



## *Ph<sup>1</sup>*-induced transfer of leaf and stripe rust-resistance genes from *Aegilops triuncialis* and *Ae. geniculata* to bread wheat

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Received 10 January 2002; accepted 14 February 2002

**Key words:** *Aegilops geniculata*, *Aegilops triuncialis*, alien gene transfer, homoeologous recombination, *Puccinia recondita*, *Puccinia striiformis*, *Triticum aestivum*

### Summary

Leaf and stripe rusts are severe foliar diseases of bread wheat. Recently, chromosomes 5M<sup>S</sup> from the related species *Aegilops geniculata* that confers resistance to both leaf and stripe rust and 5U<sup>t</sup> from *Ae. triuncialis* conferring resistance to leaf rust have been transferred to bread wheat in the form of disomic DS5M<sup>S</sup>(5D) and DS5U<sup>t</sup>(5A) chromosome substitution lines. The objective of this study was to shorten the alien segments in these lines using *Ph<sup>1</sup>*-mediated, induced homoeologous recombination. Putative recombinants were evaluated for their rust resistance, and by genomic in situ hybridization and microsatellite analyses. One agronomically useful wheat-*Ae. geniculata* recombinant resistant to leaf and stripe rust was identified that had only a small terminal segment of the 5M<sup>S</sup>L arm transferred to the long arm of an unidentified wheat chromosome. This germplasm can be used directly in breeding programs. Only one leaf rust-resistant wheat-*Ae. triuncialis* recombinant, which consists of most of the complete 5U<sup>t</sup> chromosome with a small terminal segment derived from 5AS, was identified. This germplasm will need further chromosome engineering before it can be used in wheat improvement.

### Introduction

Among the wheat foliar diseases, leaf rust (caused by *Puccinia recondita* Rob. ex Desm. f. sp. *tritici*) and stripe rust (caused by *P. striiformis* Westend f. sp. *tritici*) are the most damaging diseases of wheat worldwide (Knott, 1989; McIntosh et al., 1995; Nayar & Bhardwaj, 1998). Although, a number of resistance genes have been transferred into wheat cultivars during the last 3–4 decades, many have become ineffective because of the emergence of new rust pathotypes, necessitating a continuous search for new sources of resistance.

Related species of wheat represent a large reservoir of useful genetic variability including rust resistance that can be exploited in wheat improvement (Knott & Dvorak, 1976; Sharma & Gill, 1983; Gale & Miller,

1987; Knott, 1993; Jiang et al., 1994; Friebe et al., 1996). Although a large number of transfers with useful alien genes have been produced, few have been exploited commercially. Most of the introduced alien segments from wild relatives into wheat either do not compensate well for the loss of wheat chromatin or contain undesirable genes causing depression in yield and quality (Jiang et al., 1994; Friebe et al., 1996).

After the discovery of the role of the *Ph<sup>1</sup>* gene (Okamoto, 1957; Sears & Okamoto, 1958; Riley & Chapman, 1958; Sears 1977), there has been much interest in overcoming or bypassing its effect, so that desirable genes from alien species can be transferred to homoeologous wheat chromosomes. Induced homoeologous recombination between wheat and alien chromosomes has been demonstrated, by using differ-

ent techniques such as *ph1* mutant, nulli-5B stocks, and by crossing with some accessions of *Ae. speltoides* that carry genes epistatic to *Ph1*. However, these strategies require elaborate breeding schemes and cytological analysis to achieve the desired gene transfer (Riley et al., 1968; Sears 1976; Lukaszewski, 1995, 2000). Recently, the dominant gene *Ph<sup>1</sup>* (*Ph* inhibitor) was transferred from *Ae. speltoides* to the hexaploid wheat cultivar Chinese Spring (Chen et al., 1994). This gene suppresses the effect of the *Ph1* locus and permits homoeologous recombination between wheat and alien chromosomes in F<sub>1</sub> hybrids (Chen et al., 1994; Aghaee-Sarbarzeh et al., 2000).

Evaluation of wild *Triticum* and *Aegilops* species at the Punjab Agricultural University has identified *Aegilops* species with C, U, and M genomes as good sources of resistance to leaf and stripe rusts (Dhaliwal et al., 1993; Gill et al., 1995; Singh & Dhaliwal, 2000). Here, we report the transfer and characterization of leaf and stripe rust-resistance genes from *Ae. triuncialis* L. and *Ae. geniculata* Roth via *Ph<sup>1</sup>*-induced homoeologous recombination.

