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ORIGINAL PAPER

Development and characterization of wheat-*Ae. searsii* Robertsonian translocations and a recombinant chromosome conferring resistance to stem rust

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Abstract The emergence of a new highly virulent race of stem rust (*Puccinia graminis tritici*), Ug99, rapid evolution of new Ug99 derivative races overcoming resistance of widely deployed genes, and spread towards important wheat growing areas now potentially threaten world food security. Exploiting novel genes effective against Ug99 from wild relatives of wheat is one of the most promising strategies for the protection of the wheat crop. A new source of resistance to Ug99 was identified in the short arm of the *Aegilops searsii* chromosome $3S^s$ by screening wheat- *Ae. searsii* introgression libraries available as individual chromosome and chromosome arm additions to the wheat genome. For transferring this resistance gene into common wheat, we produced three double-monosomic chromosome populations (3A/$3S^s$, 3B/$3S^s$ and 3D/$3S^s$) and then applied integrated stem rust screening, molecular maker analysis, and cytogenetic analysis to identify resistant wheat-*Ae. searsii* Robertsonian translocation. Three Robertsonian translocations (T3AL·$3S^sS$, T3BL·$3S^sS$ and T3DL·$3S^sS$) and one recombinant (T3DS-$3S^sS$·$3S^sL$) with stem rust resistance were identified and confirmed to be genetically compensating on the basis of genomic in situ hybridization, analysis of 3A, 3B, 3D and $3S^s$S-specific SSR/STS-PCR markers, and C-banding. In addition, nine SSR/STS-PCR markers of $3S^sS$-specific were developed for marker-assisted selection of the resistant gene. Efforts to reduce potential linkage drag associated with $3S^sS$ of *Ae. searsii* are currently under way.

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Introduction

Stem rust or black rust of wheat is caused by the fungus *Puccinia graminis* Pers. F. sp. *tritici* Eriks. & E. Henn. The fungus grows primarily on the leaf sheath or stem tissues of a wheat plant and can block the vascular system, leading to lodging, shriveled grains, and total crop loss during severe epidemic years. Stem rust is often the most damaging of the three wheat rust diseases due to the potential for complete crop loss. For over 30 years, epidemics of stem rust have been effectively controlled in most wheat growing regions because of the worldwide deployment of effective stem rust resistance genes in wheat varieties and removal of important alternate hosts, such as *Barberis vulgaris* L. from the proximity of wheat fields (Singh et al. 2006, 2008a, b; Jin et al. 2006, 2009a).

However, stem rust has again become a major threat to the world wheat production with the emergence of Ug99 (TTKSK), a new race of the stem rust fungus reported in Uganda in 1999 (Pretorius et al. 2000; Wanyera et al. 2006; Jin et al. 2008a). Ug99 pathotypes defeat most of the race-

specific resistance genes currently deployed worldwide, and is considered to be the most virulent strain of stem rust to emerge in the last 50 years (Stokstad 2007). Ug99 is virulent to *Sr31* (derived from chromosome 1RS of rye, *Secale cereale* L.), a gene widely deployed in winter and spring wheat varieties in China, Europe, India and USA, and *Sr38* (derived from 2NS of *Aegilops ventricosa* Tausch), a gene deployed in some European, American and Australian cultivars (Singh et al. 2006, 2008a, b). Further concern has grown with the discovery of additional variants in the Ug99 lineage. Two new variants, TTKST and TTTSK, which were reported in 2006–2007 to be virulent to other widely deployed genes *Sr24* and *Sr36* (both were effective against race Ug99 or TTKSK) (Jin et al. 2008b, 2009a). In addition, Ug99 has migrated from East Africa to Sudan and Yemen in 2006 (Jin et al. 2008a), and Iran in 2007 (Nazari et al. 2009). The proximity of Ug99 to highly vulnerable and vast wheat crops in the Indian subcontinent and China is concerning.

Breeding of genetic resistance is considered to be the most effective approach to prevent or slow the spread of stem rust caused by Ug99 (Singh et al. 2008a). At present, among the 46 catalogued resistant genes against stem rust, only less than half of them are effective to Ug99 (McIntosh et al. 2008; Singh et al. 2006, 2008a). There are a total of 23 stem rust resistant genes derived from common wheat, only three (*Sr28*, *Sr29* and *SrTmp*) are resistant to Ug99, and the effects of these genes are moderate under heavy disease pressure. Among the 23 catalogued genes conferring some level of resistance against Ug99, 20 genes were introduced into wheat from its wild relatives. Because of limited resistance in the wheat gene pool, the discovery of novel resistance in wild relatives and its transfer to wheat by chromosome engineering is an effective strategy of disease control. New sources of Ug99 resistance in alien wheat species have been reported (Xu et al. 2008, 2009; Jin et al. 2009b) and a resistance gene from *Aegilops speltoides* Tausch has been transferred into wheat (Faris et al. 2008).

