

# Characterization and mapping of cryptic alien introgression from *Aegilops geniculata* with new leaf rust and stripe rust resistance genes *Lr57* and *Yr40* in wheat

Vasu Kuraparthi · Parveen Chhuneja ·  
Harcharan S. Dhaliwal · Satinder Kaur ·  
Robert L. Bowden · Bikram S. Gill

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**Abstract** Leaf rust and stripe rust are important foliar diseases of wheat worldwide. Leaf rust and stripe rust resistant introgression lines were developed by induced homoeologous chromosome pairing between wheat chromosome 5D and 5M<sup>g</sup> of *Aegilops geniculata* (U<sup>g</sup>M<sup>g</sup>). Characterization of rust resistant BC<sub>2</sub>F<sub>5</sub> and BC<sub>3</sub>F<sub>6</sub> homozygous progenies using genomic in situ hybridization with *Aegilops comosa* (M) DNA as probe identified three different types of introgressions; two cytologically visible and one invisible (termed cryptic alien introgression). All three types of introgression lines showed similar and complete resistance to the most prevalent pathotypes of leaf rust and stripe rust in Kansas (USA) and Punjab (India). Diagnostic polymorphisms between the alien segment and recipient parent were identified using physically mapped RFLP probes. Molecular mapping revealed that cryptic alien

introgression conferring resistance to leaf rust and stripe rust comprised less than 5% of the 5DS arm and was designated T5DL·5DS-5M<sup>g</sup>S(0.95). Genetic mapping with an F<sub>2</sub> population of Wichita × T5DL·5DS-5M<sup>g</sup>S(0.95) demonstrated the monogenic and dominant inheritance of resistance to both diseases. Two diagnostic RFLP markers, previously mapped on chromosome arm 5DS, co-segregated with the rust resistance in the F<sub>2</sub> population. The unique map location of the resistant introgression on chromosome T5DL·5DS-5M<sup>g</sup>S(0.95) suggested that the leaf rust and stripe rust resistance genes were new and were designated *Lr57* and *Yr40*. This is the first documentation of a successful transfer and characterization of cryptic alien introgression from *Ae. geniculata* conferring resistance to both leaf rust and stripe rust in wheat.

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V. Kuraparthi · B. S. Gill (✉)  
Wheat Genetic and Genomic Resources Center,  
Department of Plant Pathology, Kansas State University,  
Manhattan, KS 66506-5502, USA  
e-mail: bsgill@ksu.edu

P. Chhuneja · S. Kaur  
Department of Plant Breeding, Genetics & Biotechnology,  
Punjab Agricultural University, Ludhiana,  
141 004 Punjab, India

H. S. Dhaliwal  
Department of Biotechnology,  
Indian Institute of Technology, Roorkee 247667,  
Uttaranchal, India

R. L. Bowden  
Plant Science and Entomology Research Unit, USDA-ARS,  
Kansas State University, Manhattan, KS 66506-5502, USA

## Introduction

Leaf rust or brown rust (caused by *Puccinia triticina* Eriks.) and stripe rust or yellow rust (caused by *Puccinia striiformis* Westend. f. sp. *tritici*) are important foliar diseases of wheat worldwide. The most economical and environmentally friendly way to reduce losses due to rust diseases in wheat is through deployment of host-plant genetic resistance. There are more than 50 leaf rust resistance genes and 35 stripe rust resistance genes designated so far (McIntosh et al. 2005), most of which condition a hypersensitive reaction and interact with the pathogen in a gene-for-gene fashion (Flor 1942). Virulence in the pathogen population has been detected following the deployment of many such resistance genes. This necessitates a constant search and transfer of new and effective sources of rust resistance to counter balance the continuous evolution of rust populations. The replacement of highly variable land races by

high yielding, pure-line varieties in many parts of the world has narrowed the genetic base for disease resistance in the wheat gene pool. Wild *Triticum*, *Aegilops* and other *Triticeae* species related to wheat have been found to be invaluable sources of additional resistance genes (Dvorak 1977; Sharma and Gill 1983; Gale and Miller 1987; Jiang et al. 1994; Friebe et al. 1996; Harjit-Singh et al. 1998). Many genes conferring resistance to rust diseases were transferred from *Aegilops* species into wheat (see the review by Jiang et al. 1994; Friebe et al. 1996; Marais et al. 2005).

Very few genes for resistance to diseases and other traits transferred from non-progenitor (other than A, B and D genome diploids) species have been used in wheat germplasm enhancement due to undesirable linkage drag and yield reduction (Jiang et al. 1994; Friebe et al. 1996). With the availability of sensitive detection techniques involving in situ hybridization and densely mapped molecular markers it is now possible to detect, map and estimate the size of the alien introgressions conferring resistance to reduce linkage drag as much as possible (Young and Tanksley 1989; Jiang et al. 1993, 1994; Friebe et al. 1996; Chen et al. 1998, 2005; Dubcovsky et al. 1998; Lukaszewski et al. 2005).

The ovate goat grass *Aegilops geniculata* Roth (syn. *Aegilops ovata* L.) was found to be an excellent source of resistance genes against various pests and diseases (Dhaliwal et al. 1991, 1993; Gale and Miller 1987; Harjit-Singh and Dhaliwal 2000; Harjit-Singh et al. 1993, 1998). Rust resistance of *Ae. geniculata* was transferred to wheat by induced homoeologous chromosome pairing between chromosomes 5M<sup>S</sup> of *Ae. geniculata* and 5D of wheat (Aghaee-Sarbarzeh et al. 2002). Previous attempts to characterize a few of the introgression lines with genomic in situ hybridization (GISH) and simple sequence repeat (SSR) markers showed that the *Ph<sup>1</sup>*-mediated, induced homoeologous recombination

resulted in the transfer of 5M<sup>S</sup>L to an unidentified chromosome of wheat (Aghaee-Sarbarzeh et al. 2002). We selected five other introgression lines derived from the same crosses, all showing similar resistance reaction to both stripe rust and leaf rust, for backcrossing and further cytogenetic and molecular characterization. In this paper, we report the cytogenetic, molecular and genetic characterization of BC<sub>2</sub>F<sub>5</sub> and BC<sub>3</sub>F<sub>6</sub> derived homozygous introgression lines with resistance to both leaf rust and stripe rust.