## Materials and methods

### Plant material

One rust-resistant, disomic substitution (DS) line DS5M<sup>g</sup>(5D) was derived from a cross of the highly leaf and stripe rust-susceptible hexaploid wheat (*Triticum aestivum* L.) cultivar WL711 and *Ae. geniculata* (2n=4x=28, U<sup>g</sup>U<sup>g</sup>M<sup>g</sup>M<sup>g</sup>) accession #3547. Another rust-resistant line DS5U<sup>t</sup>(5A) was derived from the cross of WL711 with *Ae. triuncialis* (2n=4x=28, U<sup>t</sup>U<sup>t</sup>C<sup>t</sup>C<sup>t</sup>) accession #3549. DS5M<sup>g</sup>(5D) and DS5U<sup>t</sup>(5A) were crossed with Chinese Spring (CS) wheat with the *Ph<sup>1</sup>* gene, CS(*Ph<sup>1</sup>*), and their derivatives were evaluated at the seedling stage for their reactions to individual pathotypes of leaf rust 77A-1 (avirulent on *Lr9*, *Lr20*, *Lr23*, and *Lr26* and virulent on *Lr1*, *Lr3*, *Lr10*, *Lr13*, and *Lr15*), 77-2 (avirulent on *Lr9* and *Lr26* and virulent on *Lr1*, *Lr3*, *Lr10*, *Lr13*, *Lr15*, *Lr20*, and *Lr23*), 77-5 (avirulent on *Lr9* and *Lr21* and virulent on *Lr1*, *Lr3*, *Lr10*, *Lr13*, *Lr15*, *Lr20*, *Lr23*, *Lr26*, and *Lr30*) and 77-6 (avirulent on *Lr9* and *Lr20* and virulent on *Lr1*, *Lr3*, *Lr10*, *Lr13*, *Lr15*, *Lr21*, *Lr23* and *Lr26*) and stripe rust (M). The avirulence/virulence formulae of each of the pathotypes against standard rust differentials can facilitate comparison of the rust resistance

of the derivatives against the prevalent pathotypes in different regions of the world. To evaluate the derivatives for seedling resistance to individual pathotypes leaves of 5 to 7 day-old seedlings were first inoculated with urediospores using lancet needle. The inoculated seedlings were incubated for 24 h at appropriate temperature and high humidity for the rusts before moving them to a greenhouse. Data on rust infection types was recorded according to a modification of the scale given by Stakman et al. (1962) as follows: 0; immune, no visible infection/; no uredia, hypersensitive flecks present/ 1 resistant, minute nonsporulating uredia surrounded by distinct necrotic areas/ 2 resistant to moderately resistant, small uredia with slight sporulation surrounded by chlorotic or necrotic areas/ 3 moderately resistant to moderately susceptible, medium sized sporulating uredia, chlorotic areas may be present/ 4 susceptible, large sporulating uredia with no chlorosis or necrosis/ X resistant, heterogeneous, variable sized uredia distributed over leaves.

### Cytological studies

Previous studies have identified chromosomes 5M<sup>g</sup> of *Ae. geniculata* and 5U<sup>t</sup> of *Ae. triuncialis* as sources of resistance to leaf and stripe rusts (Dhaliwal, unpublished). Genomic in situ hybridization (GISH) using genomic DNA of the diploid progenitor species *Ae. comosa* Sm. in Sibth. & Sm. and *Ae. umbellulata* Zhuk. allows the detection of M<sup>g</sup>- and U<sup>t</sup>-genome chromatin in a wheat background. GISH was performed according to Friebe et al. (2000). Briefly, total genomic DNA was extracted from CS wheat, *Ae. comosa*, and *Ae. umbellulata* using the CTAB method (Ried & Anderson, 1996). The DNA from the diploid species was sheared using a needle to an average size of 200 bp and CS DNA was autoclaved for 12 min. The genomic *Ae. comosa* and *Ae. umbellulata* DNA was labeled with fluorescein (FITC) dUTP using nick-translation. A ratio of 35–40: 1 of wheat competitor DNA to labeled probe (*Ae. comosa* or *Ae. umbellulata* DNA) was used in the hybridization mixture. Chromosomes were counterstained with propidium iodide (PI) and mounted in Vectashield (Vector Laboratories). Slides were analyzed with an epifluorescence Zeiss Axioplan 2 microscope. Images were captured using a SPOT CCD (charge-coupled-device) camera operated with SPOT 2.1 software (Diagnostic Instruments) and processed with Photoshop 5.5 (Adobe Systems).

