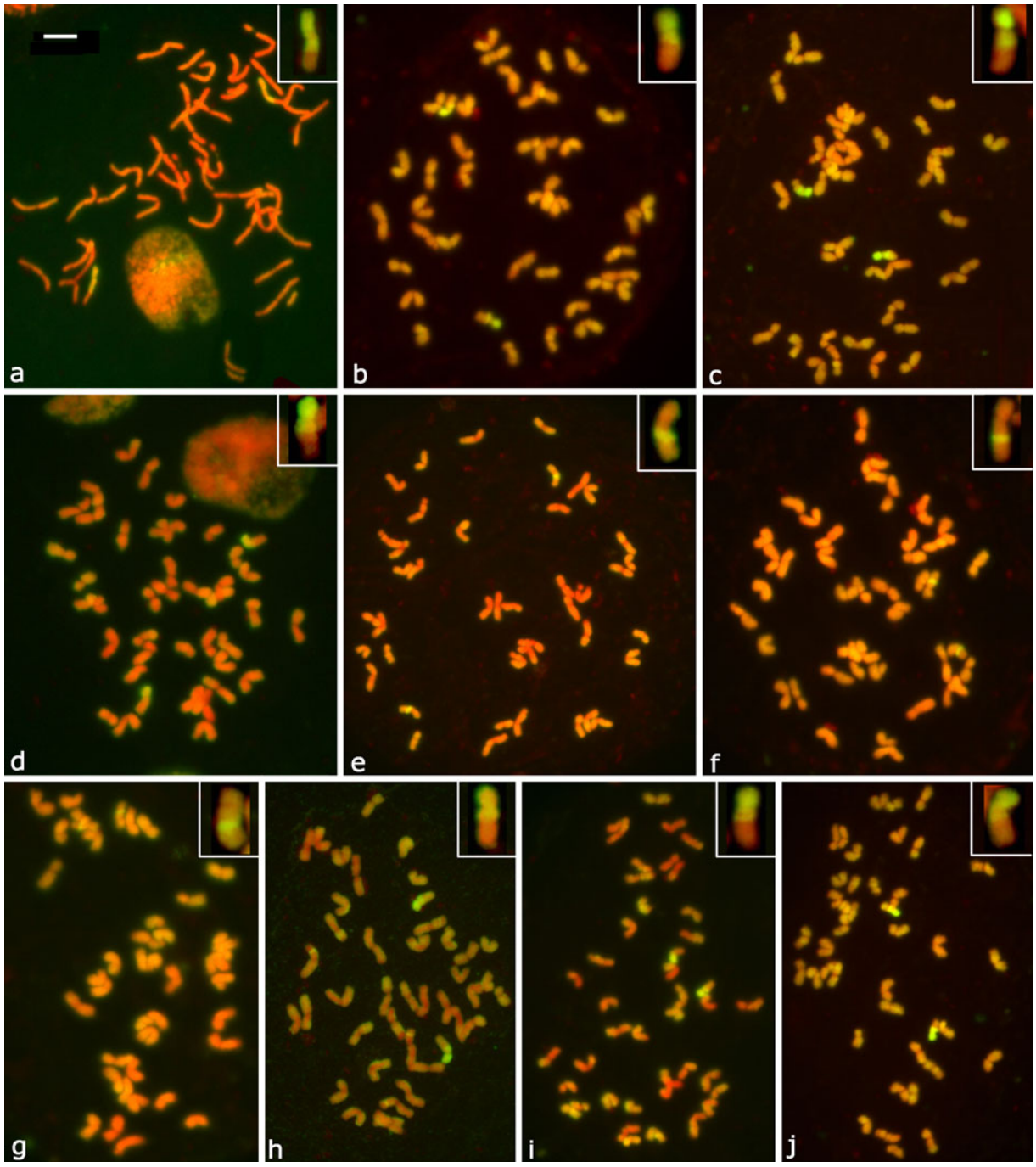


Fig. 3 Genomic in situ hybridization images of wheat–*Ae. geniculata* recombinants produced in this study. *Ae. geniculata* chromatin was visualized by yellow-green FITC florescence; wheat chromosomes were counterstained with propidium iodide and fluoresced red. The *arrows* indicate the wheat–*Ae. geniculata* translocated chromosomes. **a** TA5599 original translocation stock; **b** U6154-42; **c** U6200-64; **d** U6154-2; **e** U6154-124; **f** U200-128; **g** U6200-113; **h** U6154-139; **i** U6200-95; **j** U6200-117. *Scale bar*, 10 μm

six 5DS SSR markers and three markers (CFD40, GDM63, and CFD52) that mapped to the deletion bin C-5DL1-0.60 are missing, and the remaining 11 5DL markers are present in the original translocation donor

TA5599. Thus, the crossover in the TA5599 translocation chromosome is located between markers CFD40, GDM63, CFD52 and GWM583, GWM654, GDM138 in the deletion bin