Aegilops searsii Feldman & Kislev ex Hammer is a diploid S-genome species ($2n = 2x = 14$, S^sS^s), native to the sub-Mediterranean regions of Israel, Jordan, southwestern Syria, and southeastern Lebanon. Feldman et al. (1979) produced a Chinese Spring wheat- *Ae. searsii* amphiploid ($2n = 8x = 56$, $AABBDDS^sS^s$). Wheat-*Ae. searsii* introgression libraries where individual *Ae. searsii* chromosome or arms are added to the chromosome complement or substitute for a homoeologous chromosome of wheat, including 7 disomic chromosome additions, 14 ditelosomic chromosome additions, 21 disomic chromosome substitutions and 31 ditelosomic substitution lines were developed by Friebe et al. (1995). Several genes controlling high-molecular weight glutenin subunits have been identified and cloned from *Ae. searsii* (Sun et al. 2006; Garg et al. 2009). By screening our set of wheat-alien chromosome addition lines, we identified a novel source of resistance to Ug99 in the short arm of chromosome $3S^s$ of *Ae. searsii*. In this paper, we describe the production of compensating whole arm Robertsonian translocation T3AL·3S^{s}S, T3BL·3S^{s}S and T3DL·3S^{s}S conferring resistance to UG99. The recovery of a spontaneous T3DS-3S^{s}S·3S^{s}L recombinant allowed us to map the resistance gene to the proximal 75% of the 3S^{s}S arm.

Materials and methods

Plant materials

Three wheat-*Ae. searsii* disomic chromosome substitution lines DS3S^s(3A) (TA6555), DS3S^s(3B) (TA6556), and DS3S^s(3D) (TA6557) developed by Friebe et al. (1995) were used in this study. Each of the DS line was crossed as pollen donor with the *ph1b* mutant stock (TA3809). The F_1 plants were allowed to self to produce F_2 populations each segregating for double-monosomic chromosome combinations 3S^s/3A, 3S^s/3B, and 3S^s/3D. The chromosomes in monosomic condition are prone to misdivide during meiosis-1 and broken chromosomes reunite during interkinesis to form Robersonian translocations (Friebe et al. 2005). DNA was isolated from all F_2 progeny, assayed with molecular markers for the identification of Robersonian translocations, which were later verified by cytology.

The wheat-*Ae. searsii* disomic, ditelosomic addition lines and disomic substitution lines together with nullisomic-tetrasomic and ditelosomic lines of Chinese Spring were used for the molecular characterization of wheat-*Ae. searsii* translocations (Table 1). All stocks are maintained at the Wheat Genetic and Genomic Resources Center at Kansas State University.

Molecular markers

Wheat group-3 SSR markers and 3S^s-specific EST-STS markers were used for genotyping. The wheat group-3-specific markers, included 170 SSR primers (60 for 3A, 63 for 3B, and 47 for 3D) for assaying wheat chromosomes and 144 STS-PCR primers (71 for the short arm and 73 for the long arm of 3S^s) were used to select 3S^s-specific PCR markers. The SSR primers were selected based on the SSR physical map of Sourdille et al. (2004) and consensus SSR map of Somers et al. (2004). STS-PCR primers specific for group 3 were designed by Qi et al. (2007, 2008) on the basis of wheat expressed sequence tags (EST) mapped to wheat group 3 (http://wheat.pw.usda.gov/NSF/project/mapping_data.html). Genomic DNA was isolated from 5 to 10 cm long segments of young leaves using a BioSprint96 workstation following the protocol as described

Table 1 Plant materials used in the study

TA #[a]	Chromosome constitution	Description
Chinese spring (CS)		
TA3809	*ph1b* mutant	
TA3582	DA3S^s	CS-*Ae. searsii* disomic addition
TA6555	DS3S^s(3A)	CS-*Ae. searsii* disomic subtitution
TA6556	DS3S^s(3B)	CS-*Ae. searsii* disomic subtitution
TA6557	DS3S^s(3D)	CS-*Ae. searsii* disomic subtitution
TA7533	Dt3S^{s}S	CS-*Ae. searsii* ditelosomic addition
TA7534	Dt3S^{s}L	CS-*Ae. searsii* ditelosomic addition
TA3269	N3A-T3B	CS Nullisomic 3A–tetrasomic 3B
TA3270	N3A-T3D	CS Nullisomic 3A–tetrasomic 3D
TA3271	N3B-T3A	CS Nullisomic 3B–tetrasomic 3A
TA3272	N3B-T3D	CS Nullisomic 3B–tetrasomic 3D
TA3273	N3D-T3A	CS Nullisomic 3D–tetrasomic 3A
TA3274	N3D-T3B	CS Nullisomic 3D–tetrasomic 3B
TA3104	Dt3AS	CS Ditelosomic 3AS
TA3105	Dt3AL	CS Ditelosomic 3AL
TA3115	Dt3BS	CS Ditelosomic 3BS
TA3116	Dt3BL	CS Ditelosomic 3BL
TA3192	Dt3DL	CS Ditelosomic 3DS
TA3193	Dt3DS	CS Ditelosomic 3DL

[a] WGGRC collection accession number

by BioSprint DNA Plant Handbook (Cat. no. 941558, QIAGEN Inc., Valencia, CA, USA).

For SSR primers, PCR was performed with 25 μl of reaction mixture containing 1× PCR buffer (Bioline USA Inc., Taunton, MA, USA), 2 mM $MgCl_2$, 0.25 mM dNTPs, 0.5 pmol forward primer and reverse primer, respectively, 0.02 unit/μl of *Taq* DNA polymerase (Bioline USA Inc., Taunton, MA, USA), and 90 ng of genomic DNA. PCR was amplified with a Touch-down program as described by Wu et al. (2009).

