A Cryptic Wheat–*Aegilops triuncialis* Translocation with Leaf Rust Resistance Gene *Lr58*

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ABSTRACT

Genes transferred to crop plants from wild species are often associated with deleterious traits. Using molecular markers, we detected a cryptic introgression with a leaf rust resistance gene transferred from *Aegilops triuncialis* L. into common wheat (*Triticum aestivum* L.). One agronomically desirable rust-resistant introgression line was selected and advanced to BC_3F_{11} from a cross of hexaploid wheat and *A. triuncialis*. In situ hybridization using *A. triuncialis* genomic DNA as a probe failed to detect the alien introgression. The translocation line was resistant to the most prevalent races of leaf rust in India and Kansas. Genetic mapping in a segregating $F_{2:3}$ population showed that the rust resistance was monogenically inherited. Homeologous group 2 restriction fragment length polymorphism markers *XksuF11*, *XksuH16*, and *Xbg123* showed diagnostically polymorphic alleles between the resistant and susceptible bulks. The alien transfer originated from homeologous chromosome recombination. The *A. triuncialis*-specific alleles of *XksuH16*, *XksuF11*, *Xbg123*, and one simple sequence repeat marker *Xcfd50* cosegregated with the rust resistance, suggesting that the wheat–*A. triuncialis* translocation occurred in the distal region of chromosome arm 2BL. This translocation was designated T2BS·2BL-2^{t}L(0.95). The unique source and map location of the introgression on chromosome 2B indicated that the leaf rust resistance gene is new and was designated *Lr58*.

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Abbreviations: BSA, bulk segregant analysis; CS, Chinese Spring; GISH, genomic in situ hybridization; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeat.

Leaf rust or brown rust (caused by *Puccinia triticina* Eriks.) is one of the most common diseases affecting wheat production worldwide. Development and deployment of resistant cultivars has been the most successful, environmentally sound, and economically viable approach to combat leaf rust. Incorporating host genetic resistance to this pathogen into adapted elite germplasm lines is therefore a major objective of most wheat breeding programs. Numerous resistance genes have been identified and introgressed into released cultivars (McIntosh et al., 1995, 2005), yet the continuous emergence of new races of the pathogen has been a substantial challenge to breeders attempting to produce cultivars with durable resistance. Thus, it is necessary to continue to identify further sources of resistance and incorporate them into elite breeding lines. Wheat has a narrow genetic base, and its wild relatives can be used as a source of new genes for disease resistance (Dvorak, 1977; Sharma and Gill, 1983; Gale and Miller, 1987; Jiang et al., 1994; Friebe et al., 1996).

Published in Crop Sci. 47:1995–2003 (2007).
doi: 10.2135/cropsci2007.01.0038

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Several strategies have been used for transferring alien segments that are smaller than complete chromosome arms into wheat from nonprogenitor wild species. Sears (1956) used radiation treatment to transfer a leaf rust resistance gene (*Lr9*) from *Aegilops umbellulata* Zhuk. to wheat. Recently, Masoudi-Nejiad et al. (2002) exploited the action of gametocidal genes to transfer alien chromosome segments to wheat. Both ionizing radiation treatment and gametocidal genes induce random chromosome breakage and fusion of the broken segments, resulting in translocation chromosomes. The majority of translocations were between nonhomeologous chromosomes, which leads to duplication or deficiencies and thus were noncompensating and agronomically undesirable. Alien genes from nonprogenitor species have been transferred to wheat through induced homeologous chromosome pairing between wheat and alien chromosomes (see the reviews by Friebe et al., 1996; Jiang et al., 1994) by making the *Ph1* gene ineffective. This was done either by using mutant or null alleles of the *Ph1* gene (Sears, 1972, 1981) or by using *Ph*^{I}, an epistatic inhibitor of the *Ph1* gene from *Aegilops speltoides* Tausch (Riley et al., 1968a, 1968b). These transfers were genetically compensating because they involved homeologous recombination. Their agronomic desirability, however, depended on the size of the alien segments transferred, which determined the degree of linkage drag (Jiang et al., 1994; Friebe et al., 1996). Small interstitial secondary recombinants could be isolated by further chromosome engineering using the primary recombinants (Sears, 1972, 1981; Lukaszewski, 2000, 2006). The identification and characterization of a cytologically undetectable primary recombinant, with a cryptic wheat–*Aegilops geniculata* Roth introgression, suggested that it is feasible to transfer small alien segments without linkage drag (Kuraparthy et al., 2007a).

Aegilops triuncialis L. ($2n = 4x = 28$, $U^tU^tC^tC^t$), a nonprogenitor tetraploid species, was found to be an excellent source of resistance to various pests and diseases (Dhaliwal et al., 1991; El Bouhssini et al., 1998; Romero et al., 1998; Harjit-Singh and Dhaliwal, 2000; Martin-Sanchez et al., 2003). Previously, rust resistance of *A. triuncialis* was transferred to wheat using the induced homeologous pairing effect of the *Ph*^{I} gene (Aghaee-Sarbarzeh et al., 2002). Genomic in situ hybridization (GISH) and simple sequence repeat (SSR) marker analysis identified only one leaf rust resistant wheat–*A. triuncialis* recombinant, consisting of most of the complete 5U^t chromosome with a small terminal segment derived from 5AS (Aghaee-Sarbarzeh et al., 2002).