C-5DL1-0.60. The recombinants U6200-64, U6200-117, and U6154-2 are missing the maker CFD40 and GDM63, which mapped to the deletion bin C-5DL1-0.60, but are positive for CFD52. U6200-42, U6200-95, and U6154-139 are missing CFD40, but positive for GDM63 and CFD52, which mapped to the deletion bin C-5DL1-0.60. Thus, the 5M^{g}L segments in U6200-64, U6200-117, U6154-2 U6200-42, U6200-95, and U6154-139 are shorter compared with the original donor TA5599 (Fig. 4). The interstitial recombinant U6154-124 was designated as Ti5DS 5DL-5M^{g}L(0.15–0.47)-5DL; U6154-128 as Ti5DS 5DL-5M^{g}L(0.25–0.44)-5DL; U6200-113 as Ti5DS·5DL-5M^{g}L(0.33–0.53)-5DL; U6200-42 and U6200-95 as T5DL-5M^{g}L·5M^{g}S(0.32); U6154-139 as T5DL-5M^{g}L·5M^{g}S (0.34); and U6200-64 as T5DL-5M^{g}L·5M^{g}S(0.46). U6200-117 and U6154-2 were both designated as T5DL-5M^{g}L·5M^{g}S(0.33), but they differed for the presence of marker BE443201/*Mse*I and reaction to stem rust (Fig. 4).

