Molecular characterization of two Triticum speltoides interstitial translocations carrying leaf rust and greenbug resistance genes

Permalink
https://escholarship.org/uc/item/9v70g3gr

Journal
Crop Science, 38(6)

ISSN
0011-183X

Authors
Dubcovsky, J
Lukaszewski, AJ
Echaide, M
et al.

Publication Date
1998

DOI
10.2135/cropsci1998.0011183X003800060040x

Peer reviewed
CELL BIOLOGY & MOLECULAR GENETICS

Molecular Characterization of Two *Triticum speltoides* Interstitial Translocations Carrying Leaf Rust and Greenbug Resistance Genes

J. Dubcovsky,* A. J. Lukaszewski, M. Echaide, E. F. Antonelli, and D.R. Porter

**ABSTRACT**

Resistance genes for leaf rust (*Puccinia recondita* Rob. ex Desm.) and greenbug (*Schizaphis graminum* Rondani) were transferred from chromosome 7S of *Triticum speltoides* (Tausch) Gren. to chromosome 7A of hexaploid wheat (*Triticum aestivum* L.) by means of the *phi*1 mutation that promotes homeologous recombination. The chromosome segments from *T. speltoides* were characterized by C-banding and restriction fragment length polymorphisms (RFLP). Since the segments of *T. speltoides* chromosome 7S do not recombine with wheat chromosome 7A in the presence of the wild-type *Phi* locus only one molecular marker per chromosome segment is required to monitor the introgressed genes in marker assisted selection programs. The new leaf rust resistance gene, designated *Lr*47, and the greenbug resistance gene *Gb*5 were located on interstitial chromosome segments from *T. speltoides* translocated to wheat chromosome arms 7AS and 7AL, respectively. Physically, both were located in the distal one third of the arms, but genetically the *Lr*47 segment was 2 to 10 centimorgans (cM) from the centromere and was 20 to 30 cM long; the *Gb*5 segment was 18 to 22 cM from the centromere and was 40 to 50 cM long.

Since the per-acre value of wheat is lower than that of many alternative crops, wheat must be grown efficiently with minimum application of pesticides. The use of disease resistance genes is the method of choice for controlling diseases in this crop and has been proven repeatedly to be an effective and environmentally sound method of controlling serious yield-reducing pathogens. Unfortunately, the gene pool of cultivated wheat for resistance to pests and pathogens is inadequate to respond to the evolution of different pathogen populations. Replacement of highly variable land races by higher yielding, pure-line varieties in many parts of the world has further reduced the wheat gene pool. In this context, it is important to import alternative genes from other sources.

Diploid species *T. monococcum* L., *T. speltoides* (Taush) Gren., and *T. tauschii* (Cosson) Schmaltz., with genomes closely related to the A, B, and D genomes of bread wheat, offer a pool of genes for resistance that can contribute to crop genetic diversity. Genes from these species have been incorporated into bread wheat conferring resistance against leaf rust (*Puccinia recondita* Rob. ex Desm.), stem rust (*Puccinia graminis* Pers.:Pers.), Hessian fly (*Mayetiola destructor* (Say)), Russian wheat aphid (*Diuraphis noxia* Mordvilko), powdery mildew (*Blumeria graminis* (DC.) E.O. Speer (syn. *Erysiphe graminis* DC.),) greenbug, and root knot nematode (*Meloidogyne incognita* (Kofoid & White) Chitwood) (reviewed by [McIntosh, 1991; Friebe et al., 1996]).

In most experiments aiming at such interspecific transfers, recombination is induced by the *phi*1 mutation and primary recombinant chromosomes are recovered. Most often, these primary recombinants are single breakpoint translocations (Lukaszewski, 1995; Dubcovsky et al., 1996a) carrying large segments of alien chromosomes. Linkage between the targeted genes and undesirable genes on the alien segment usually result in yield and/or quality penalties and in a limited use of such alien transfers in practical breeding (Friebe et al., 1996). Consequently, to be deployed in agriculture the alien chromosome segments must be as short as possible. In this paper, we report the development of two interstitial translocations of *T. speltoides* chromosome 7S in common wheat. These translocations carrying leaf rust and greenbug resistance genes are characterized by C-banding and RFLP markers.