Rust resistance of *A. triuncialis* also was transferred to wheat without inducing homeologous pairing between chromosomes of wheat and *A. triuncialis* (Harjit-Singh et al., 2000; Aghaee-Sarbarzeh et al., 2001). In one leaf rust resistant line, an introgressed *A. triuncialis* segment was identified on chromosome arm 4BS (Aghaee-Sarbarzeh et al., 2001).

We selected one leaf rust resistant introgression line derived from the wheat–*A. triuncialis* cross of Harjit-Singh et al. (2000) for further backcrossing, molecular characterization, and mapping of the alien introgression. We identified and mapped the cryptic wheat–*A. triuncialis* rust-resistant translocation using cytogenetic and molecular mapping in a segregating population.

MATERIALS AND METHODS

Plant Material

The introgression line was developed by crossing the susceptible hexaploid wheat cultivar WL711 with rust-resistant *A. triuncialis* (TA10438, PAU no. 3549) and backcrossing the resultant F_1 with WL711 (Harjit-Singh et al., 2000; Aghaee-Sarbarzeh et al., 2001). Leaf rust resistant BC_1F_1 plants were selected, backcrossed further to WL711 and selfed to develop BC_3F_{11} lines. In the BC_2F_1 and BC_3F_1 generations, leaf rust resistant plants with the full complement of wheat chromosomes were selected for further selfing. In the backcross and segregating generations, selection for rust resistance was made by screening the seedling progenies using the Indian races 77–5 (avirulent on plants with *Lr9*, *Lr19*, *Lr24*, and *Lr25*, and virulent on *Lr1*, *Lr3*, *Lr10*, *Lr13*, *Lr15*, *Lr20*, *Lr23*, *Lr26*, *Lr30*, *Lr33*, *Lr36*, *Lr48* and *Lr49*) and 104–2 (avirulent for *Lr9*, *Lr15*, *Lr19*, *Lr24*, and *Lr25*, and virulent for *Lr1*, *Lr3*, *Lr10*, *Lr13*, *Lr14*, *Lr16*, *Lr17*, *Lr18*, *Lr20*, *Lr23*, and *Lr26*). The same plants were screened as adults under artificial rust epiphytotic conditions at Punjab Agricultural University, Ludhiana, India. The BC_3F_{11} resistant introgression line with normal plant growth and development was selected and further screened for resistance to five U.S. leaf rust races (for virulence/avirulence formulae, see Long et al., 2000) at Kansas State University, Manhattan (Table 1).

One leaf rust resistant wheat–*A. triuncialis* introgression line (TA5605), along with the original *A. triuncialis* accession (TA10438), 'Chinese Spring' (CS), and the parental cultivars WL711 and Jagger were used for cytogenetic and molecular genetic analysis.

The hard red winter wheat cultivar Jagger (seedling susceptible to U.S. leaf rust races PRTUS25 and MCDL) was crossed as a female with the introgression line (TA5605). A total of 118 F_2 plants were used for genetic analysis and molecular mapping of leaf rust resistance. From each F_2 plant, 18 to 20 F_3 seedlings were screened for leaf rust reaction at the seedling stage. All the plants were grown in square pots filled with Scotts Metro Mix 200 (Scotts Miracle-Gro Co., Marysville, OH).

Screening the Plants for Leaf Rust Reaction

The seedling and adult reactions of the parental lines inoculated with the five leaf rust races are shown in Table 1. Rust inoculations, incubation of the infected plants, and rust scoring followed Browder (1971). All F_2 plants, their parents, and line TA5605 were inoculated with race PRTUS25 at the two-leaf seedling stage to screen for segregation of rust reaction. For progeny testing, 18 to 20 F_3 seedlings from each F_2 plant were grown and screened with the same race.

Molecular Characterization and Mapping

Genomic in situ hybridization was used to determine the size of the alien introgression in line TA5605, as described in Zhang et al. (2001), using *A. triuncialis* genomic DNA as a probe and CS genomic DNA as a blocker.

Table 1. Seedling† and adult plant‡ reactions of TA5605 and parents to five races of leaf rust.

Cultivar or line (source of resistance)	Reaction									
	PRTUS6		PRTUS25		PRTUS35		MCDL		PNMQ	
	Seedling	Adult plant	Seedling	Adult plant	Seedling	Adult plant	Seedling	Adult plant	Seedling	Adult plant
TA5605 (T2BS·2BL-2^{I}L(0.95))	;	5MS	;	5MS	4	90S	;1	5MS	3+C	5MS§
'WL711'	3+	90S	4	90S	4	90S	4	90S	3+	5MS§
'Jagger'	nt	nt	3+C	nt	3+C	nt	3+	80MS	3C	nt
'Wichita' (control)	4	90S	4	90S	4	90S	4	90S	4	90S

†Infection types (ITs) of seedlings were scored according to the modified Stakman scale of Roelfs et al. (1992) as illustrated in McIntosh et al. (1995). 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.