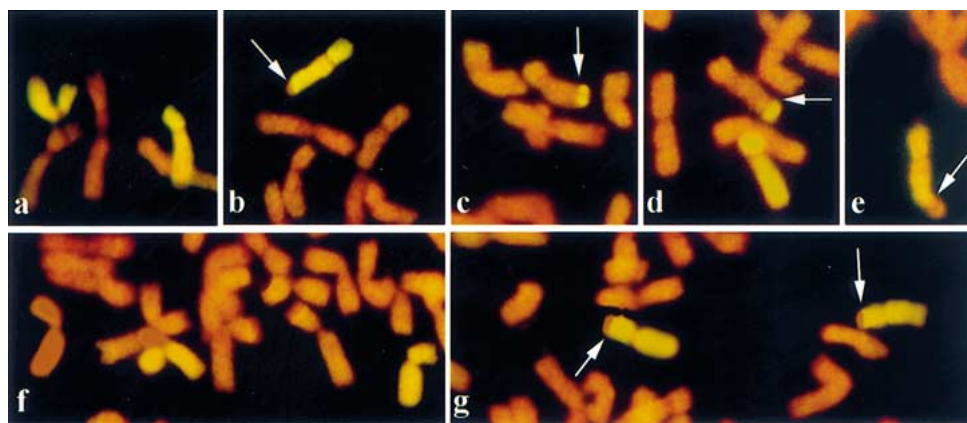


Figure 1. GISH pattern of wheat-*Ae. geniculata* (a-e) and wheat-*Ae. triuncialis* (f, g) introgression lines: (a) DS5M<sup>6</sup>(5D), (b) 2K-9-2, (c) 2K-11-1, (d, e) 2K-8-2, (f) DS5U<sup>1</sup>(5A), and (g) 2K-69-4. M<sup>6</sup>-genome chromatin of *Ae. geniculata* and U<sup>1</sup>-genome chromatin of *Ae. triuncialis* is visualized by yellow-green FITC fluorescence, whereas wheat chromosomes are counterstained with PI and fluoresce red. Arrows point to the breakpoints.

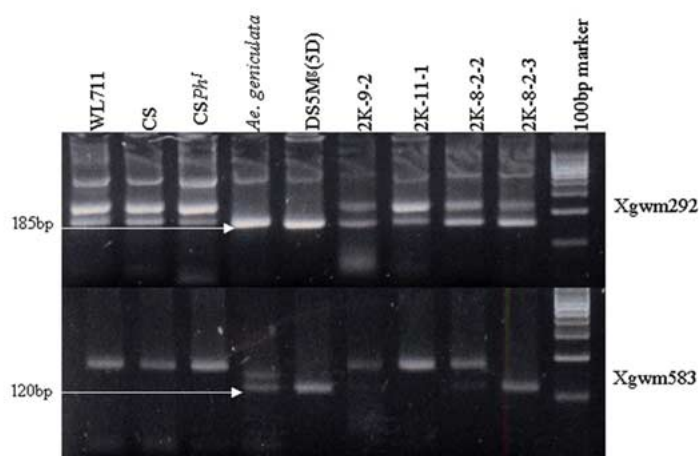


Figure 2. PCR analysis of the parents and introgression lines with microsatellite markers GWM292 and GWM583 specific to chromosomes 5D. These markers amplified 185-bp and 120-bp DNA fragments specific to *Ae. triuncialis* and are also present in the substitution line and derivatives.

#### DNA isolation and PCR amplification

Total genomic DNA was isolated from the parents and introgression lines following the CTAB method of Ried & Anderson (1996). Seven microsatellite markers (GWM154, GWM156, GWM186, GWM291, GWM293, GWM415, and GWM595) specific to chromosome 5A and eight (GWM182, GWM190, GWM192, GWM269, GWM292, GWM358, GWM565, and GWM583) specific to chromosome 5D (Röder et al., 1998) were used for molecular analysis of introgression lines along with the parental lines.

The PCR reactions were made in a volume of 25ul in a MJ thermocycler (Watertown, MA, USA) as de-

scribed previously (Röder et al., 1998) with minor modifications. The reaction mixture contained 250 nM of each primer, 0.2 mM of each dNTPs, 1.5 mM MgCl<sub>2</sub>, 1U Taq polymerase, and 50 ng of template DNA. After 3 min at 94 °C, 35 cycles were made for 1 min at 94 °C, 1 min at 50°, 55°, and 60 °C (as reported for the individual microsatellite by Röder et al., 1998), and 2 min at 72 °C followed by a final extension step at 72 °C for 10 min. PCR products were separated on 2.3% Metaphor agarose gel in 1X Tris borate buffer. Gels were stained with ethidium bromide, and DNA was visualized with UV light.