For STS-PCR primers, 75 μl of reaction mixture containing 1× PCR buffer (Bioline USA Inc. Taunton, MA), 2 mM $MgCl_2$, 0.3 mM dNTPs, 0.4 pmol each of forward primer and reverse primers, 0.02 unit/μl of *Taq* DNA polymerase (Bioline USA Inc., Taunton, MA, USA), and 270 ng of genomic DNA was subjected to PCR under the program of "Touch-down 63" as described by Qi et al. (2007). PCR-amplified products were then allocated into 10 μl of aliquots and each digested with 9 four-base restriction enzymes (*Alu*I, *Hae*III, *Mse*I, *Msp*I, *Rsa*I, *Mbo*I, *Taq*$^\alpha$I, *BstU*I, and *Hha*I) for 2 h at 37°C by adding 5 μl of enzyme mixture composed of 3.25 μl of ddH_2O, 1.5 μl of NEB buffer 2 or 4, 0.15 μl of 100× BSA, 0.1 μl of enzyme stock solution. PCR products were resolved on 2.5% agarose gels in 1× TBE for SSR-PCR; 1.5% agarose gels for STS-PCR and visualized by ethidium bromide staining under UV light.

Stem rust assays

Stem rust response assays were conducted at Kansas State University, Manhattan, Kansas. Stem rust inoculation with race RKQQC was applied as described by Wu et al. (2009). Parental lines, F_1 progeny, single F2 plants, and homozygous translocation lines were inoculated with RKQQC and stem rust infection types were scored 12–14 days post-inoculation based on a 0–4 scale as described by Roelfs and Martens (1988). Plants with infection type of "2+" or less were considered to be resistant to stem rust, whereas an infection type of "3–4" was scored as susceptible. Homozygous translocation lines developed in this study were inoculated with stem rust race TTKSK (Ug99) and scored at the USDA-ARS Cereal Disease Laboratory at the University of Minnesota, St. Paul.

Development of translocation lines

Three F_2 populations derived from 3S^s double-monosomic F_1 hybrids 3S^s/3A, 3S^s/3B, and 3S^s/3D were used to develop wheat-*Ae. searsii* Robertsonian translocations. F_2 seedlings were screened for stem rust and young leaves were collected from stem rust-resistant individuals to extract genomic DNA for PCR amplification. Four STS-PCR markers (primer–restriction enzyme combinations) marking the distal and proximal regions of each arm of chromosome 3S^s were used to identify putative Robertsonian translocations (Table 2). Because the stem rust resistance was located on the short arm of 3S^s, only those plants with at least one 3S^s short arm-specific PCR marker and missing at least one long arm-specific marker were selected for further genomic in situ hybridization (GISH) analysis.

GISH and C-banding analysis of translocation lines

Genomic DNA for probe labeling was extracted using a DNeasy Plant Mini Kit following manufacturer's instructions (QIAGEN Inc. Valencia, CA, USA). GISH probes were prepared by labeling 1 μg of genomic DNA with Green 496 dUTP (5-Fluorescein dUTP) (ENZ-42831, Enzo Life Sciences International Inc., Plymouth Meeting, PA, USA) and 30–40 mU of DNase I (Cat. No. 18010-017, Invitrogen, Carlsbad, CA, USA) in a 50-μl reaction volume by nick translation.

GISH was conducted according to Zhang et al. (2001) with some modifications. Squash preparations were made after staining with acetocarmine. After hybridization at 37°C overnight, the slides were washed in 2× SSC twice at RT for 5 min, twice at 42°C for 10 then 5 min, and once at RT for 5 min. A drop (25–30 μl) of Vectashield mounting medium containing 1 μg/ml of PI (Cat.No.H-1400, Vector Laboratories Inc, Burlingame, CA, USA) was added to

Table 2 Primer sequences of *Ae. searsii* $3S^s$ polymorphic SSR and STS-PCR markers derived from wheat ESTs on wheat group-3 chromosomes and primer/enzyme combinations producing $3S^s$ polymorphism

Marker	Forward primer 5′–3′	Reverse primer 5′–3′	Chr. location[a]	Enzyme for polymorphism	Specificity
cfa2170-SSR	TGGCAAGTAACATGAACGGA	ATGTCATTCATGTTGCCCCT	3A 145.6		$3S^SL/3A$
wmc674-SSR	TTTGAAAACTCCTCGGGTCGTC	CACGAGCTCGAGGTGTTTGTAG	3B 4.4		$3S^SS$
BE443404-STS	TTGTTGACAGCGTACCGAAG	AACTGCCCAATCACACCATC	3BS8-0.78-1.00	*Hae*III	$3S^SS/3D$
BE443202-STS	CGTACGGGAACCTAGAGCAC	TCACTTGAGGTAAATAAATCAACCA	3BS8-0.78-1.00	*Msp*I	$3S^SS/3B$
BE443960-STS	GTGGCGAATGTTGAAAGGAT	AAATCACTTGGCAGGAATGC	3DS3-0.24-0.55	*Mse*I	$3S^SS$
BE495182-STS	AATGCTGGGACAAACAAAGC	TTGAAAGCCTCGACTCCTGT	3DS3-0.24-0.55	*Mbo*I	$3S^SS/3A/3D$
BE442782-STS	GTTGCTGAAGCTGAGGAAGG	TGAGGGGTACTACGGAATCG	3DS3-0.24-0.55	*Mbo*I	$3S^SS/3B$
BE490739-STS	GTCGACAACTGTGCTCCAAC	GAAAGGCCACCCGTTTTTAT	C-3DS3-0.24	*Rsa*I	$3S^SS$
BG605144-STS	GTGGCAGTCGCTAACAATGA	CATGCGCATTGGATGATAAC	C-3DS3-0.24	*Taq*[a]I	$3S^SS$
BE442715-STS	GCAGGCTTATTGGAAATGGA	TGCTTAACAAGGTGCCTTCA	C-3DS3-0.24	*Mbo*I	$3S^SS/3D$
BE404709-STS	CGCAATGGTTTGGTTTCAGT	TGCTTTTGCCCTATGTTTCC	C-3DL2-0.27	*Rsa*I	$3S^SL/3A$
BE403201-STS	TGCATCAAAAGCAGATGTCC	AGAGCAAAGTTCGTGCAAAT	C-3DL2-0.27	*Hae*III	$3S^SL/3B$
BE443753-STS	CGATGCCCTAAATTTGCAGT	GCCATGATGAAAGGCCTAAG	C-3DL2-0.27	*Msp*I	$3S^SL$
BE498661-STS	CACTGGCGAATCGAACATAG	CGTCGAAACAGACCAGTGAA	3DL3-0.81-1.00	*Rsa*I	$3S^SL$
BG263906-STS	GTACTGGAAGCCCATCTTCG	ACAGGCAAGCACAATCACAA	3DL3-0.81-1.00	*Hae*III	$3S^SL$
BM138635-STS	ATGTTTGCCGTTGCTCTCTT	TAGTATGGCCGGACGTTTTT	3DL3-0.81-1.00	*Hae*III	$3S^SL/3A/3D$
BF428994-STS	GTCCCAGTGGTTCACCTGAT	CGCATGGGCTTTCATAAGAT	3DL3-0.81-1.00	*Hae*III, MspI	$3S^SL$
BE404125-STS	GTGGGCGAATATCTTCCAAA	GGATCGTCTTCGTCATCCAT	3DL3-0.81-1.00	*Hae*III, *Rsa*I	$3S^SL/3D$
BE443397-STS	CTCCTCGAGAACCACTGCTC	TAAGGGTCAAATGGGTGGTC	3DL3-0.81-1.00	*Alu*I	$3S^SL/3D$
BE443305-STS	CTGCCTGTGATCTGGACAAA	TTGGCATCAAATCAACACAAA	3DL3-0.81-1.00	*Rsa*I	$3S^SS$