‡Adult-plant stage ratings are based on the modified Cobb scale (Peterson et al., 1948). Numbers indicate disease severity (percentage of leaf area affected) and letters indicate 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, nt = not tested.

§Resistance due to *Lr13*.

Based on previous reports on the association of chromosomes 5A (Aghaee-Sarbarzeh et al., 2002) and 4B (Aghaee-Sarbarzeh et al., 2001) with *A. triuncialis*-derived rust resistance, we initially selected nine and 14 SSRs mapping on chromosome 5A and 4B of wheat, respectively, for characterizing the introgression line. Bulk segregant analysis (BSA) with distally mapped restriction fragment length polymorphism (RFLP) markers that detect orthologous alleles among the three genomes was then used to diagnostically identify markers and chromosomes associated with the rust resistance. Three DNA bulks each for resistant and susceptible phenotypes were made by pooling the DNA of 10 homozygous resistant and 10 susceptible F_2 plants. These DNA bulks along with DNA from the susceptible cultivars WL711 and Jagger and TA5605 were digested with six restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I). The DNA isolation, Southern blotting, and hybridizations were as reported in Kuraparthy et al. (2007b). In the first attempt, a total of 17 RFLP markers mapping distally on homoeolgous groups 1 and 2 (Appels, 1997; Sharp, 1996; GrainGenes maps for wheat available at wheat.pw.usda.gov/GG2/maps.shtml#wheat [verified 6 July 2007]), were used for BSA. To physically characterize the wheat–alien translocation in line TA5605 and determine the translocation breakpoint with respect to the fraction length of CS deletion bins, 18 RFLP markers mapping distally in homeologous group 2 chromosomes of wheat (Delaney et al., 1995; Nelson et al., 1995; Dubcovsky et al., 1996; Sharp, 1996; Erayman et al., 2004) were used. A total of 34 SSRs physically or genetically mapped on the long arms of homeologous group 2 chromosomes (Roder et al., 1998; Sourdille et al., 2004; Somers et al., 2004) were used for molecular mapping of the rust-resistant introgression to a specific chromosome in TA5605. The RFLP and SSR markers diagnostically identifying the *A. triuncialis* segment in TA5605 were mapped in the F_2 population to genetically map the leaf rust resistance.

Linkage Analysis

The computer program MAPMAKER (Lander et al., 1987) Version 2.0 for Macintosh was used to calculate linkage using the Kosambi mapping function (Kosambi, 1944) with a logarithm of odds threshold of 3.00.

RESULTS

Rust Reaction of the Introgression Line

At the seedling stage, the introgression line showed a clear, hypersensitive resistant reaction (Fig. 1a) to leaf rust races PRTUS6, PRTUS25, and MCDL, and a susceptible reaction to races PNMQ and PRTUS35 (Table 1). The recipient wheat cultivar WL711 was highly susceptible at the seedling stage (Table 1, Fig. 1a). Line TA5605 was resistant (Fig. 1b) to races PRTUS6, PRTUS25, MCDL, and PNMQ at the adult plant stage but was susceptible to race PRTUS35 (Table 1). Cultivar WL711 (having *Lr13*) was highly susceptible to all races of leaf rust except PNMQ (avirulent on *Lr13*) at the adult plant stage (Table 1).

Segregation for leaf rust reaction was analyzed by screening the F_2 and F_3 populations at the seedling stage using race PRTUS25. The F_2 plants and progenies showed clearly different infection types for resistance (; to ;1C) and susceptibility (3+ to 4) (data not shown).

Genetic Analysis of Rust Resistance

The F_2 population of 118 plants developed from Jagger × TA5605 segregated 86 resistant and 32 susceptible plants, a good fit for dominant monogenic (3:1) segregation. Progeny of these F_2 plants when tested with race PRTUS25 gave 34 homozygous resistant, 52 heterogygous resistant, and 32 homozygous susceptible lines. This further indicated that leaf rust resistance in the introgression line was monogenically inherited.

Molecular Characterization of the Alien Introgression

No signal could be detected in the introgression line TA5605 when *A. triuncialis* DNA was used as a probe in the in situ hybridization experiments (data not shown). This indicated that the introgressed *A. triuncialis* chromatin in the leaf rust resistant translocation TA5605 was very small and cytologically undetectable.