## Materials and methods

### Plant material

All of the introgression lines were developed by crossing disomic substitution line DS 5M<sup>S</sup>(5D) with the Chinese Spring (CS) *Ph<sup>1</sup>* stock (Chen et al. 1994) and crossing the F<sub>1</sub> with susceptible bread wheat cultivar WL711 (Aghaee-Sarbarzeh et al. 2002). Resistant BC<sub>1</sub>F<sub>1</sub> plants from the above crosses were backcrossed to WL711 again and some selected BC<sub>2</sub>F<sub>1</sub> plants that had no obvious effect on plant growth and development were selfed to develop BC<sub>2</sub>F<sub>5</sub> (TA5599, TA5600, TA5601, TA5603) lines and a few others were further backcrossed and selfed to generate BC<sub>3</sub>F<sub>6</sub> (TA5602) lines (Table 1).

Introgression lines with resistance against leaf rust and stripe rust were selected in each generation by screening the progenies under artificial rust epidemic conditions in the field at the Punjab Agricultural University, Ludhiana, India. The selected BC<sub>2</sub>F<sub>5</sub> and BC<sub>3</sub>F<sub>6</sub> resistant introgression lines were further screened for their resistance reaction against the most virulent races of leaf rust and stripe rust (Table 2) at the Kansas State University, Manhattan, USA.

**Table 1** Description of wheat stocks used in the present study

TA number	PAU number	Generation	Designation	Description
TA6675	BTC 3, 11	Not known	DS5M(5D) <sup>a</sup>	Substitution line
TA5599	T550	BC <sub>2</sub> F <sub>5</sub>	T5MS-5ML-5DL	Translocation line
TA5600	BTC91	BC <sub>2</sub> F <sub>5</sub>	DS5M(5D) <sup>a</sup>	Translocation line
TA5601	T598	BC <sub>2</sub> F <sub>5</sub>	T5DL-5DS-5M <sup>S</sup> S(0.75) <sup>b</sup>	Translocation line
TA5602	T756	BC <sub>3</sub> F <sub>6</sub>	T5DL-5DS-5M <sup>S</sup> S(0.95) <sup>b</sup>	Translocation line
TA5603	BTC102	BC <sub>2</sub> F <sub>5</sub>	T5DL-5DS-5M <sup>S</sup> S(0.95) <sup>b</sup>	Translocation line
TA10437	Acc3547	–	U <sup>S</sup> U <sup>S</sup> M <sup>S</sup> M <sup>S</sup> (2n = 2x = 28)	<i>Aegilops geniculata</i> (donor parent)
TA2009	–	–	–	Wichita
TA4325-152	–	–	–	WL711
TA3812	–	–	–	Chinese Spring with <i>Ph<sup>1</sup></i> gene
TA2761	–	–	MM (2n = 2x = 14)	<i>Ae. comosa</i> (diploid M-genome species)

<sup>a</sup> It is not confirmed whether the missing A-genome marker alleles of probe GSP in these lines are due to nullisomy for chromosome 5A or due to homoeologous translocation between 5A and 5B

<sup>b</sup> Numerical letters in the brackets indicate the fraction length of chromosome arm 5DS estimated based on CS deletion bin based physical map of Gill and Raupp (1996) and Qi and Gill (2001)

**Table 2** Seedling and adult plant infection types (ITs) of wheat-*Ae. geniculata* introgression lines and cultivars inoculated with five races of *Puccinia triticina* and one race of *Puccinia striiformis*

Cultivar/line (source of resistance)	Leaf rust										Stripe rust		
	PRTUS6		PRTUS25		PRTUS35		MCDL		PNMQ		KS2005		
	Seedling	Adult plant	Seedling	Adult plant	Seedling	Adult plant	Seedling	Adult plant	Seedling	Adult plant	Seedling	Adult plant	
TA6675 (DS5M(5D))	NT	tR	2+C	tR	TR	2+C	tR	2+C	tR	;2C	tR	;N	OR
TA5599 (T5M <sup>5</sup> S-5M <sup>5</sup> L-5DL)	NT	tR	2+C	tR	TR	2C	tR	2C	tR	;1C	tR	;N	20R
TA5600 DS5M <sup>5</sup> (5D)	NT	tR	12	tR	TR	2+C	tR	2+C	tR	;1C	tR	;1C	OR
TA5601 (T5DL-5DS-5M <sup>5</sup> S(0.75))	NT	tR	2C	tR	TR	2C	tR	1+C	tR	;2C	tR	;IN	OR
TA5602 (T5DL-5DS-5M <sup>5</sup> S(0.95))	NT	tR	2C	tR	TR	2C	tR	2C	tR	;1C	tR	;IN	OR
TA5603 (T5DL-5DS-5M <sup>5</sup> S(0.95))	NT	tR	2C	tR	TR	2+C	tR	1+C	tR	;1C	tR	0;IN	OR
TA10437	NT	tR	1C	tR	TR	X	tR	2+C	tR	;1C	tR	1+	OR
TA2009	NT	90S	4	90S	90S	4	90S	4	90S	4	90S	4	70MS
TA4325-152	NT	90S	4	NT	90S	4	90S	4	90S	33C	5MR	3	70MS
TAM107 (susceptible wheat cultivar)	NT	90S	4	90S	90S	4	90S	4	90S	4	90S	4	90MS-S