[a] Chromosome location of SSR markers are based on Somers DJ et al. (2004); chromosome location of STS markers are based on the deletion bins where the corresponding EST clones located

each slide after 15–20 min, then covered with a 24 × 30-cm glass cover slip. Fluorescent images were captured with a SPOT2.1 charge-coupled device (CCD) camera (Diagnostic Instruments, Sterling Heights, MI, USA) using an epifluorescence Zeiss Axioplan 2 microscope. Images were processed with Adobe Photoshop CS3 (Version 10.0.1) (Adobe Systems Incorporated, San Jose, CA, USA).

C-banding and chromosome identification of the translocation lines was according to Gill et al. (1991).

Development and molecular characterization of homozygous translocations

Group 3-specific SSR (Sourdille et al. 2004) and $3S^s$-specific STS-PCR markers were used to determine the genome allocation of the wheat-*Ae. searsii* translocations. PCR amplification was performed as described above.

Results

Selection of *Ae. searsii* $3S^s$-specific molecular markers

Among the 170 wheat group-3 SSR primers screened, only wmc674 and cfa2170 produced polymorphic markers for $3S^SL$ and $3S^SS$, respectively. A total of 18 out of 144 STS-PCR primers screened, 9 for each arm, produced $3S^s$ polymorphic markers after the PCR products were digested with 9 different restriction enzymes (Table 2). Among the $3S^s$ polymorphic PCR markers, BE404709/*Rsa*I and cfa2170 are co-dominant for 3A and $3S^s$; BE442782/*Mbo*I is co-dominant for 3B and $3S^s$; BE495182/*Mbo*I and BE404125/*Hae*III are co-dominant for 3D and $3S^s$; BE442715/*Mbo*I and BM138635/*Hae*III are co-dominant for 3A, 3D and $3S^s$. SSR marker wmc674 and 12 other STS-PCR markers are dominant being polymorphic only for *Ae. searsii* chromosome $3S^s$. Primer/enzyme combination BE443305/*Rsa*I produced a specific marker for the short arm of $3S^s$, but its corresponding EST (BE443305) was mapped in the deletion bin of 3DL3-0.81-1.00 in the long arm of wheat chromosome 3D. Based on the location and stability of the markers: BE443404/*Hae*III was selected as a distal marker and BE490739/*Rsa*I as a proximal marker for the short arm of $3S^s$; BE443753/*Msp*I was selected as a proximal and BE498661/*Rsa*I as the most distal marker for the long arm of $3S^s$ (Fig. 1). These four primer/enzyme combinations were used as molecular markers to screen and select putative wheat-*Ae. searsii* translocation chromosomes.

Development of wheat-*Ae. searsii* translocation lines

A total of 1,549 F_2 seedlings from double monosomic populations (562 for $3S^s$/3A, 489 for $3S^s$/3B and 498 for