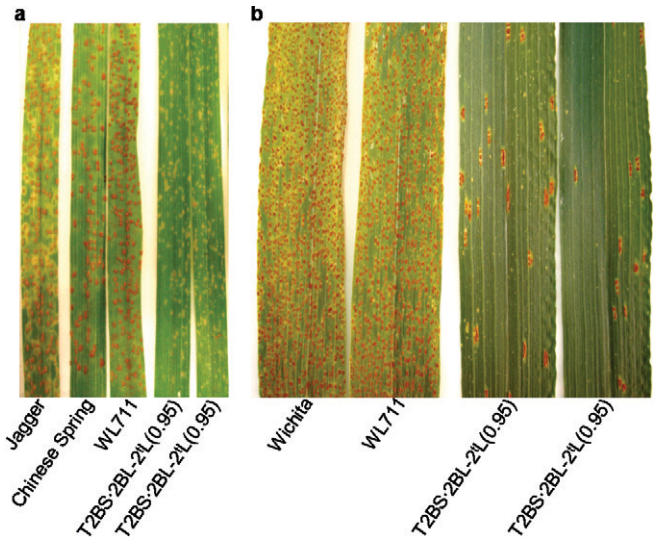

Figure 1. Rust resistance in introgression line TA5605 with T2BS.2BL-2^{t}L(0.95): (a) leaf rust (race PRTUS25) reaction of the parents and TA5605 at the seedling stage; (b) leaf rust (race MCDL) reaction of the parents and TA5605 line at the adult stage.

None of the tested 23 SSRs of chromosomes 5A and 4B identified the *A. triuncialis*-specific introgression in the translocation line TA5605 (data not shown). This suggested that either the leaf rust resistant introgression in TA5605 is different from previous reports of Aghaee-Sarbarzeh et al. (2001, 2002) or the marker density might not be enough to detect the leaf rust resistant introgression of *A. triuncialis* in TA5605. Bulked segregant analysis of the homozygous resistant and susceptible F_2 bulks was used for chromosome mapping and tagging of the leaf rust resistant introgression using distally mapped RFLP markers. Of the initial 17 RFLP markers for homeologous groups 1 and 2 used in the BSA, 13 were polymorphic between Jagger and TA5605 with one or more restriction enzymes.

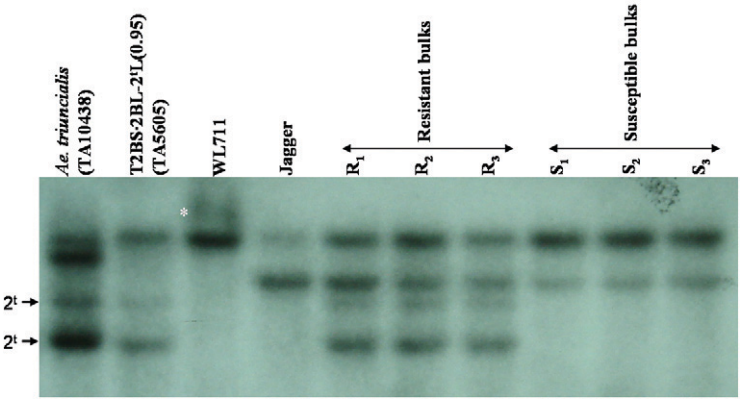

Figure 2. Molecular mapping of rust-resistant introgression T2BS·2BL-2^{t}L(0.95) using bulked segregant analysis. Southern hybridization pattern of *Eco*RV-digested genomic DNA of parents and bulks from homozygous resistant and susceptible F_2 plants using probe KSUH16. The restriction fragment length polymorphism fragments diagnostically polymorphic between resistant and susceptible bulks are indicated by arrows. The WL711 allele of *XksuH16* replaced by an *Aegilops triuncialis*-specific allele is indicated with an asterisk.

None of the seven polymorphic RFLP probes of homeologous group 1 chromosomes identified the diagnostic polymorphism between resistant and susceptible bulks. Probe KSUF11, mapped on homeologous group 2 chromosomes of wheat, identified diagnostically polymorphic alleles between the resistant and susceptible bulks. The polymorphic fragments detected by probe KSUF11 in the resistant bulks were specific to *A. triuncialis* with all the six enzymes used in the BSA (Table 2). This indicated the presence of *A. triuncialis* chromatin and its association with rust resistance in the translocation line TA5605.

We further selected 18 additional RFLP markers that were genetically or physically mapped on the homeologous chromosome arm 2L for characterizing the translocation in TA5605. The diagnostic marker patterns of some of the informative probes are given in Table 2. From this set of markers, two RFLP probes KSUH16 and BG123 further showed *A. triuncialis*-specific diagnostic polymorphism between resistant and susceptible bulks with all six enzymes used (Table 2). Probe KSUH16 detected the replacement of one of the wheat group 2L chromosome alleles by the *A. triuncialis* homeologous chromosome (2^t) in line TA5605 (Fig. 2). Furthermore, *A. triuncialis*-specific alleles of RFLP probes KSUH16, BG123, and KSUF11 cosegregated with leaf rust resistance in the F_2 mapping population. This unequivocally indicated that the rust resistance of the translocation line TA5605 was derived from homeologous group 2 chromosomes of *A. triuncialis,* and the introgression occurred onto the homeologous chromosome arm 2L of wheat through homeologous recombination. The diagnostically polymorphic alleles between resistant and susceptible bulks generated by RFLP markers *XksuD23*, *Xbcd410*, and *Xpsr609* were not specific to *A. triuncialis* (Table 2), suggesting that these markers were linked with the rust resistance and physically mapped proximal to the breakpoint of the wheat–*A. triuncialis* translocation.