**MATERIAL AND METHODS**

A chromosome of *T. speltoides* was found in some stocks of wheat originating from Kansas State University (Lukaszewski, 1995). The origin of the material, identical C-banding patterns and the presence of a gene conferring resistance to biotypes C and E of greenbug, *Schizaphis graminum* Rondani, suggest that this may be chromosome 7S1 described by Friebe et al. (1991). This chromosome was originally transferred from *T. speltoides* to bread wheat by irradiating hybrid seed (*CI18502 * / *T. speltoides* // 'Fletcher' / 3 / 5*Centruck') with fast neutrons (Wells et al., 1982). The dominant greenbug resistance gene present in the derived translocation lines (*CI17882, CI17884, and CI17885*) was designated *Gb*5 (Tyler et al., 1987) and was later transferred to line KS90H450 from Kansas State University (Friebe et al., 1991). Tests at University of California, Riverside, showed that this chromosome also carried a gene for resistance to leaf rust and, possibly, to blackpoint. The causal agent of black point was identified as *Fusarium proliferatum* (T. Matsushima) Nirenberg (syn.

Abbreviations: cM, centimorgans; *Gb*, greenbug resistance gene; *Lr*, leaf rust resistance gene *Phi* (gene controlling homeologous pairing); RFLP, restriction fragment length polymorphisms.

after inoculation, following McIntosh et al. (1995). A race
tative assessment of the seedling response was done about 2 wk
spores with talc when the third leaf was expanded. A qualita-
ternational reference stocks (McIntosh et al., 1995). Seedlings
were grown in a greenhouse and inoculated by dusting dry
characteristics were established by means of near-isogenic in-
Institute "Edwald A. Favret" and their avirulence-virulence
ferring resistance to leaf rust was further screened with nine
seedlings. The line carrying a recombinant chromosome con-
T7AS-7S#1S.7AL respectively. Leaf rust resistance testing of
stetical inserts of alien chromatin into wheat chromosomes
combination. Secondary recombinant chromosomes with in-
were intercrossed and allowed to recombine in the presence
combinants with the breakpoints flanking the locus of interest

Cephalosporium proliferatum

<table>
<thead>
<tr>
<th>Race</th>
<th>Polymorphism</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>T7AS-7S#1S-7AS-7AL</td>
<td>1996, personal communication.</td>
<td>Pavon Virulence/avirulence pattern</td>
</tr>
</tbody>
</table>

Phl

V = virulent, av = avirulent, - = moderately resistant,
MS = moderately susceptible,
S = susceptible.

Twelve lines showed recombination between chromo-
tions, were identified among 165 BC1 (Fig. 1 and 2). The re-
tionship between RFLP markers present on chromosome 7Am
DraI, EcoRI, HindIII, XbaI. The presence of

RESULTS

CROP SCIENCE, VOL. 38, NOVEMBER-DECEMBER 1998
Fig. 1. Primary recombinant chromosomes obtained from the phh1lb-induced recombination between chromosome 7A of wheat and 7S#1 of T. speltoides. Upper row: recombinant chromosomes with 7A centromere. From left to right, two long arm recombinants and one short arm recombinant (T~AS-~S#~S-~ASS~AL). Far right, centric translocations (T7AS-7S#lS-7AL and T7AS*7S#lL). Bottom row: chromosomes with 7S#l centromeres and terminal segments of 7A. From left to right, three long arm recombinants and two short arm recombinants. Arrowheads point to the location of translocation breakpoint.