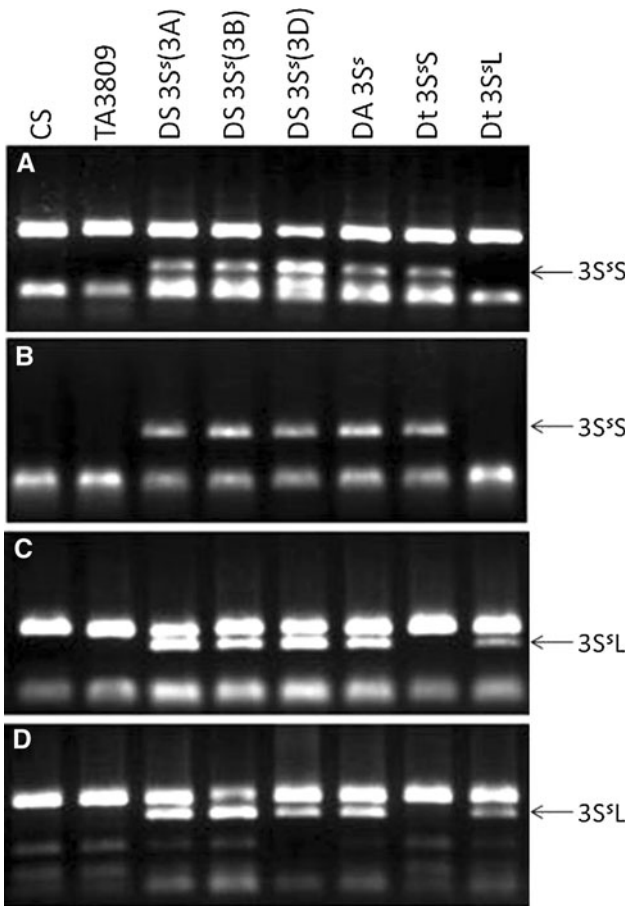

Fig. 1 STS-PCR patterns of Chinese spring, TA3809 (*ph1b* mutant), DS3S^s(3A), DS3S^s(3B), DS3S^s(3D) disomic substitution lines, DA3S^s disomic addition line, Dt3S^{s}S, Dt3S^{s}L ditelosomic addition lines amplified by STS primers: **a** BE443404, product digested with *Hae*III, **b** BE490739, product digested with *Rsa*I, **c** BE443753, product digested with *Msp*I, **d** BE498661, product digested with *Rsa*I; *arrows* indicate the 3S^s-specific fragments

3S^s/3D) were screened for stem rust resistance; 774 (45.7% for 3S^s/3A, 52.6% for 3S^s/3B and 52.4% for 3S^s/3D) were resistant to race RKQQC with infection type "0 to 2$^+$". Plants derived from the 3S^s/3A population showed intermediate infection types of "2 to 2$^+$", while 3S^s/3B and 3S^s/3D families had lower infection types of "1$^+$ to 2" (Table 3). Of interest, 3S^{s}S lines had consistently lower infection frequencies (LIF) in repeated tests.

Only resistant plants were used for isolation of genomic DNA for STS-PCR marker screening with primer/enzyme combination BE443404/*Hae*III, BE490739/*Rsa*I, BE443753/*Msp*I, and BE498661/*Rsa*I. Thirty-one plants were positive for both 3S^s short arm markers and negative for both long arm markers of 3S^s; three plants had two positive short arm markers, one positive long arm proximal marker and one negative distal long arm marker; two plants were positive for both long arm markers and negative for both short arm markers (most likely misclassified by stem rust phenotyping). These 36 plants were putative Robertsonian translocations and were further characterized by GISH analysis.

GISH identified six plants with Robertsonian translocations (U5967(4)98, U5967(5)160, U5969(5)113, U5971(3)30, U5972(4)3 and U5972(5)113) where the 3S^{s}S arm was translocated to a wheat chromosome arm (Table 3; Fig. 2a–c); One plant (U5972(3)123) with negative short arm markers and positive long arm markers had a wheat-*Ae. searsii* recombinant consisting of the long arm of 3S^s, the proximal part of the short arm of 3S^s and the distal part derived from wheat (TW-3S^{s}S·3S^{s}L) (Table 3; Fig. 2d). The remaining plants either contained a 3S^{s}S telocentric chromosome, 3S^{s}S isochromosome or a complete 3S^s chromosome.

Molecular characterization of the wheat-*Ae. searsii* translocation and recombinant lines

Fifteen SSR-PCR primers from wheat group 3 and 5 STS-PCR primers polymorphic for *Ae. searsii* 3S^s were used to perform PCR amplification with genomic DNA from six translocation lines together with wheat group 3 nullisomic-tetrasomic (NT) lines, the short and long arm ditelosomic lines, and wheat-*Ae. searsii* disomic substitution lines of

Table 3 Summary of STS-PCR markers and GISH analysis of putative wheat-*Ae. searsii* translocation lines

Plant no.	BE443404/*Hae*III (3BS6-0.78-1.00)	BE490739/*Rsa*I (C-3DS3-0.24)	BE443753/*Msp*I (C-3DL2-0.27)	BE498661/*Rsa*I (3DL3-0.81-1.00)	GISH analysis	Derived from
U5967(4)98	+	+	−	−	Robertsonian	3A/3S^s
U5967(5)160	+	+	−	−	Robertsonian	3A/3S^s
U5969(5)113	+	+	−	−	Robertsonian	3B/3S^s
U5971(3)30	+	+	−	−	Robertsonian	3B/3S^s
U5972(4)3	+	+	−	−	Robertsonian	3D/3S^s
U5972(5)113	+	+	−	−	Robertsonian	3D/3S^s
U5972(3)123	−	−	+	+	Recombinant	3D/3S^s