Evaluation of the recombinants for stem rust resistance

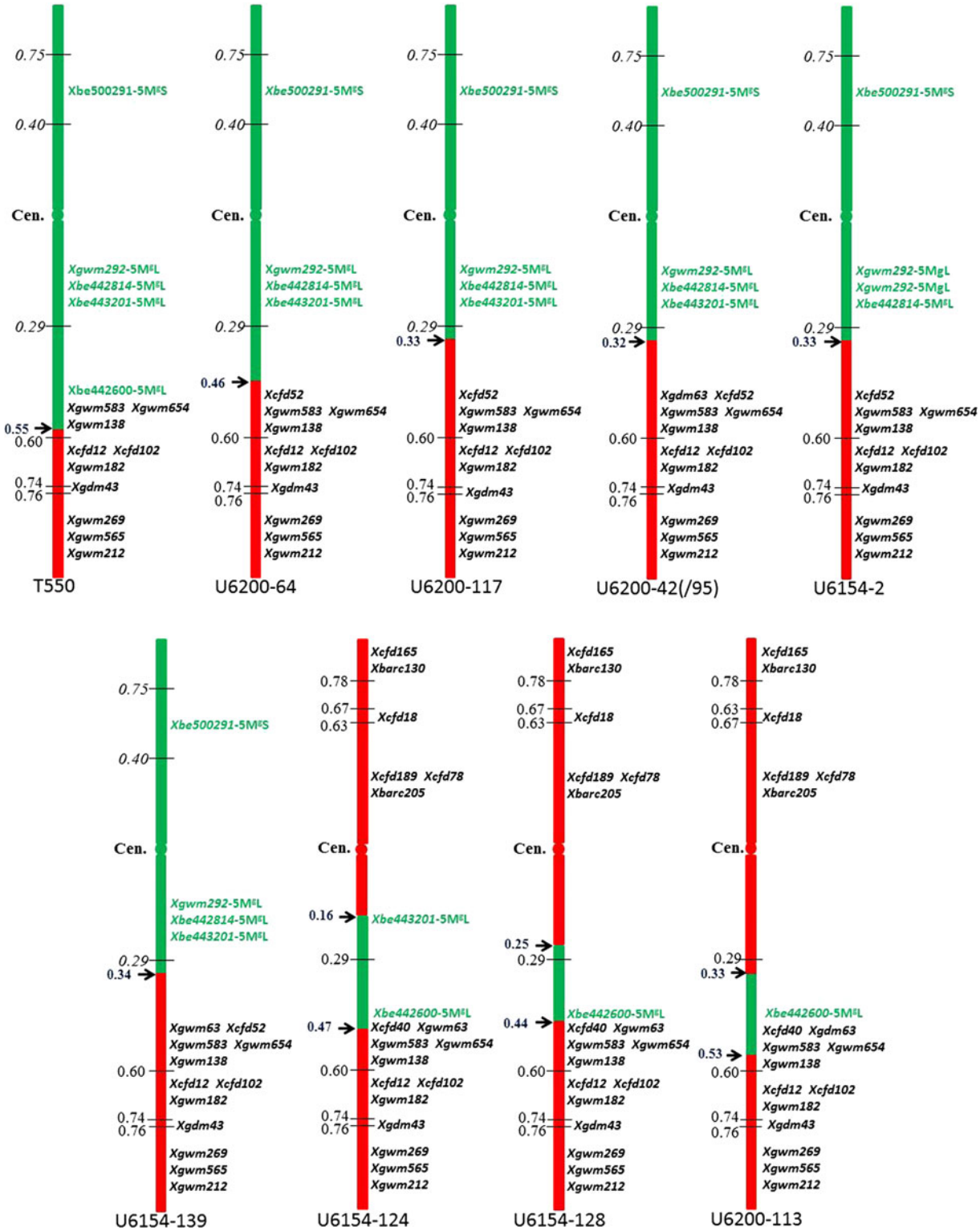
Of the nine recombinants, U6154-124 (Ti5DS 5DL-5M^{g}L-5DL, TA5630), U6200-64 (T5DL-5MgL 5M^{g}S, TA5625), and U6200-117 (T5DL-5MgL 5M^{g}S, TA5643) have low infection types to both stem rust race RKQQC and TTKSK with ITs of 2 to 2+, similar to the resistance gene donor TA5599. The remaining six recombinants, WL711, Lakin, and TA3809, are susceptible to RKQQC and TTKSK with infection types of 3+ to 4 (Table 5).

Discussion

We discovered a new stem rust resistance gene on a segment from the long arm of chromosome 5M^g from *Ae. geniculata*. Although the resistance was initially discovered in disomic addition line TA7659, we employed other existing *Ae. geniculata* translocation stocks to further manipulate and characterize resistance on 5M^{g}L. It is uncertain whether or not the 5M^{g}L resistance from the different *Ae. geniculata* accessions used to produce TA7659 and TA5599 represent the same resistance locus. Translocation stock TA5599 (Kuraparthy et al. 2007) is a T5M^{g}S·5M^{g}L-5DL translocation with a long arm FL of 0.55 that carries this newly discovered resistance gene. We were able to further define the position of *Sr53* to approximately FL 0.33 of 5M^{g}L by the development of interstitial recombinants, aided by new molecular markers, the development and characterization of additional populations, and genomic in situ hybridization. The only other wheat stem rust resistance gene reported on the long arm of chromosome 5D is *Sr30*, which was previously mapped distal to SSR markers CFD12 and GWM182 on 5DL (Kaur et al. 2009; Hiebert et al. 2010a), and represents a different locus.