ITs of seedlings were scored according to the modified Stakman scale of Roelfs et al. (1992) as illustrated in McIntosh et al. (1995). The seedling ITs are 0 = no uredinia or other macroscopic sign of infection, ; = no uredinia but small hypersensitive necrotic or chlorotic flecks present, ;N = necrotic areas without sporulation, 1 = small uredinia surrounded by necrosis, 2 = small to medium uredinia surrounded by necrosis or chlorosis (green islands may be surrounded by necrotic or chlorotic border), 3 = medium uredinia with or without chlorosis, 4 = large uredinia without chlorosis, X = heterogeneous, similarly distributed over the leaves, C = more chlorosis than normal for the IT, + = uredinia somewhat larger than normal for the IT, NT = not tested. A range of variation between ITs is recorded, with the most prevalent IT listed first (1C, 12, or ;2C)

At the adult plant stage ratings are based on the modified Cobb scale (Peterson et al. 1948) and include disease severity (percent leaf area affected) and infection type; 0 = no uredinia or other macroscopic sign of infection (immune); t = traces (small hypersensitive necrotic or chlorotic flecks); R = resistant, MR = moderately resistant; MS = moderately susceptible; S = susceptible

The five rust resistant wheat-*Ae. geniculata* introgression lines along with the resistant substitution line TA6675 (DS5M<sup>g</sup>(5D)), the susceptible recurrent parent WL711, the original rust resistant donor accession (TA10437) of *Ae. geniculata* ( $2n=28$ , U<sup>g</sup>U<sup>g</sup>M<sup>g</sup>M<sup>g</sup>) and Chinese Spring were used for cytogenetic and molecular characterization using GISH and Restriction Fragment Length Polymorphisms (RFLPs).

For genetic analysis and molecular mapping of leaf rust and stripe rust resistance, the highly susceptible hard red winter wheat cultivar Wichita was crossed as the female with the introgression line (TA5602) with smallest alien segment T5DL-5DS-5M<sup>g</sup>S(0.95). An F<sub>2</sub> population of 111 plants derived from one F<sub>1</sub> plant was used for genetic and molecular mapping of the rust resistance. Nullitetrasonic stocks of group-5 chromosomes of CS wheat (Sears 1954, 1966a) were used to map the rust resistant introgressions to specific chromosomes. All the plants were grown in the square pots filled with Scotts Metro Mix 200 (Sun Gro Horticulture Canada CM Ltd).

#### Leaf rust and stripe rust screening

The leaf and stripe rust reaction of all the introgression lines and parental lines was tested by screening the plants at two-leaf seedling and adult-plant stages. For testing the leaf rust response, five pathotypes (PRTUS6, PRTUS25, PRTUS35, PNMQ, MCDL) (for virulence/avirulence formulae see Long et al. 2000) of *P. tritici* Erikss were used. Isolate KS2005 of *P. striiformis* Westend. f. sp. *tritici* was used for screening the plants for stripe rust reaction. Isolate KS2005 belongs to race PST-100 (virulent on Lehmi, Heines VII, Produra, Yamhill, Stephens, Lee, Fielder, Express, Yr8-AVS, Yr9-AVS, Clements, and Compair).

The F<sub>2</sub> population, along with the parents, Wichita and the introgression line TA5602, were inoculated with stripe rust isolate KS2005 at the two-leaf seedling stage and the same plants were inoculated with leaf rust race MCDL at adult plant stage, to study the stripe rust and leaf rust resistance segregation.

Urediniospores for each race suspended in Soltrol-170 mineral oil (Chevron-Phillips chemical company) were atomized onto the plants. For stripe rust test, inoculated seedlings and adult plants were kept in the dark dew chamber for 24 h at  $12 \pm 2^\circ\text{C}$ . After inoculation, plants were kept in growth chambers that were set at  $16^\circ\text{C}$  day and  $14^\circ\text{C}$  night temperatures with 16 h photoperiod. For leaf rust test, inoculated seedlings and adult plants were incubated in a dew chamber for 18 h at  $18^\circ\text{C}$ . Plants were then placed in a greenhouse at  $19\text{--}21^\circ\text{C}$ , with supplemental sodium vapor lighting. The infection types (ITs) of stripe rust were scored 20 days after inoculation. For leaf rust the IT scoring was done 10–12 days after inoculation. Infection

types for leaf rust and stripe rust reaction at seedling stage was scored according to the modified Stakman scale of Roelfs et al. (1992) and at adult-plant stage, the rust reaction was scored according to the modified Cobb scale (Peterson et al. 1948) as illustrated in McIntosh et al. (1995).

#### Molecular characterization and mapping

Genomic *in situ* hybridization was used to monitor the size of the alien introgression in the rust resistant translocation lines. GISH was done according to Zhang et al. (2001) using *Ae. comosa* ( $2n = 14 = \text{MM}$ ) genomic DNA as probe and CS genomic DNA as blocker.

RFLP probes that detect orthologous alleles among 5A, 5B, 5D and 5M<sup>g</sup> chromosomes were used to identify and map the introgressed segments. DNA isolation, and Southern hybridizations were done according to Kuraparthy et al. (2007). A total of 11 RFLP clones and one cDNA of grain soft protein (GSP) were used to identify and map the rust resistant introgressions from *Ae. geniculata* in wheat. RFLP markers were selected based on the previously published map locations in the genetic and physical maps (<http://www.wheat.pw.usda.gov/GG2/maps.shtml#wheat>; Dubcovsky et al. 1996; Gill and Raupp 1996; Nelson et al. 1995; Qi and Gill 2001).

#### RFLP clones

All BCD and CDO clones were provided by Dr. M.E. Sorrells (Cornell University, Ithaca, NY, USA); PSR clones were from Dr. M.D. Gale (John Innes Centre, Norwich, UK); and ABC and ABG clones were provided by Dr. A. Kleinhofs (Washington State University, Pullman, WA, USA). FBB clones and cDNA of GSP were obtained from Dr. P. Leroy (INRA, France).