+ present for the 3S^s-specific marker, − absent for the 3S^s-specific marker

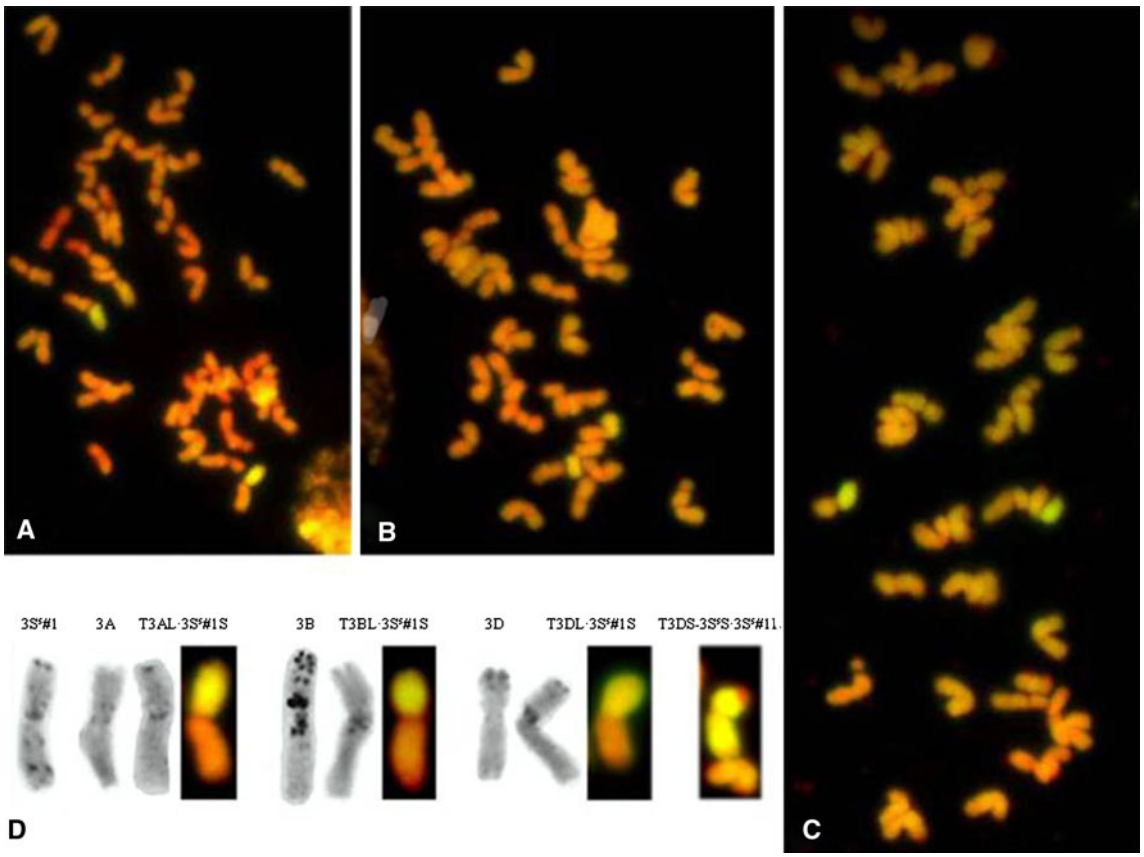

Fig. 2 GISH and C-banding patterns of wheat-*Ae. searsii* translocations: **a** GISH pattern of the T3AL·3S^{s}S translocation stock, **b** GISH pattern of the T3BL·3S^{s}S translocation stock, **c** GISH pattern of the T3DL·3S^{s}S translocation stock, **d** C-banding and GISH patterns of the translocation chromosomes, from *left* to *right* T3AL·3S^{s}S, T3BL·3S^{s}S, T3DL·3S^{s}S and T3DS-3S^{s}S·3S^{s}L. *Ae. searsii* chromatin was visualized by yellow-green FITC florescence and wheat chromosomes were counterstained with propidium iodide and fluoresce red

DS3S^s(3A), DS3S^s(3B), and DS3S^s(3D) as template DNA. All of the tested 3S^s short arm STS-PCR markers were present, whereas all 3S^s long arm markers were all absent in the six wheat-*Ae. searsii* translocation lines. All group 3 long arm SSR markers of were present in the six wheat-*Ae. searsii* translocation lines, but short arm SSR markers specific for wheat chromosome 3A, 3B or 3D were absent in U5967(4)98 and U5967(5)160, U5969(5)113 and U5971(3)30, U5972(4)3 and U5972(5)113, respectively (Fig. 3). Thus, U5967(4)98 and U5967(5)160 were identified as T3AL·3S^{s}S Robertsonian translocation lines. U5969(5)113 and U5971(3)30 were identified as T3BL·3S^{s}S; U5972(4)3 and U5972(5)113 were identified as T3DL·3S^{s}S translocation lines, and the stocks were designated as TS1, TS2, TS3, TS4, TS5, and TS6, respectively.

The recombinant line U5972(3)123 was selected from the 3S^s/3D double monosomic population and, thus, the distal wheat segment most likely is derived from 3DS. A total of 21 3D-specific SSR markers were used to identify the source of the distal wheat segment. All the 10 SSR markers at the long arm of 3D were absent; 7 of the 10 SSR makers mapped in the deletion bin 3DS6-0.55-1.00 of 3DS were present, but other 3 markers in the deletion bin 3DS6-0.55-1.00 and the marker GWM341 in the deletion bin 3DS3-0.24-0.55 were absent (Fig. 3). Thus, the recombinant was identified as T3DS-3S^{s}S·3S^{s}L. The breakpoint was mapped by SSR markers in the deletion bin 3DS6-0.55-1.00, being in accordance with the chromosome measurements estimating the size of the distal *Ae. searsii* segment of 25% the arm replaced by a wheat chromosome segment. This stock was designed as TS8.