The wheat–alien translocation in line TA5605 was physically characterized and the translocation breakpoint was determined with respect to the fraction length of the CS deletion bins based on the presence or absence of diagnostic polymorphisms between chromosomes 2^{t}L of *A. triuncialis* and group 2 chromosomes of wheat using physically mapped RFLP markers. None of the markers, physically mapped in the deletion bins 2L-0.69–0.70, 2L-0.70–0.76, and 2L-0.76–0.85, diagnostically identified the *A. triuncialis* segments (Table 2, Fig. 3) in line TA5605. Only three (*XksuH16*, *XksuF11*, and *Xbg123*) out of 10 informative RFLP markers mapped in the deletion bin 2L-0.89–1.00 diagnostically identified the *A. triuncialis*-specific chromatin in translocation line TA5605. This suggested that the breakpoint of the translocation in line TA5605 was located in the deletion bin 2L-0.89–1.00 of the consensus physical map

and that the size of the introgressed segment was <10% of the long arm of wheat chromosome 2L (Fig. 3). Because the deletion bin 2L-0.89–1.00 of the consensus map was the same as the deletion bin 2BL6-0.89–1.00 of 2B (Delaney et al., 1995), the introgressed segment is actually <10% of the long arm of wheat chromosome 2BL.

To identify and establish the specific wheat chromosome involved in the wheat–*A. triuncialis* translocation in TA5605, physically and genetically mapped homeologous group 2, chromosome-specific SSR markers were used. Only *Xcfd50* of 34 SSR markers surveyed diagnostically identified the *A. triuncialis*-specific segment in TA5605. Marker *Xcfd50* amplified a single, high-molecular-weight band specific to *A. triuncialis* in TA5605, and a low-molecular-weight band in Jagger. Molecular mapping in the F_2 population showed that the *A. triuncialis*-specific allele of *Xcfd50* cosegregated with the leaf rust resistance gene. Previously, *Xcfd50* was mapped physically and genetically to the distal region of chromosome arms 2BL and physically in 2DL of wheat (Sourdille et al., 2004). To allocate the *Xcfd50* allele associated with rust resistance to a specific homeologous group 2 chromosome, SSR markers were further used for molecular mapping. Of the 34 SSRs surveyed for polymorphism between TA5605 and Jagger, three were codominant, six were dominant, and 24 were not polymorphic. Four dominant (*Xgwm365*, *Xgwm265*, *Xgwm501*, and *Xcfd267*) and the three codominant SSR markers (*Xgwm311*, *Xgwm294*, and *Xbarc76*) and four RFLP markers (*XksuD23*, *XksuH16*, *XksuF11*, and *Xbg123*) were then mapped in the F_2 population to identify linkage of the rust resistance gene with chromosome-specific SSRs. None of the SSRs specific to chromosome 2A (*Xgwm365*, *Xgwm265*, *Xgwm294*, or *Xbarc76*) or 2D (*Xgwm311*) showed linkage with the leaf rust resistance gene or with the diagnostic markers *XksuF11*, *XksuH16*, or *Xbg123*, thus suggesting that the diagnostically polymorphic allele of *Xcfd50* was associated with chromosome 2B. Hence, the rust resistance gene from *A. triuncialis* in TA5605 was in chromosome arm 2BL. The identity of the *A. triuncialis* chromosome arm (2U^{t}L or 2C^{t}L) involved in the translocation T2BS·2BL-2^{t}L(0.95) is unknown.

Table 2. Diagnostic restriction fragment length polymorphism marker patterns in the resistant and susceptible bulks and introgression line TA5605.

Clone	'Chinese Spring' deletion bin location	Diagnostic polymorphism†		Wheat–*Aegilops triuncialis* introgression line T2BS·2BL-2^{t}L(0.95)†
		Resistant bulk	Susceptible bulk	
KSUD8	2L-0.69–0.70	W/J	W/J	W
KSUF15	2L-0.70–0.76	W/J	W/J	W
KSUE16	2L-0.76–0.85	W/J	W/J	W
KSUD22	2L-0.76–0.85	W/J	W/J	W
BCD135	2L-0.76–0.85	W/J	W/J	W
KSUH9	2L-0.89–1.00	W/J	W/J	W
KSUF41	2L-0.89–1.00	W/J	W/J	W
CDO678	2L-0.89–1.00	W/J	W/J	W
FBA8	2L-0.89–1.00	W/J	W/J	W
BCD410	2L-0.89–1.00	W	J	W
KSUD23	2L-0.89–1.00	W	J	W
PSR609	2L-0.89–1.00	W	J	W
KSUH16	2L-0.89–1.00	2^{t}L	J	2^{t}L
KSUF11	2L-0.89–1.00	2^{t}L	J	2^{t}L
BG123	2L-0.89–1.00	2^{t}L	J	2^{t}L

†W, a 'WL711' allele; J, a 'Jagger' allele; 2^{t}L, an *A. triuncialis* specific allele; W/J, either Jagger or WL711 allele(s) (diagnostically not polymorphic between resistant and susceptible bulks).