Short arm of 7s-7A. All seven short-arm recombinants with 7S#1 centromeres were resistant suggesting that the translocation breakpoints in this group of recombinants were distal to the $Lr$ locus. Among the five reciprocal short-arm recombinants with 7A centromeres only one, labeled T7AS-7S#lS-7AS-7AL (Fig. 1, upper row, third from right) conferred resistance to leaf rust. This chromosome had the most proximal translocation break-point among all short arm recombinants. Additional three backcrosses of recombinant chromosome VAS-7S#lS-7AS.7AL to Pavon wheat were performed and plants homozygous for the interstitial translocation were selected by C-banding. Homozygous T7AS-7S#lS-7AS-7AL line showed resistant response to leaf rust races PRTUS 06 and 17 used in the original screening of the 7s-7A substitution line and the primary recombinants, as well as to nine leaf rust races from Argentina with simultaneous virulence on $Lr_1$ and $Lr_{10}$ genes (Table 1). The $Lr$ resistance gene present in the T7AS-7S#lS-7AS7AL line is designated $Lr_{47}$.

The position and length of the interstitial T. speltoides chromosome segment present in homozygous line T7AS-7S#lS-7AS-7AL, was characterized with 14 RFLP clones previously mapped in the short arm of chromosome 7A"S (Dubcovsky et al., 1996 b). Loci Xcdo57 and Xubc455, located close to the centromere, showed an additional restriction fragment (from T. spel-7SS) and an absent fragment (presumably from the replaced T. uestivum 7AS) in substitution line 7s-7A. These RFLPs were not detected in line T7AS-7S#lS-7AS-7AL indicating that this line originated by a recom-bination event that occurred between loci Xcdo57 and Xubc465.2 to 9 cM from the centromere (Fig. 3). Physi-7cally, the T. speltoides segment in chromosome T7AS-7S#lS-7AS7AL is located in the distal third of the arm. The distal 7AS-7S#lS breakpoint is at FL (fractional length) 0.85 (Friebe et al., 1996).

The seven loci tested for the chromosome segment including Xubc465 and Xwg834 were polymorphic be-tween Pavon and the substitution line 7s-7A (Fig. 3). Each of these loci showed a polymorphic T. speltoides fragment present in substitution line 7s-7A and in recombinant line T7AS-7S#lS-7S#lL-7AL that was absent in Pavon and in the recombinant line for the long arm T7AS-7AL-7S#lL-7AL (Fig. 4). On the basis of the distances between these markers in T. morzococcum (Dubcovsky et al., 1996b), it was inferred that the length of this T. speltoides segment was between 27 cM (Xubc465 to Xwg834 in 7A") and 43 cM (Xcdo57 to Xbcd93 in 7A").

Fig. 2. Transfer of the Gb5 locus from chromosome 7S#1 of T. speltoides to 7A of wheat. From left to right: original chromosomes 7A and 7S-7A; two reciprocal recombinants T7AS.7AL-7S#lL and T7AS-7S#lS-7S#lL-7AL both of which carry Gb5; crossing over within the segment of 7S#1 common to the two primary recombinants results in chromosome T7AS-7AL-7S#lL-7AL with an interstitial insert of 7S#1. Arrowheads point to the translocation breakpoints.
Fig. 3. RFLP analysis of chromosome 7A of *T. monococcum* and inferred location of the *T. speltoides* chromosome segments (in gray) on the basis of RFLPs in substitution line 7S-7A, and recombinant lines T7AS-7S#1S-7AS-7AL and T7AS.7AL-7S#1L-TAL. Distances are in centimorgans and the arrowhead points to the centromere. Locus Xabc455 is in the short arm and locus Xpsr311 is in the long arm.

The five loci distal to Xwg834 (Xbcd93 to Xabg704, Fig. 3) showed no polymorphisms present simultaneously in substitution line 7S-7A and T7AS-7S#1S-7AS-7AL. This contrasts with the high level of polymorphism observed for the rest of chromosome 7S-7A and suggests that these markers are included in the 7A region of chromosome 7S-7A. A low level of polymorphisms in this region was expected, because the distal 7A segment present in the original 7S-7A had numerous opportunities for recombination with chromosome 7A from Pavon during the backcrossing process.