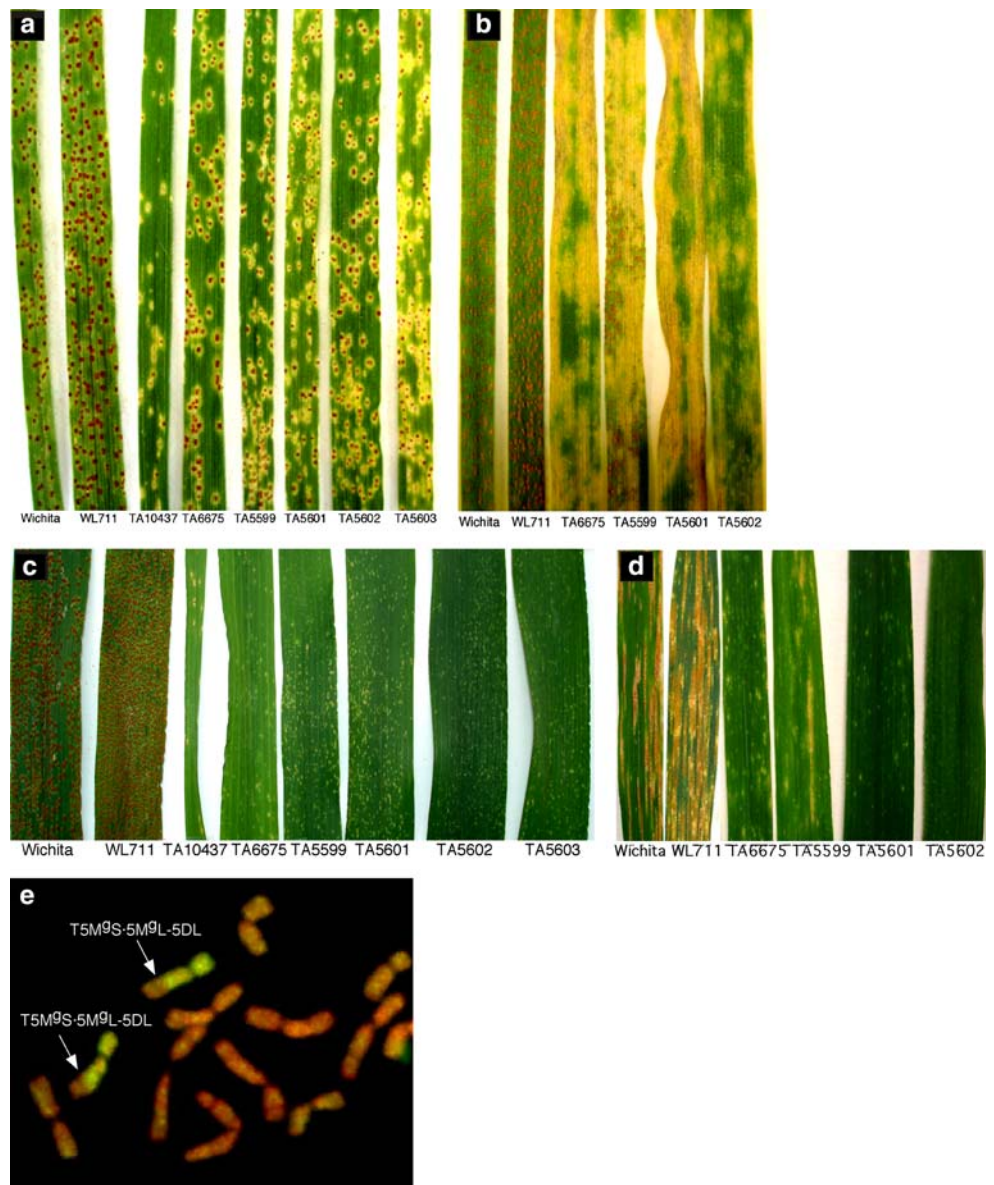
## Results

#### Rust reaction of the introgression lines

At the seedling stage, the *Ae. geniculata* accession TA10437, disomic substitution line DS5M<sup>g</sup>(5D) (TA6675) and all the introgression lines showed resistant to moderately resistant reaction to leaf rust, whereas the recipient wheat cultivar WL711 and Wichita were susceptible (Table 2; Fig. 1a). All the introgression lines and the parents showed a clear hypersensitive resistant reaction to the isolate KS2005 of stripe rust at the seedling stage, whereas the wheat cultivars WL711 and Wichita were highly susceptible (Table 2; Fig. 1b).

At the adult-plant stage, the parental accession of *Ae. geniculata*, DS5M<sup>g</sup>(5D) and all the introgression lines were

**Fig. 1** **a** Leaf rust (race: MCDL) reactions of the parents and wheat-*Ae. geniculata* introgression lines at the seedling stage. **b** Stripe rust (race: KS2005) reactions of the parents and the wheat-*Ae. geniculata* introgression lines at the seedling stage. **c** Leaf rust (race: MCDL) reactions of the parents and the wheat-*Ae. geniculata* introgression lines at the adult-plant stage. **d** Stripe rust (race: KS2005) reactions of the parents and the wheat-*Ae. geniculata* introgression lines at the adult-plant stage. **e** GISH pattern of mitotic metaphase chromosomes of translocation T5M<sup>S</sup>S·5M<sup>L</sup>L-5DL in introgression line TA5599 using total genomic DNA of *Ae. comosa* as probe. M<sup>S</sup>-genome chromatin of *Ae. geniculata* is visualized by green FITC fluorescence, whereas wheat chromosomes counterstained with Propidium Iodide (PI) fluoresce red. *Arrows* point to the translocation breakpoint between the 5M<sup>S</sup> and 5D chromosomes



completely resistant (hypersensitive flecks) to all the leaf rust races tested (Table 2, Fig. 1c). Cultivars WL711 and Wichita were highly susceptible to the above races of leaf rust except that WL711, having *Lr13*, showed a resistant reaction to PNMQ (avirulent on *Lr13*) (Table 2). Introgression lines and their resistant donor parents (TA10437, TA6675) were completely resistant (as revealed by their hypersensitive reaction), whereas the parental cultivars WL711 and Wichita were highly susceptible to isolate KS2005 of stripe rust (Table 2, Fig. 1d). All the introgression lines showed similar ITs typical of substitution line (TA6675) to both leaf rust and stripe rusts at adult-plant stage (Table 2; Fig. 2a, b).