DISCUSSION

In this study, we identified and mapped a small alien translocation with a leaf rust resistance gene transferred from *A. triuncialis* to wheat without disruption of the normal bivalent pairing control. Because the *Ph1* gene suppresses homeologous pairing between wheat and alien chromosomes, such a transfer of alien chromatin was unexpected. There are two possible mechanisms for the origin of wheat–alien translocations. One is through spontaneous breakage and reunion of wheat and alien chromosomes during introgressive hybridization. The second is through homeologous pairing and recombination between homeologous chromosomes. The molecular marker data showed that wheat homoeoloci were substituted by alien homoeoloci in a precise recombination-like manner (Fig. 2).

Spontaneous transfers due to a low level of pairing were frequently observed in hybrids of hexaploid wheat and *A. triuncialis* (Romero et al., 1998; Harjit-Singh et al., 1993), and of hexaploid or tetraploid wheat and *A. peregrina* (Hack.) Maire & Weiller (Yu et al., 1990; Spetsov et al., 1997). Although wide variation existed among various homeologous chromosomes and genotypes, meiotic pairing frequencies, as high as 80 to 85%, were observed in wheat–*A. geniculata* hybrids (Cifuentes et al., 2006). This low level of pairing in wheat–alien hybrids was used to transfer Hessian fly resistance (Martin-Sanchez et al., 2003) and cereal cyst nematode resistance (Romero et al., 1998) genes from *A. triuncialis*, and powdery mildew resistance (Spetsov et al., 1997) and root-knot nematode resistance (Yu et al., 1990) genes from *A. peregrina* to wheat. Such low levels of chromosome pairing in wheat wide crosses could be due to partial homology between wheat and alien chromosomes, or to ineffectiveness of the *Ph1* gene in preventing homeologous chromosome pairing in the distal high recombination gene-rich regions of wheat. Romero et al. (1998) and Martin-Sanchez et al. (2003) speculated that transfers derived from wheat–*A. triuncialis*

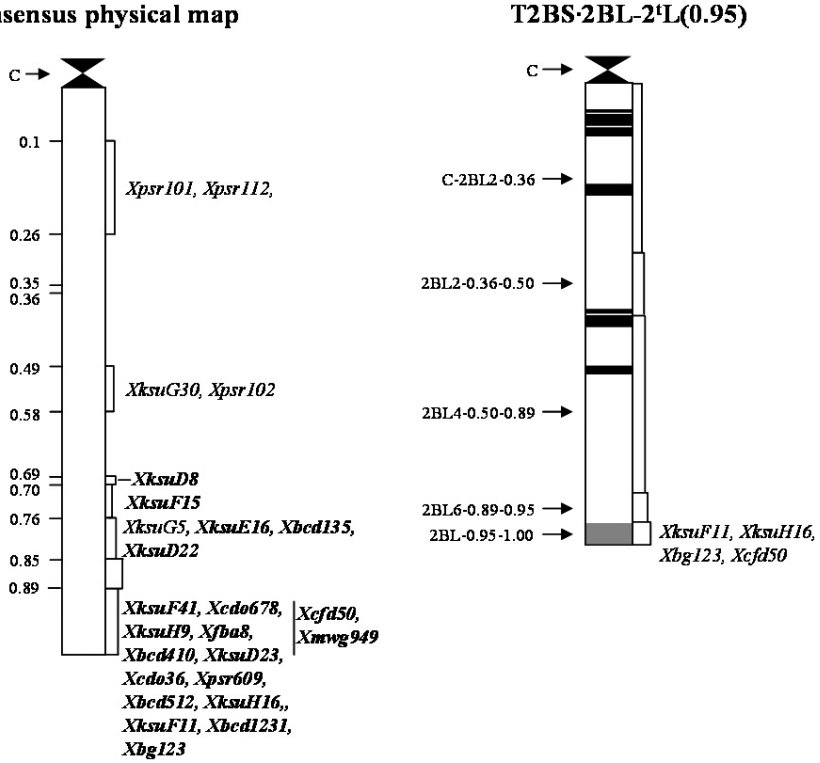

Figure 3. Physical map of chromosome 2B of wheat and inferred genomic in situ hybridization, restriction fragment length polymorphism, and simple sequence repeat marker-based physical map of recombinant wheat–*Aegilops triuncialis* chromosomes 2B and 2t in WL711 background. In the inferred physical map of the introgression line T2BS·2BL-2^{t}L(0.95), *A. triuncialis* 2t chromatin is indicated in gray. The solid black bands represent the C-banding pattern of chromosome 2B. The 2L consensus physical map was based on Delaney et al. (1995), Nelson et al. (1995), Dubcovsky et al. (1996), Sharp (1996), Sourdille et al. (2004), and Erayman et al. (2004). Markers used in the present study are shown in bold.

hybrids could be due to the ability of the C genome to suppress the *Ph1* diploidization mechanism (Kimber and Feldman, 1987). Such an effect might not be the case in this study, however, because a low level of chromosome pairing was observed in the wheat–*A. triuncialis* hybrid originally used to transfer the rust resistance (Harjit-Singh et al., 2000). Previously, Jena et al. (1992) reported the spontaneous introgression of chromosomal segments conferring resistance to brown planthopper from *Oryza officinalis* Wall. ex G. Watt chromosomes to those of rice (*O. sativa* L.).