## Results

### *Wheat-Ae. geniculata* introgression lines

GISH and molecular analysis of DS5M<sup>g</sup>(5D) confirmed the presence of a pair of a complete 5M<sup>g</sup> chromosomes in this line (Figure 1a). Four of the eight microsatellite markers (GWM192, GWM292, GWM358, and GWM583) specific to chromosome 5D revealed polymorphism between WL711 and *Ae. geniculata*. However, only microsatellites GWM292 and GWM583 amplified 185-bp and 120-bp PCR products specific to chromosome 5M<sup>g</sup> of *Ae. geniculata* in DS5M<sup>g</sup>(5D) (Figure 2). DS5M<sup>g</sup>(5D) and all the backcross derivatives except 2K-11-1 also amplified 5M-specific DNA fragments. The 120-bp DNA fragment was faint in line 2K-9-2. The GISH pattern of the 2K-9-2 line showed a wheat-*Ae. geniculata* recombinant chromosome where the complete short arm and most of the long arm was derived from chromosome 5M<sup>g</sup> and the distal part of the long arm was derived from wheat (Figure 1b).

The derivative 2K-11-1 was segregating for a wheat-*Ae. geniculata* recombinant chromosome where the short arm and most of the long arm was derived from wheat and the distal region of the long arm was derived from chromosome 5M<sup>g</sup> (Figure 1c).

Two types of wheat-*Ae. geniculata* recombinant chromosomes, in addition to a complete 5M<sup>g</sup> chromosome, are present in the derivative 2K-8-2. One of the recombinants is derived mostly from wheat except for the distal 15% of the long arm, which is derived from chromosome 5M<sup>g</sup> (Figure 1d) like that of 2K-11-1. The other recombinant is mostly derived from 5M<sup>g</sup> except for the distal 10% of the long arm, which is derived from wheat (Figure 1e) like that of 2K-9-2.

The *Ae. geniculata* parent accession #3547, DS5M<sup>g</sup>(5D) and its backcross derivatives 2K-9-2, 2K-11-1, and 2K-8-2 were resistant to the most virulent leaf rust pathotypes 77-5 and 77-6 and race M of stripe rust at the seedling stage, whereas the recipient wheat cultivar WL711 is susceptible to both rusts (Table 1).

### *Wheat-Ae. triuncialis* introgression lines:

GISH analysis of DS5U<sup>t</sup>(5A) revealed the presence of a complete U<sup>t</sup>-genome chromosome in this line. The *Ae. triuncialis* chromosome has a satellite in the short arm identifying it as either 1U<sup>t</sup> or 5U<sup>t</sup> (Figure 1f). The group-5, long-arm specific, microsatellite markers GWM156 and GWM186 detected a polymorphism between WL711 and *Ae. triuncialis*, but

Table 1. Reactions to leaf rust pathotypes 77A-1, 77-2, 77-5 and 77-6 and stripe rust pathotype M at the seedling stage of the wheat-*Ae. geniculata* and wheat-*Ae. triuncialis* introgression derivatives and parental lines

Line	77A-1	77-2	77-5	77-6	M
WL711	3 <sup>+</sup>	3 <sup>+</sup>	3 <sup>+</sup>	3 <sup>+</sup>	3 <sup>+</sup>
CS( <i>Ph1</i> )	3 <sup>+</sup>	3 <sup>+</sup>	3 <sup>+</sup>	3 <sup>+</sup>	3 <sup>+</sup>
<i>Ae. geniculata</i>	–	–	0;	0;	0;
DS5M <sup>g</sup> (5D)	–	–	0;	0;–1	0;
2K-11-1	–	–	2 <sup>–</sup>	2	0;
2K-8-2	–	–	2	2	2 <sup>+</sup>
2K-9-2	–	–	2	2	0;
<i>Ae. triuncialis</i>	0;	0;	–	–	–
DS5U <sup>t</sup> (5A)	0;	0;–1	–	–	–
2K-69-4	0;	0;	–	–	–

0; 1 and 2: resistant/ 3 and 3<sup>+</sup>: susceptible/ +: more than the average of the class.