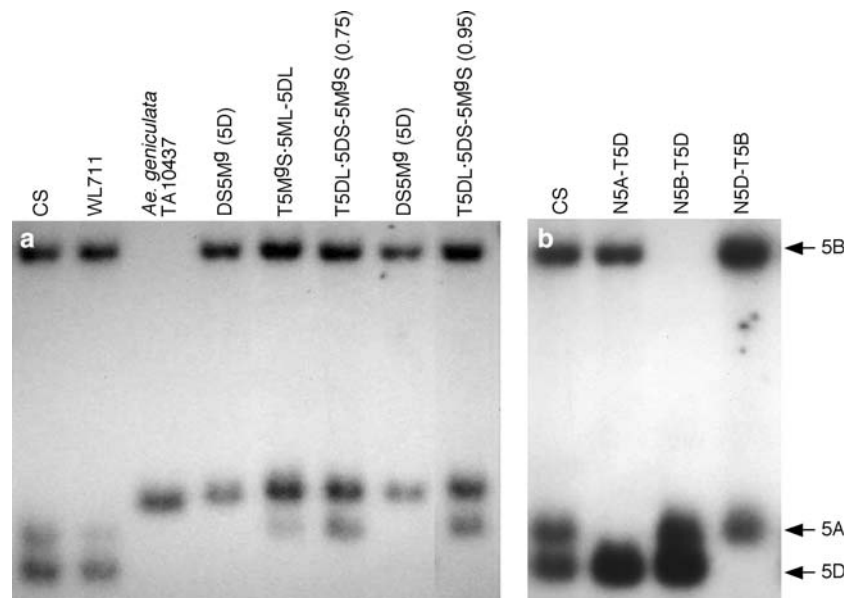
The F<sub>2</sub> population derived from the cross Wichita × TA5602 (T5DL·5DS·5M<sup>S</sup>S(0.95)) was inoculated with stripe rust at the two-leaf seedling stage, and the

same plants were screened with leaf rust race MCDL at the adult-plant stage. The segregating F<sub>2</sub> population showed clear ITs of resistance and susceptibility to stripe rust isolate KS2005 at seedling stage and ITs of resistance and susceptibility to leaf rust race MCDL at adult plant stage. All the stripe rust resistant F<sub>2</sub> plants were resistant to leaf rust and all the susceptible plants to stripe rust were susceptible to leaf rust.

#### Characterization of leaf rust and stripe rust resistant introgression lines

Cytogenetic characterization of introgression lines using *Ae. comosa* DNA as probe in the GISH experiments showed that the lines showing both leaf rust and stripe rust resistance had three types of introgressions of chromosome

**Fig. 2** RFLP analysis of introgression lines. **a** Southern hybridization pattern of *Eco*RI-digested genomic DNA of parents and introgression lines probed with a cDNA of wheat grain soft protein (*GSP*). **b** Southern hybridization pattern of probe *GSP* to *Eco*RI-digested genomic DNA of homoeologous group-5 aneuploids of Chinese Spring



5M<sup>S</sup> of *Ae. geniculata* in wheat. In the first type, a complete chromosome arm and part of the other arm was derived from 5M<sup>S</sup> of *Ae. geniculata* (Fig. 1e). The second type of rust resistant introgression line possessed only a part of the chromosome 5M<sup>S</sup>. No introgression of chromosome 5M<sup>S</sup> could be detected using GISH in the third type of resistant introgression lines. All three types of introgressions, including those with progressively smaller introgressed segments showing similar ITs to different races of leaf rust and stripe rust in the translocation lines, tentatively indicated that the leaf rust and stripe rust resistance genes are located in a contiguous and very small introgressed segment from chromosome 5M<sup>S</sup> of *Ae. geniculata* in wheat.

In order to establish the nature of the rust resistant introgression, previously-mapped RFLP probes (<http://www.wheat.pw.usda.gov/GG2/maps.shtml#wheat>; Dubcovsky et al. 1996; Gill and Raupp 1996; Nelson et al. 1995; Qi and Gill

2001), which detect orthologous alleles among the A, B, D and M genomes were selected for chromosome mapping. Based on the published map positions, 11 RFLP probes and one cDNA of *GSP* mapping to homoeologous group-5 chromosomes, were used. All three types of introgression lines, parental lines, CS (*Ph<sup>1</sup>*) and *Ae. geniculata* (TA10437) were digested with five different restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hind*III, *Xba*I) to identify introgressed segments in each resistant line using diagnostic polymorphisms between wheat and chromosome 5M<sup>S</sup>. RFLP marker FBB323 mapped at the distal end of chromosome 5DL, although producing multiple bands, showed distinct polymorphism between 5M<sup>S</sup> and wheat homoeologous group-5 chromosomes. Only one introgression line (TA5600) out of five showed the chromosome arm 5M<sup>S</sup>L specific alleles with RFLP probe FBB323 (Table 3). This indicated that the rust resistance in all the lines was due to

**Table 3** Characterization of introgression lines using physically mapped RFLP and cDNA wheat clones (“+” and “–” indicates the presence and absence of diagnostically polymorphic bands between wheat and chromosome 5M<sup>S</sup> of *Ae. geniculata*)