We identified one rust-resistant alien translocation line (TA5605) with a cytologically undetectable alien segment from *A. triuncialis*. Based on GISH and molecular mapping, we previously described a method for estimating the size of alien introgressions (Kuraparthy et al., 2007a). The translocation in the present study was described as T2BS·2BL-2^{t}L(0.95). Because the specific homeologous group 2 chromosome of *A. triuncialis* involved in the translocation was unknown, we identified the donor chromosome as 2^{t}L, where superscript t refers to an *A. triuncialis* chromosome. We termed this small introgression, undetected by cytological analysis, as a "cryptic alien introgression" (Kuraparthy et al., 2007a). The "cryptic" nature of the *A. triuncialis* introgression in T2BS·2BL-2^{t}L(0.95) was supported by molecular mapping, where only three of 10 otherwise informative RFLPs in deletion bin 2BL6-0.89–1.00 diagnostically identified the *A. triuncialis* chromatin (Table 2, Fig. 3). An unconventional recombination mechanism was speculated for such introgressions in rice (Jena et al., 1992). It is not known if a cryptic alien introgression can occur in the absence of a chiasmatic meiotic association in wheat, but the precise exchange indicates a recombination-like event.

Previously, *XksuH16* was placed in bin 2L-0.85–0.89 and *XksuF41* was mapped in the distal deletion bin 2L-0.89–1.00 in a consensus physical map of homeologous group 2 chromosomes (Delaney et al., 1995). This order is highly unlikely because most of the genetic maps indicated that *XksuF41* was proximal to *XksuH16* (Nelson et al., 1995; Gill et al., 1991; Gale and Miller, 1987; Sharp, 1996) and *XksuH16* incorrectly was placed in the deletion bin apparently due to the lack of polymorphism between homoeoalleles in the physical mapping experiments of Delaney et al. (1995). The absence of diagnostically polymorphic alleles between the resistant and susceptible bulks for *XksuF41* and the identification of *A. triuncialis*-specific alleles by *XksuH16*, and by the most terminally mapped marker *Xbg123* of Dubcovsky et al. (1996), suggest that the wheat–*A. triuncialis* introgression in TA5605 is a terminal transfer. Our results also suggest that *XksuF41* should be proximal to *XksuH16* and that *XksuH16* is in deletion bin 2L-0.89–1.00.

Identification of cryptic alien introgressions with disease resistance from *A. triuncialis* in the present study and from *A. geniculata* reported previously (Kuraparthy et al., 2007a) suggest that it is feasible to transfer disease resistance genes with minimal linkage drag from wild species by selecting rust-resistant backcross derivatives with no obvious effect on plant growth, and by characterizing lines using GISH and terminally mapped molecular markers from genetic and physical maps. Using this strategy, we showed conclusively that cryptic wheat–alien introgressions with rust resistance can be produced in wheat.

Previously, for the detection and characterization of critical recombinants in targeted chromosome engineering, diagnostic cytological or molecular markers specific to the chromosome or chromosome arm targeted for

alien gene transfer were used (Lukaszewski and Xu, 1995; Lukaszewski, 2000; Iqbal et al., 2000; Qi et al., 2007). Characterization and mapping of cytologically undetectable cryptic alien introgressions from wheat–alien direct crosses, however, require rapid and efficient strategies such as BSA (Michelmore et al., 1991). Using BSA, we not only quickly detected the wheat chromosome involved in TA5605, but also identified the *A. triuncialis*-specific segment associated with leaf rust resistance gene *Lr58* in T2BS·2BL-2^{t}L(0.95).