Long Arm Recombinants

Screening of the 7S-7A-substitution line of Pavon showed that chromosome 7S#1 conferred resistance to greenbug biotype C. Centric translocation T7AS-7S#1S.TAL was susceptible indicating that the resistance gone was located on the long arm. To confirm this result, backcross progenies with all 25 recombinant chromosomes were screened for greenbug resistance with biotype C. Since the screened progenies segregated for normal chromosomes 7A and the primary recombinants, segregation for resistance to biotype C was taken as a sign that the given recombinant carried the Gb5 locus. Uniform susceptible progenies indicated lack of the locus.

The resistant response of all recombinants with complete 7S#1L arms and the susceptible response of all recombinants with complete 7AL arms confirmed the long arm location of Gb5. Among the long arm recombinants with the centromere of 7A, only one chromosome, T7AS.7AL-7S#1L, conferred resistance. This recombinant had the most proximal translocation breakpoint and encompasses the three distal C-bands of 7S#1L (Fig. 2). Among the plants with reciprocal recombinant chromosomes with the 7S#1 centromere one was susceptible and the remaining ones were resistant. Chromosome T7AS-7S#1S.7S#1L-7AL was selected among the long arm recombinants with 7S#1 centromeres and Gb5 for the production of the interstitial segment of 7S#1L on the long arm of chromosome 7A.
Fig. 4. Southern blot hybridization of the ABC465 and PSRl29 clones speltoides 59 progeny from the double heterozygote (20" bine in the presence of the wild-type

The primary recombinant T7AS-7AL-7S#lL was origi-
mend between T7AS-7AL-7S#lL and T7AS-7S#lS. The

7AL-7S#lL

to the 7A segment in T7AS.7AL-7S#lL and that

7S#lL-7AL, was further characterized using 10 RFLP

recombinant chromosomes gives a crossover frequency

located in the chromosome segment adjacent to the

centromere, [Image 0x0 to 624x803]

The seven loci analyzed for the distal region of the

Xpsr680 and XucdlOl (Esi2),

The methodology to reduce the length of these seg-

DISCUSSION

Many wheat derivatives carrying resistance genes

were

chromosome (Sears, 1981) is tedious and slow. C-band-

number of primary recombinants increase the probabil-

markers provide a more precise way to characterize the

primary recombinants for the production of the second-

mosomes with interstitial segments of

inserts in the secondary recombinants were of consider-

some segments transferred by homeologous recombina-

but the size of the alien segment may still be a problem.

The size of the alien segment may still be a problem.
sufficient to indicate the simultaneous presence of the corresponding resistance gene.

Orthologous relationships between Lr47 and leaf rust resistance genes Lr29 and Lr34 located in the short arm of homeologous group 7 (McIntosh et al., 1995) cannot be conclusively demonstrated with the available information. Lr29 has a similar pattern of avirulence reactions to Lr47 but its location within the short arm of homeologous group 7 is not known. The map position of Lr34, close to Xwg834 on 7DS (Nelson et al., 1995), is within the limits of T. speltoides segment in T7AS-7S#1S-7AS-7AL. However, Lr34 differs from Lr47 in that it is mainly an adult plant resistance gene (McIntosh et al., 1995).

There is only one additional greenbug resistance gene reported on homeologous group 7 (Hollenhorst and Joppa, 1983). This gene, labeled Gb3, is present in variety Largo chromosome 7D and confers resistance to greenbug biotypes C, E, H, I, and K but not to biotypes B, F, and G. Gb3 differs from Gb5 only in its resistance to biotype H (Porter et al., 1997). More precise mapping information of Gb3 and Gb5 is required to elucidate the relationship between these two genes.

ACKNOWLEDGMENTS

J. Dubcovsky acknowledges financial support from USDA-Fund for Rural America competitive grant 97-36200-5272 and for a Faculty Research Grant (1997-1998) from University of California, Davis.