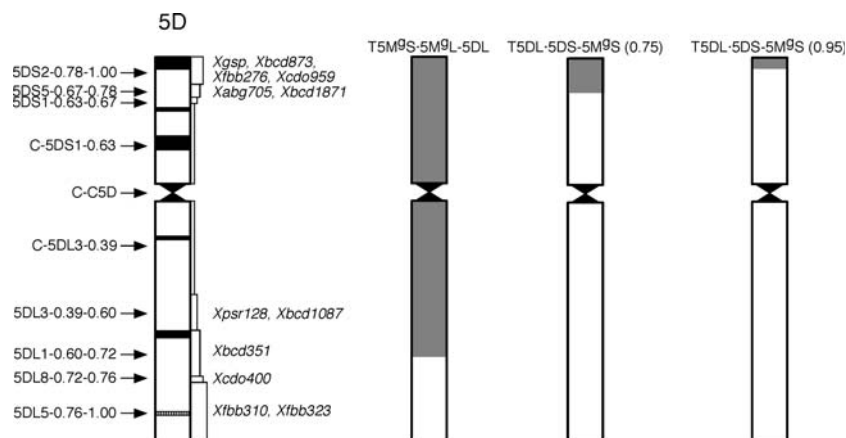
Clones	Wheat- <i>Ae. geniculata</i> introgression lines				
	TA6675 DS5M <sup>S</sup> (5D)	TA5600 DS5M <sup>S</sup> (5D)	TA5599 T5M <sup>S</sup> S-5M <sup>S</sup> L-5DL	TA5601 T5DL-5DS-5M <sup>S</sup> S(0.75)	TA5602, TA5603 T5DL-5DS-5M <sup>S</sup> S(0.95)
GSP	+	+	+	+	+
FBB276	+	+	+	+	+
BCD873	+	+	+	+	+
BCD1871	+	+	+	+	–
BCD1087	+	+	+	–	–
PSR128	+	+	+	–	–
BCD351	+	+	+	–	–
CDO400	+	+	–	–	–
FBB323	+	+	–	–	–

the introgression of short arm of 5M<sup>S</sup> to one of the chromosomes in wheat. The RFLP markers FBB276 and GSP mapped at the telomeric end of chromosome arm 5S (Dubcovsky et al. 1996; Gill and Raupp 1996) showed the diagnostically polymorphic marker alleles of chromosome 5M<sup>S</sup> in all three types of introgression lines (Fig. 2a, Table 3) which confirmed that the rust resistance in all translocation lines was due to the introgression of a part of chromosome arm 5M<sup>S</sup> of *Ae. geniculata* in wheat.

Previous reports indicated that CS contains three copies of GSP, one in each of the A, B and D genomes (Jolly et al. 1996; Tranquilli et al. 1999; Turner et al. 1999). However, the absence of two low molecular weight marker alleles of GSP in the substitution line (TA6675) (Fig. 2a) showed that the disomic substitution line was nullisomic for two group-5 homoeologous chromosomes, either 5B and 5D or 5A and 5D or 5B and 5A. This was also evident from the absence of multiple marker alleles of FBB323 in the substitution line TA6675. To identify the specific wheat chromosome involved in the gene transfer nullitetrasomics of CS were used. Because the introgression lines were in a WL711 background and the nullitetrasomics are in a CS background, the marker that showed diagnostic polymorphic alleles between WL711 and translocation line TA5602 (T5DL·5DS·5M<sup>S</sup>S(0.95)), yet remained monomorphic between WL711 and CS, was used to map the rust resistant introgression of 5M<sup>S</sup> to specific wheat chromosome using CS nullitetrasomics. Of the two markers that diagnostically identified the 5M<sup>S</sup> segment in line TA5602 (T5DL·5DS·5M<sup>S</sup>S(0.95)), GSP showed three monomorphic alleles between WL711 and CS (Fig. 2a), whereas FBB276 showed polymorphic and variable number of marker alleles between CS and WL711. Southern hybridizations of group-5 nullitetrasomics with the GSP probe showed that the lowest molecular weight allele belonged to 5D, the allele with a

slightly higher molecular weight is from chromosome 5A and the allele with the highest molecular weight belonged to chromosome 5B (Fig. 2b). The absence of the lowest molecular weight allele and presence of 5M<sup>S</sup> specific allele suggest that the rust resistance of *Ae. geniculata* was, in fact, transferred to chromosome 5D of wheat in all three types of introgression lines (Fig. 2a).

Rust resistant introgressions were characterized with respect to the fraction length of CS chromosomal deletions using physically mapped RFLP markers of homoeologous group-5 chromosomes. The chromosome bin location of all the markers has been reported previously (Gill and Raupp 1996; Qi and Gill 2001) except FBB323 and ABC310 previously genetically mapped on 5D and 5B, respectively, were placed in the distal deletion bins 5DL-5 (FL 0.76) by combining the maps of Gill and Raupp (1996) and Nelson et al. (1995). Translocation breakpoints in the introgression lines were determined based on the presence or absence of diagnostic polymorphisms between chromosomes 5M<sup>S</sup> of *Ae. geniculata* and 5D of wheat for the physically mapped RFLP markers and cDNA of GSP (Table 3, Fig. 3). Introgression line TA5599 showed diagnostically polymorphic alleles for all markers except CDO400 and FBB323, which were mapped distally in the physical map (Fig. 3, Table 3). Because BCD351 showed the diagnostic polymorphism and it was mapped in the CS deletion bin 5DL1-0.60-0.72, the breakpoint of the translocation T5M<sup>S</sup>L-5DL in line TA5599 was, present in this deletion bin (Fig. 3). Likewise, the breakpoint of the translocation T5DL·5DS·5M<sup>S</sup>S in line TA5601 was present in CS deletion bin 5DS5-0.67-0.78, because only the proximal marker (BCD1871) on the short arm diagnostically identifying the introgression was mapped in this deletion bin (Fig. 3, Table 3). The third type of alien introgression, which could not be detected using GISH, showed diagnostic polymorphism only for markers



**Fig. 3** Physical map of chromosome 5D of wheat and inferred GISH and RFLP marker-based physical maps of recombinant wheat-*Ae. geniculata* chromosomes 5M<sup>S</sup> and 5D in a WL711 background. In the inferred physical maps of the introgression lines, *Ae. geniculata*

5M<sup>S</sup> chromatin is indicated by grey blocks. Empty blocks represent the 5D chromosome. The 5D physical map constructed based on Gill and Raupp (1996), Nelson et al. (1995), Qi and Gill (2001)

GSP, BCD873 and FBB276 (Table 3). Because all three of these markers were mapped in the deletion bin 5DS2-0.78-1.00, the breakpoint of the translocation between 5M<sup>S</sup> and 5D in introgression lines TA5602 and TA5603 was present in the deletion bin 5DS2-0.78-1.00 (Fig. 3). To distinguish the alien introgression in TA5601 from those in TA5602 and TA5603 we used the fraction length of their introgressed segment of 5M<sup>S</sup> to designate the translocation. Thus, the translocations were designated in introgression line TA5601 as T5DL·5DS-5M<sup>S</sup>S(0.75) and in TA5602 and TA5603 as T5DL·5DS-5M<sup>S</sup>S(0.95) (Fig. 3, Table 1).