Evaluation of the translocation lines for stem rust resistance

Five wheat-*Ae. searsii* translocation lines and three wheat-*Ae. searsii* 3S^s substitution lines together with Chinese Spring as a control were inoculated by race RKQQC and by TTKSK (Ug99) in St. Paul, MN, USA. All the T3BL·3S^{s}S, T3DL·3S^{s}S and T3DS-3S^{s}S·3S^{s}L translocation lines and 3S^s(3B), 3S^s(3D) substitution lines were resistant

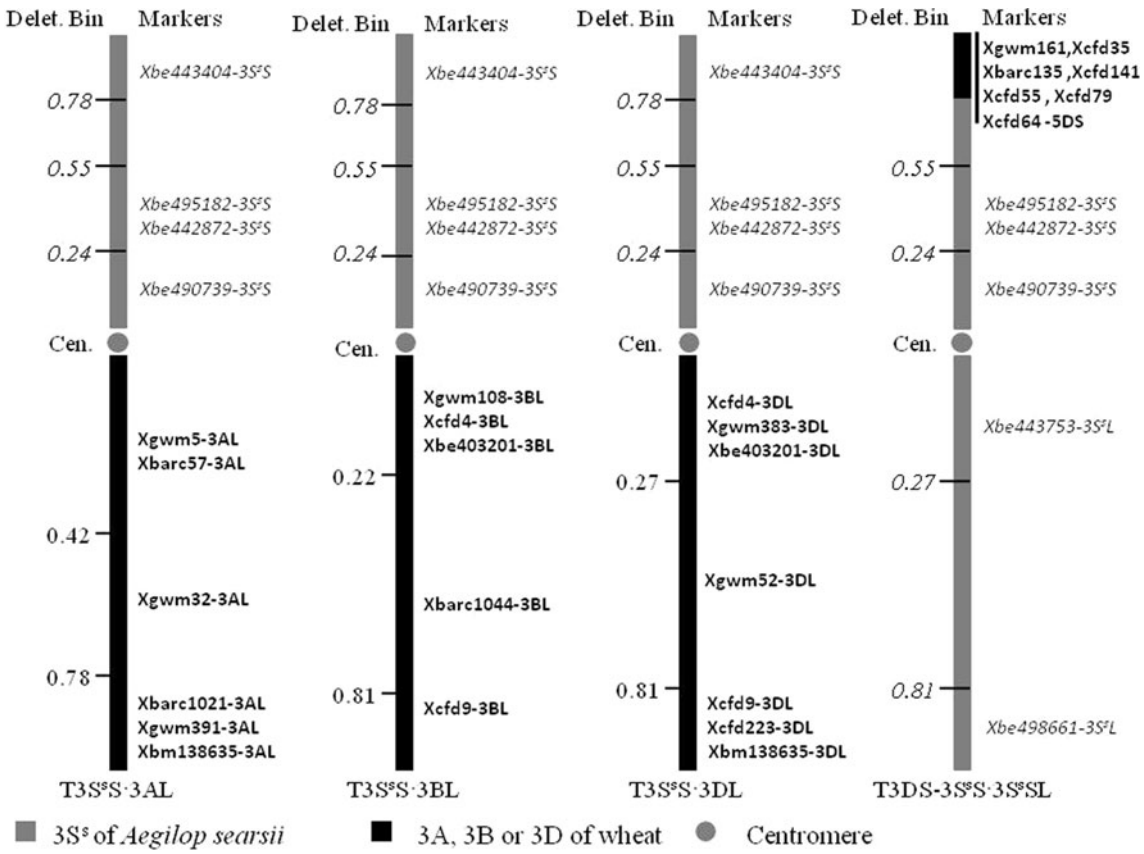

Fig. 3 Genotyping of wheat-*Ae. searsii* translocation lines by SSR and STS markers. Markers in *italics* are 3S^{s}S-specific STS-PCR markers; markers in *bold black* are wheat 3A, 3B and 3D long arm specific. Physical maps of SSR markers of 3A, 3B and 3D are derived from Sourdille et al. (2004); deletion bins in *italic number* on 3S^{s}S are according to the locations of markers derived EST on wheat chromosome group 3 (Qi et al. 2007, 2008). All the translocations present all the long arm-specific markers of wheat 3A, 3B or 3D and 3S^s short arm markers, but are missing all the tested short arm-specific markers of wheat 3A or 3B or 3D and 3S^s long arm markers, thus, TS1 and TS2 are designated as T3AL·3S^{s}S; TS3 and TS4 are designated as T3BL·3S^{s}S; TS5 and TS6 are designated as T3DL·3S^{s}S. Seven 3DS-specific SSR makers mapped in the deletion bin 3DS6-0.55-1.00 and STS-PCR markers of 3S^s long arm specific were present but all the 3DL-specific SSR markers were absent in the recombinant U5972(3)123, thus, TS8 is designed as T3DS-3S^{s}S·3S^{s}L

to RKQQC, showing ";2- LIF" infection types and resistant to TTKSK with ";1 + LIF" infection types most prevalent (Table 4; Fig. 4). The Roberstonian translocation stocks developed in this study are novel sources for resistance to Ug99.

Discussion

In a wheat-alien double monosomic plant, both the alien and its homoelogous wheat univalent have a chance to break at the centromere and fusion of the broken arms results in the formation of Robertsonian translocations (Sears 1952). The frequency of compensating wheat-alien Robertsonian translocations in progenies of double monosomic plants depends on the targeted chromosomes, genetic background and environmental conditions and ranges from 4% to almost 20% (Lukaszewski et al. 1983, 1997; Friebe et al. 2005). In this study, a total of 6 compensating Robertsonian translocations lines and 12 3S^s telosomes or isochromosomes were identified in 724 analyzed progenies of double monosomic plants [257 for (3S^s/3A), 257 for (3S^s/3B), and 260 for (3S^s/3D)]. The ratio of centric breakage that causes telocentric chromosomes, isochromosomes and translocation chromosomes was 2.3% and the frequency of Robertsonian translocation was 0.8% irrespective of the double monosomic populations characterized. The frequency of 3S^s centric breakage fusion in this study is lower than those reported earlier. However, the real frequency of 3S^s centric breakage fusion is higher because we only monitored the 3S^s arm in this study.