Among the three resistant recombinants, U6200-117 has the shortest 5M^{g}L alien chromatin with a FL of 0.33; U6200-64 has a 5M^{g}L FL of 0.46; and U6154-124 has an interstitial translocation with FL 0.16–0.47. The alien chromatin between FL 0.16 and 0.33 is shared by these resistant recombinants, whereas the other susceptible recombinants except U6154-128 have 5M^{g}L FL of 0.32–0.34. Thus, the stem rust resistance gene is most likely around FL 0.33 of 5M^{g}L. U6154-128, an interstitial recombinant with crossovers at FL 0.25 and FL 0.44 of the 5M^{g}L, is the only line involving FL 0.33 of 5M^{g}L that is susceptible to stem rust. We suspect that the limited resolution of GISH, a chromosome rearrangement, further recombination, or mutation has affected the classification of this recombinant or the resistance gene. The development of additional interstitial wheat–*Ae. geniculata* translocation lines from the BC_2F_2 of TA5599/CS-*ph1b* is in process. These recombinants will provide useful stocks for the fine molecular and physical mapping of *Sr53*.

GISH and molecular marker analysis grouped the recombinants into two different types: interstitial Ti5DS·5DL-5M^{g}L-5DL recombinants and terminal T5DL-5M^{g}L·5M^{g}S recombinants with shortened long arm segments of 5M^g. Although interstitial recombinants U6154-128 and U6200-113 came from two different populations, they had a similar 5M^{g}L segment length (19–20% the long arm), were missing the same 5D SSR and 5M^{g}L-specific STS-PCR markers, and were susceptible to stem rust. The recombinants U6200-42, U6200-95, and U6154-139 also shared similar crossover locations, molecular


0.75
Xbe500291-5MfS
0.40
Cen.
Xgwm292-5MfL
Xbe442814-5MfL
Xbe443201-5MfL
0.29
Xbe442600-5MfL
Xgwm583 Xgwm654
Xgwm138
0.55
0.60
Xcfd12 Xcfd102
Xgwm182
0.74
0.76
Xgdm43
Xgwm269
Xgwm565
Xgwm212
T550
0.46
Xcfd52
U6200-64
0.33
U6200-117
0.32
Xgdm63 Xcfd52
U6200-42(/95)
Xgwm292-5MgL
Xbe442814-5MfL
U6154-2
0.78
Xcfd165
Xbarc130
0.67
0.63
Xcfd18
Xcfd189 Xcfd78
Xbarc205
0.16
Xbe443201-5MfL
Xbe442600-5MfL
0.47
Xcfd40 Xgwm63
U6154-124
0.25
0.44
U6154-128
U6200-113
0.53

◀ **Fig. 4** Genotyping of wheat–*Ae. geniculata* recombinant lines by SSR and STS markers. Chromosome fragments from $5M^g$ of *Ae. geniculata* (*green*) and 5D of wheat (*red*) are indicated. *Markers in green* are $5M^g$-specific STS-PCR makers; *markers in black* are 5D-specific. Physical maps of SSR markers of 5D are derived from Sourdille et al. (2004). Deletion bins in *italic numbers* for $5M^g$ are based on the reported locations of ESTs in wheat group 5 chromosomes. *Arrows* indicate the breakpoints of recombinants, and the *numbers to the left of an arrow* indicate the distance (fraction length) from the breakpoint to the centromere

marker patterns, and susceptibility to stem rust, although they occurred independently. Additional molecular markers are needed to more accurately characterize those recombinants, but it seems likely that there is a recombination hot spot near FL 0.33.