#### Genetic and molecular analysis of rust resistance

An F<sub>2</sub> population of 111 plants from Wichita x T5DL·5DS-5M<sup>S</sup>S(0.95) was screened at the two-leaf seedling stage with stripe rust isolate KS2005 and at adult plant stage with leaf rust race MCDL. The F<sub>2</sub> population segregated 81 resistant and 30 susceptible plants, which was a good fit for monogenic segregation ratio of 3:1. This indicated that the stripe rust and leaf rust resistance in T5DL·5DS-5M<sup>S</sup>S(0.95) was monogenically inherited and that the rust resistance was dominant. In addition, all the stripe rust resistant F<sub>2</sub> plants were resistant to leaf rust and vice versa. This indicated that the leaf rust and stripe rust resistance in 5M<sup>S</sup>S segment was conferred either by two independent closely linked genes or by a single gene with a pleiotropic effect. Molecular mapping of the RFLP clone FBB276 and cDNA clone GSP in the F<sub>2</sub> population showed the co-segregation of *Ae. geniculata* specific marker alleles for both the markers with the leaf rust and stripe rust resistance. This suggested that the translocated segment of *Ae. geniculata* in introgression line TA5602 (T5DL·5DS-5M<sup>S</sup>S(0.95)) did not recombine with wheat chromosome arm 5DS, further confirming the association of rust resistance with 5M<sup>S</sup>S translocation and its map location on chromosome arm 5DS.

#### Discussion

The present study reports the genetic and molecular mapping of the leaf rust and stripe rust resistant introgression in an F<sub>2</sub> population. GISH and molecular characterization using physically mapped RFLP markers showed that the alien transfers conferring rust resistance were of three different types, based on the size of introgression of 5M<sup>S</sup> chromosome into chromosome 5D of wheat. The specific wheat chromosome involved in translocation was determined by mapping the diagnostic polymorphic alleles in CS nullitetrasonics of group-5 homoeologous chromosomes. All the rust resistant translocation lines showed the introgression of 5M<sup>S</sup> was to the chromosome 5D of wheat and

the smallest introgression of 5M<sup>S</sup>S with leaf rust and stripe rust resistance in line T5DL·5DS-5M<sup>S</sup>S(0.95) (TA5602) was less than 5% of the chromosome arm 5DS of wheat. The unique and new map location of the alien introgression on chromosome 5DS suggested that the leaf rust and stripe rust resistance genes reported here were new and were designated *Lr57* and *Yr40*, respectively.

Molecular mapping of the smallest translocation with rust resistance using physically and/or genetically mapped RFLP markers revealed that the novel introgression with rust resistance in line T5DL·5DS-5M<sup>S</sup>S(0.95) maps in less than 20% of the distal region of the short arms of group-5 chromosomes of wheat (Fig. 3, Table 3). Because the smallest genomic DNA segment that could be resolved using GISH is 25 million base (Mb) pairs (Mukai et al. 1993), the absence of *Ae. comosa* (MM) GISH signals in the introgression line T5DL·5DS-5M<sup>S</sup>S(0.95) suggests that the alien introgression conferring novel rust resistance in this line is less than 25 Mb pairs of DNA. Because hexaploid wheat contains 17,000 Mb of DNA (Bennet and Leitch 1995) and the total length of all the wheat chromosomes is 235.4  $\mu$ m (Gill et al. 1991), 1  $\mu$ m a wheat chromosome corresponds to about 72 Mb of DNA (Mukai et al. 1991). Considering that the length of wheat 5D chromosome is 10.4  $\mu$ m with an arm ratio of 1.9 (Gill et al. 1991), the total amount of DNA of chromosome 5D corresponds to 748.8 Mb. The lack of *Ae. comosa* GISH signal in introgression lines TA5602 and TA5603 suggested that the alien introgression in T5DL·5DS-5M<sup>S</sup>S(0.95) was less than 3.5% of the distal chromosome arm 5DS (Fig. 3). The observation and estimation of the introgressed alien segment size in the present study is also supported by the resolution limits of fluorescent GISH which is estimated to be about 3–4% of the recombinants in wheat (Lukaszewski et al. 2005). Localization of recombination breakpoint in the distal part of the chromosome arm 5DS in T5DL·5DS-5M<sup>S</sup>S(0.95) was further supported by the physical and genetic map positions of the diagnostic RFLP markers BCD873, FBB276 and GSP on the distal telomeric end of 5DS (<http://www.wheat.pw.usda.gov/GG2/maps.shtml#wheat>, Dubcovsky et al. 1996; Gill and Raupp 1996, Qi and Gill 2001, Nelson et al. 1995, Tranquilli et al. 1999) (Fig. 3).

From the U- and M-genome cluster species of the *Triticeae*, only the diploid U-genome (*Aegilops umbellulata*, 2n = 2x = UU) and M-genome (*Ae. comosa*) donors of *Ae. geniculata* were used to transfer novel rust resistance genes into wheat. In a ground-breaking alien gene transfer for germplasm enhancement in crop plants, Sears (1956) transferred *Lr9* from *Ae. umbellulata* into wheat using irradiation. This compensating translocation was later found to be a homoeologous chromosome transfer T6BS·6BL-6UL (Sears 1961, 1966b; Friebe et al. 1995). *Yr8* and *Sr34* were transferred from *Ae. comosa* into wheat by utilizing



induced homoeologous pairing effect of *Ae. speltoides* (Riley et al. 1968a, b). This transfer was later found to be of the non-compensating type, with structurally rearranged chromosome segments of chromosome 2M translocated onto chromosomes 2D or 2A of wheat (Friebe et al. 1996; Nasuda et al. 1998). Although diploid U and M genome *Aegilops* species were used in alien gene transfers for germplasm enhancement, resistance gene transfers of *Ae. geniculata* in wheat were not unequivocally characterized and catalogued for germplasm release. Results from the present study showed the successful transfer and characterization of three different *Ph<sup>1</sup>* induced genetically compensating homoeologous transfers of chromosome 5M<sup>S</sup> of *Ae. geniculata* to chromosome 5D of wheat. The present study also showed the precise transfer of novel leaf rust and stripe rust resistance genes from *Ae. geniculata* to bread wheat in translocation line T5DL-5DS-5M<sup>S</sup>(0.95).