The stem rust resistance gene from *Ae. searsii* was originally located at the short arm of chromosome 3S^s. In this study, we developed a recombinant line (T3DS-3S^{s}S·3S^{s}L), which contained a pair of wheat-*Ae. searsii* recombinant chromosomes, where the 25% distal region of the short arm of 3S^s is replaced by a small segment of 3DS (Fig. 2d). This line is resistant to stem rust race RKQQC

Table 4 Reaction to stem rust races RKQQC and TTKSK for the wheat-*Ae.searsii* translocation and substitution lines

Entry name	Type	Infection type		
		RQKKC	TTSSK(Ug99)	
		2010[a]	2010	2011[b]
TA6555	DS3S^s(3A)	;12+	;1LIF[c]/;1+LIF	;/;1
TA6556	DS3S^s(3B)	;LIF/2-LIF	;LIF/;1+LIF	0;
TA6557	DS3S^s(3D)	;LIF/2-LIF	;LIF/;1+LIF	0;/;1LIF
TS1	T3AL·3S^{s}S	;12+	2;/;	1;/;1LIF
TS3	T3BL·3S^{s}S	;LIF/2-LIF	;13-LIF	0;
TS4	T3BL·3S^{s}S	;LIF/2-LIF	;1LIF/;1+LIF	0;
TS5	T3DL·3S^{s}S	;LIF/2-LIF	;1+LIF/;LIF	0;/;1LIF
TS6	T3DL·3S^{s}S	;LIF/2-LIF	;1LIF/;1+LIF	0;
TS8	T3DS-3S^{s}S·3S^{s}L	;LIF/2-LIF	–[d]	;11+LIF
Chinese Spring		4	4	33+/3

[a] Test in 2010

[b] Test in 2011

[c] *LIF* low infection frequency

[d] Not evaluated

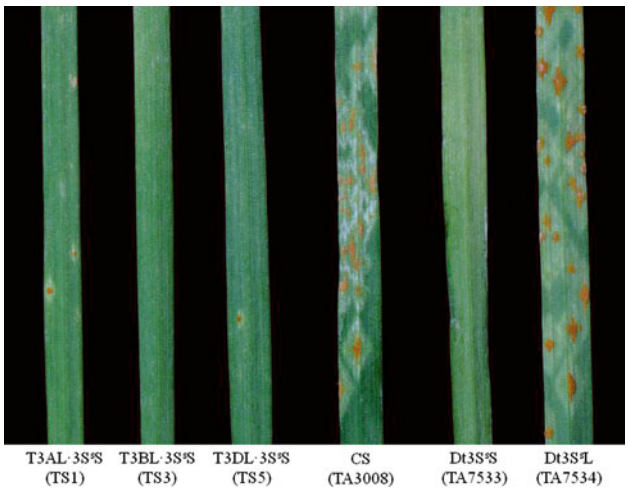

Fig. 4 Infection types of wheat-*Ae. searsii* substitution lines and derived compensating translocation lines. Infection types were scored 14 days post inoculation with stem rust culture TTKSK (Ug99)

with a consistent infection type (Table 4), thus, mapping the resistance gene to the 75% proximal region of 3S^{s}S.

The seedling infection type when challenged with stem rust race RKQQC is different among the three 3S^s substitution lines. The DS3S^s(3A) substitution line and the T3AL·3S^{s}S translocation line are intermediate with infection types of "2 to 2$^+$", whereas DS3S^s(3B) and DS3S^s(3D) substitution lines, and T3S^{s}S·3BL and T3S^{s}S·3DL translocation lines and the recombinant T3DS-3S^{s}S·3S^{s}L stock have lower infection types of "; to 2" (Table 4; Fig. 4). Each wheat-*Ae. searsii* substitution line and translocation line has the same genetic background (Chinese Spring) except they are lacking chromosomes 3A, 3B, 3D in the substitution lines and are lacking 3AS, 3BS and 3DS in the derived translocation lines. Thus, there is likely a complimentary or modifying locus on chromosome 3AS of Chinese Spring, which interacts with the resistance on 3S^{s}S. We are presently investigating this effect by assaying stem rust resistant F_1-hybrids between different 3AS deletion lines, the DS3S^s(3A) substitution line, and the T3AL·3S^{s}S translocation line.

We successfully developed three wheat-*Ae. searsii* compensating translocation stocks (T3AL·3S^{s}S, T3BL·3S^{s}S, and T3DL·3S^{s}S) and one recombinant (T3DS-3S^{s}S·3S^{s}L) with seedling resistance to Ug99, which may have impact in wheat improvement. The recombinant line may be useful for further directed chromosome engineering aimed at producing an interstitial recombinant stock. Research aimed at developing interstitial recombinants with shortened 3S^s fragment of *Ae. searsii* is in progress. The stem rust resistance gene present in the translocation stocks T3AL·3S^{s}S (TA5619), T3BL·3S^{s}S (TA5620), T3DL·3S^{s}S (TA5621), and T3DS-3S^{s}S·3S^{s}L (TA5622) is designated as *Sr51*.

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