The present study reports a total of nine independent wheat 5DL–*Ae. geniculata* $5M^gL$ recombinants developed from 270 F_2 plants. Four recombinants are derived from F_3 families of the cross TA5599/TA3809 (*ph1b* mutant), but none of the corresponding F_2 plants are homozygous *ph1bph1b* based on *ph1b*-specific maker screening (data not shown). Thus, all recombinants reported in this study occurred spontaneously. The average spontaneous translocation frequency between $5M^gL$ and 5DL is unusually high in our results, up to 3.3%. However, our results are consistent with the observations of Cifuentes and Benavente (2009a, b) where the hybridization rates of *Ae. geniculata* with wheat were higher compared with other *Aegilops* species. Analysis of homoelogous metaphase I pairing between the hexaploid wheat D genome and *Ae. geniculata* revealed frequent pairing in the hybrids ($ABDU^gM^g$), with 3DL and 5DL showing the highest pairing rates (Cifuentes and Benavente 2009b). Based on the spontaneous translocation frequency observed in the present and previous studies and the recognized tolerance/resistance of *Ae. geniculata* accessions to various abiotic and biotic stresses, we suggest that *Ae. geniculata* could be a particularly desirable target for wheat-alien introgression efforts.

Although a large number of translocation lines with useful alien traits have been produced, only a small portion have been exploited commercially because of the non-compensation or genetic drag from the introduced alien segments causing depression in yield and quality (Jiang et al. 1994; Friebe et al. 1996). We identified three wheat–*Ae. geniculata* recombinants carrying the stem rust resistance gene *Sr53*. The interstitial translocation Ti5D·5DL-$5M^gL$ (0.15–0.47)-5DL (U6154-124, TA5630) contains about 32% of the $5M^gL$ segment including *Sr53* and should be a useful source of stem rust resistance for wheat breeding. Molecular markers developed in this study are closely linked to *Sr53*, with genetic distances of 1.7 to 4.7 cM. Although the map order of marker BE442600/*Mse*I was not in perfect agreement between the two populations, this is not uncommon in wheat genetic mapping experiments with limited population sizes. Markers BE443202/*Mbo*I and BE442600/*Mse*I were closest to the gene in both populations, with distances of 1.7 to 2.4 cM in population U6200 and 1.7 to 1.2 cM in population U6154. These markers can be used for a marker-assisted selection of *Sr53*. Small seed samples of the *Sr53* recombinant stocks are available upon request.

Table 5 Reaction to races RKQQC and TTKSK (or Ug99) of *P. graminis* f. sp. *tritici* for the wheat–*Ae.geniculata* translocation lines and parental and control stocks

Entry name	Infection type[a]	
	RKQQC	TTKSK
Chinese Spring	3+4	3+4
Lakin	3+4	3+
WL711	3+4	3+
TA3809	3+4	3+
TA5599	22+	2-2
TA7659	22+	2
U6200-64	22+	2
U6200-117	22+	2
U6200-42	33+	3+
U6200-95	33+	3+
U6154-2	33+	33+
U6154-139	33+	3+
U6154-124	22+	2+
U6200-113	33+	3
U6154-128	33+	3+

[a] Infection types were scored at 12–14 days post-inoculation based on a 0–4 scale as described by Roelfs and Martens (1988). Plants with infection types of "2 or 2+" were considered to be resistant to stem rust, whereas infection types of "3 to 4" were scored as susceptible. Infection types to race RKQQC of *P. graminis* f. sp. *tritici* were assayed in Manhattan KS, whereas infection types to race TTKSK were assayed at the USDA-ARS Cereal Disease Lab in St. Paul, MN

Acknowledgments This research was part of the project "Durable Rust Resistance in Wheat" supported by Bill and Melinda Gates Foundation through a contract to Cornell University and a special USDA-CSREES grant to the Wheat Genetic and Genomic Resources Center at Kansas State University. We thank W. John Raupp for a critical editorial review of the manuscript and Shuangye Wu for her technical assistance. This is contribution number 11-285-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS 66506-5502, USA.

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