Cryptic wheat–alien introgressions, especially terminal segments, are the most desirable and feasible translocations for transferring disease resistance genes in wheat, because disease resistance genes are mostly located in the terminal recombination-rich regions of grass chromosomes (Leister et al., 1998). The physical localization of expressed resistance gene analogs on wheat chromosomes showed that about 75% of the R genes mapped in the distal 20% of the chromosomes; most of the wheat R genes were present in the telomeric or subtelomeric regions (Dilbirligi et al., 2004). This also was supported by physical mapping of linked markers and expressed sequence tags (ESTs) in a core set of CS deletion lines (Qi et al., 2004). In wheat, recombination also is unevenly distributed; 90% of the recombination occurs in the distal regions toward the telomeric ends of the chromosomes (Gill et al., 1993; Lukaszewski and Curtis, 1993; Lukaszewski, 1995). Furthermore, homeologous recombination appears to be highly localized and occurs distal to homologous recombination (Luo et al., 2000; Lukaszewski et al., 2003, 2005). Wheat–alien transfers were mostly derived from single crossover events. Only two breakpoints were detected by a single RFLP marker in a sample of eight wheat–rye (*Secale cereale* L.) recombinants for the 1RL arm of rye probed with 36 RFLP markers (Rogowsky et al., 1993). All recombination events were restricted to the distal 18% of the arm in wheat–wheatgrass [*Thinopyrum ponticum* (Podp.) Barkworth & D. R. Dewey] recombinants with wheat streak mosaic virus resistance (Qi et al., 2007). Kuraparthy et al. (2007a) provided further evidence for such transfers where the smallest wheat–*A. geniculata* cryptic terminal introgression with *Lr57* and *Yr40* was found to be <3.5% of the chromosome arm 5DS. Identification of such terminal single-breakpoint transfers needs molecular or cytological markers mapped at or near the telomeric ends of the wheat chromosomes. Physically and genetically mapped RFLPs and SSRs (wheat.pw.usda.gov/GG2/maps.shtml#wheat [verified 10 July 2007]) and bin-mapped EST markers (wheat.pw.usda.gov/NSF/project/mapping_data.html [verified 10 July 2007]) could be an ideal resource for such markers. The rice genomic sequence also may be useful to develop markers if the wheat–rice synteny is conserved in such regions.

Although the resistance gene *Lr58* transferred from *A. triuncialis* to wheat in this study has not been deployed in any cultivar, virulence to this gene exists in races PNMQ and PRTUS35 (Table 1) of North America. These two races are virulent on *Lr58* at both the seedling and the adult plant stages (Table 1). Such virulence to genes transferred to wheat from *A. tauschii* (*Lr39* and *Lr41*) and *Triticum monococcum* L. (an unnamed gene in KS92WGRC23) has been detected before deployment of these genes in agriculture (Hussien et al., 1997; Raupp et al., 2001). In each of these cases, virulence was found in *Puccinia triticina* race PNMQ. Interestingly, the race PNMQ is also virulent to the genes *Lr9* and *Lr24* that were transferred to wheat from *Aegilops umbellulata* and *Thinopyrum ponticum*. The presence of virulence to new genes derived from wheat relatives before development of resistant cultivars will limit the usefulness of these genes unless they are deployed in combination with other effective genes for resistance to leaf rust. Identification of markers (SSR marker *Xcfd50* and RFLP markers *XksuH16*, *Xbg123*, and *XksuF11*) linked to *Lr58* provide a tool to incorporate this gene into pyramids that include other effective resistance genes.

Homeologous group 2 chromosomes of wheat contain at least 19 cataloged genes for leaf rust resistance. Except for *Lr11* (2A) and *Lr35* (2B), whose arm location is unknown, most of the resistance genes were mapped on the short arms of homeologous group 2 chromosomes (see www.ars.usda.gov/Main/docs.htm?docid=10342 [verified 10 July 2007]). Only three leaf rust resistance genes have been mapped to homeologous chromosome arm 2L of wheat, and all three were derived from wild related species. Resistance gene *Lr38* mapped on chromosome 2AL was a noncompensating translocation from *Agropyron intermedium* (Host) P. Beauv. (Friebe et al., 1993). Resistance gene *Lr54* mapped on 2DL was derived from a whole-arm translocation from *Aegilops kotschyi* Boiss. (Marais et al., 2005). Gene *Lr50* mapped on 2BL was introgressed from *Triticum timopheevii* (Zhuk.) Zhuk. ssp. *armeniacum* (Jakubz.) Slageren (Brown-Guedira et al., 2003). Furthermore, chromosome arm 2L contains at least three stripe rust resistance genes (*Yr5*, *Yr7*, and *Yr37*) and five stem rust resistance genes (*Sr9* allelic series, *Sr16*, *Sr20*, *Sr21*, and *Sr28*) (see http://www.ars.usda.gov/Main/docs.htm?docid=10342). In this study, mapping of the leaf rust resistance gene *Lr58* in the distal region of chromosome arm 2BL suggests either the presence of conserved orthologous R loci in 2L or that the genomic region of 2L is rich in resistance genes. Precise genetic mapping using linked RFLP markers that produce orthologous alleles in the chromosome arms 2L is necessary to characterize such regions.

Acknowledgments

We thank Robert McIntosh for reviewing the manuscript and providing the new gene designation for the rust-resistant gene reported in this study. We extend a special note of thanks to Duane Wilson and Jon Raupp for their excellent technical assistance. We thank Bernd Friebe for the helpful discussions and Peng Zhang for her guidance in the GISH technique. Research

supported by Kansas Wheat Commission and USDA special grant to WGGRC. This paper is Contribution no. 07-152-J from the Kansas Agricultural Experimentation Station, Kansas State University, Manhattan.

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