the alleles specific to *Ae. triuncialis* were absent in the substitution line. Line DS5U<sup>t</sup>(5A) has a speltoid-type spike confirming previous results that the missing wheat chromosome in this line is 5A (Dhaliwal, unpublished). *Ae. triuncialis* specific alleles of markers GWM156 and GWM186 may, therefore, belong to 5C<sup>t</sup> and not to 5U<sup>t</sup> of *Ae. triuncialis*. Besides chromosome 5U<sup>t</sup>, line DS5U<sup>t</sup>(5A) was segregating for a wheat-*Ae. triuncialis* recombinant chromosome where the distal part of the short arm was derived from an U<sup>t</sup>-genome chromosome (data not shown). GISH analysis of 2K-69-4 showed that this line was homozygous for a wheat-*Ae. triuncialis* recombinant chromosome where the distal part of the short arm was derived from wheat and the remaining part of the short arm and the complete long arm was derived from chromosome 5U<sup>t</sup> (Figure 1g).

The *Ae. triuncialis* accession #3549, DS5U<sup>t</sup>(5A) and line 2K-69-4 were resistant to the most virulent leaf rust pathotypes 77A-1 and 77-2 (Table 1). Moderate susceptibility (tS-5S) of line DS5U<sup>t</sup>(5A) to leaf rust under the field conditions compared to the high susceptibility (90S) of the recipient wheat parent WL711 suggests that chromosome 5U<sup>t</sup> has a gene conditioning slow rusting against the prevalent leaf rust races. The introgression line 2K-69-4 showed resistance to leaf rust under field conditions and in the seedling stage (Table 1).

## Discussion

The presented data show that the *Ae. geniculata* chromosome 5M<sup>g</sup> has genes conferring resistance to leaf and stripe rusts at the seedling stage. GISH analysis identified three wheat-*Ae. geniculata* introgression lines. GISH and microsatellite analyses of the derivatives 2K-9-2 and 2K-8-2 indicated that these lines still contain almost a complete 5M<sup>g</sup> chromosome, which makes them agronomically less desirable. However, the derivative 2K-11-1 has only a small terminal *Ae. geniculata* segment in the long arm of a wheat chromosome. The wheat chromosome involved in this recombinant chromosome is most likely 5D, because both 5D and 5M<sup>g</sup> were monosomic in the original F<sub>1</sub> hybrid, and are likely to recombine in a *Ph<sup>1</sup>* background. Because the *Ae. geniculata* segment in this translocation is small and approximately 15% of the 5M<sup>g</sup>L arm, the agronomic performance of this germplasm should not be impaired. Plants homozygous for the recombinant chromosome are presently being selected that will be useful in breeding programs.

The presented data also confirm that the *Ae. triuncialis* chromosome 5U<sup>t</sup> has a gene that confers resistance to leaf rust at the seedling stage. We recovered only one recombinant chromosome where the distal part of the short arm was derived from 5AS of wheat and the remaining part of the short arm and the complete long arm was derived from chromosome 5U<sup>t</sup>. Because this recombinant chromosome is mostly derived from 5U<sup>t</sup> and may contain many agronomically undesirable genes, further chromosome manipulations are needed before this germplasm can be exploited in wheat improvement.

Alien chromatin conditioning multiple resistance to several diseases has been reported frequently. Multiple resistance is mainly due to linkage of resistance genes on the alien chromatin. Linkage of the *Sr36/Pm6* from *T. timopheevii*, *Lr19/Sr25* and *Lr24/Sr24* from *Agropyron elongatum*, and *Lr25/Pm7* and those present in T1BL·1RS translocation lines (*Lr26/Sr31/Yr9/Pm8*) from *Secale cereale* are some of the examples (Friebe et al., 1996).

The results of the present study indicated that the induction of homoeologous pairing between wheat chromosomes and chromosomes of related species by *Ph<sup>1</sup>* gene is a good strategy to transfer desired variability into wheat genome. The use of substitution lines carrying the desirable alien gene(s) in crosses with hexaploid wheat stock carrying *Ph<sup>1</sup>* will facilitate controlled and compensated introgression of alien

genes into their wheat homoeologue. Moreover, the crossing scheme and recovery of derivatives with introgressed alien segments carrying the target gene is relatively easier and faster than crosses using the *ph1b* or nullisomic 5B stocks.

## Acknowledgements

This research has been financed in part by a grant made by the United States Department of Agriculture under U.S.-India fund and the Kansas Wheat Commission. Dr Aghaee-Sarbarzeh is grateful to the Agricultural Research, Education and Extension Organization (AREEO), Ministry of Agriculture, Government of Islamic Republic of Iran, for a grant of scholarship. We thank Peng Zhang for her help with the GISH analysis. This manuscript is contribution number 02-228-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS 66506-5502, USA.

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