Most wheat derivatives with resistance genes from alien species had limited use in practical breeding because of cytological instability of alien chromosome segments incorporated in non-homoeologous regions or because of the linkage of the undesirable genes on the large alien segments (Friebe et al. 1996; Nasuda et al. 1998). Three effective methods have been used for the intergenomic transfer of genes in wheat, irradiation (Sears 1956), induced-homoeologous pairing (Riley et al. 1968a, b) and gametocidal chromosome-induced chromosome breakage (Endo 1988, 1994; Masoudi-Nejad et al. 2002). Of the three methods, induced homoeologous pairing is the method of choice. Because chromosome segments transferred by homoeologous recombination are usually in the correct location in the genome and compensate well for the replaced original chromosome segment, transfers are more likely to be agronomically desirable. However, even with homoeologous recombination, the length of the alien segment may be large either due to non-random distribution of recombination (Lukaszewski 1995; Lukaszewski et al. 2004; Qi et al. 2007; Rogowsky et al. 1993) or due to the fact that most of the alien chromosome is highly rearranged and only a small segment is available for recombination as was the case with 2M chromosome transfer (Nasuda et al. 1998). Sears (1972, 1981) suggested a method for further reducing the length of the alien segments. In this strategy, reciprocal primary recombinants with breakpoints flanking the locus of interest were intercrossed and allowed to recombine in the presence of the wild type *Ph<sup>1</sup>* locus, which permits only homologous recombination. Secondary recombinant chromosomes with smallest interstitial inserts of alien chromatin into wheat chromosomes were then selected (Sears 1972, 1981). Lukaszewski (2000, 2006) used this method to reduce the size of rye chromatin in wheat.

The present results demonstrate the phenomenon of what may be termed as “cryptic alien introgression” that may

have gone undetected because of the methodological limitations of alien introgression research often based on cytological methods and rarely a few molecular markers. Because disease resistance genes are mostly located in the terminal recombination-rich regions of the grass chromosomes (Leister et al. 1998; Dilbirligi et al. 2004; Qi et al. 2004) the detection of the small terminal alien introgressed segments carrying disease resistance genes will be difficult using cytological methods alone. By selecting rust resistant lines which had no obvious effects on plant growth and development from primary recombinants and characterizing those lines using GISH and physically and genetically mapped RFLP probes, we detected cryptic alien introgression and identified one very small and novel transfer T5DL-5DS-5M<sup>S</sup>(0.95) (TA5602, TA5603) with leaf rust and stripe rust resistance genes. As revealed by the small size of the alien introgression (less than 3.5% of distal 5DS) on chromosome 5DS, our results suggest that it is possible to transfer novel and useful genetic variability from wild species without the usual linkage drag. Furthermore, if more than one gene is located on the alien segment as is the case here then these ‘cryptic alien introgressions’ are effective resistance pyramids that will behave as single mendelian factors in breeding.

Homoeologous group-5 chromosomes of wheat contain at least seven catalogued genes for rust resistance. Except for, *Yr19*, whose arm location is unknown on chromosome 5B, most of the resistance genes were mapped on the long arms of homoeologous group-5 chromosomes (*Lr18* and *Yr3* on 5BL, *Lr1* and *Sr30* on 5DL and *Yr34* on 5AL) (see <http://www.ars.usda.gov/Main/docs.htm/docid=10342>). Only two leaf rust resistance genes have been mapped on the homoeologous chromosome arm 5S of wheat. An uncatalogued major gene with a broad-spectrum of resistance to leaf rust at adult-plant stage was mapped 16.7 cM proximal to *Xgwm443* on chromosome arm 5BS (Obert et al. 2005). *Lr52*, a major gene conferring a broad-spectrum wheat leaf rust resistance, was mapped 16.5 cM distal to the microsatellite marker *Xgwm443* on chromosome arm 5BS of wheat (Hiebert et al. 2005). It is not known if *Lr57* is orthologous to any of the *Lr* genes mapped on the short arms of group-5 chromosomes. The other sources of resistance carrying resistance to leaf rust and stripe rust, *Lr26/Yr9* (Metten et al. 1973) and *Lr37/Yr17* (Bariana and McIntosh 1993) have been overcome by pathotypes of these two rust pathogens. *Yr40* and *Lr57* would be useful in replacing the defeated sources of resistance.

Wheat stripe rust disease caused by *P. striiformis* has become increasingly destructive since the late 1990s and severe damage to wheat caused by stripe rust was reported on all the continents (see the review by Chen et al. 2002; Chen 2005). PST-100 accounted for 33.4% of the total PST races. Furthermore, more than 96% of the isolates belonged

to the group of races with virulences to *Yr8*, *Yr9*, and other resistance genes, which caused widespread stripe rust epidemics in the US from 2000 to 2005 (Chen and Penman 2006). The isolate KS2005 used for stripe rust screening in the present study belonged to race PST-100 and was high temperature tolerant partly explaining its occurrence in the south central US (Milus et al. 2006). All the introgression lines reported in the present study gave a resistant reaction to isolate KS2005 both at seedling as well as adult plant stage (Table 2). Hence, the wheat-*Ae. geniculata* stripe rust resistant introgression lines, especially T5DL-5DS-5M<sup>6</sup>S(0.95) is an excellent germplasm that could be used in wheat breeding programs in North America for developing stripe rust resistant wheat cultivars.

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