Development of Wheat Lines Having a Small Introgressed Segment Carrying Stem Rust Resistance Gene Sr22

Eric L. Olson, Gina Brown-Guedira,* David Marshall, Ellen Stack, Robert L. Bowden, Yue Jin, Matthew Rouse, and Michael O. Pumphrey

ABSTRACT
The wheat stem rust resistance gene Sr22 confers resistance to *Puccinia graminis* f. sp. *tritici* Pers. race TTKSK (also known as Ug99) that developed in Africa and is an immediate threat to world wheat production. The resistance gene is present on a chromosomal translocation derived from *Triticum boeoticum* Boiss., which has a genome that is partially homologous to the A genome of *T. aestivum* L. Sr22 has been deployed in a limited number of cultivars due to poor agronomic performance of lines carrying the resistance gene. Linkage analysis of simple sequence repeat (SSR) markers on chromosome 7A was performed to identify loci closely linked to Sr22. The most tightly linked proximal and distal SSR marker loci were Xcfa2123 and Xwmc633, respectively. A two-step process was then used to develop resistant lines having smaller chromosome segments derived from the diploid donor. First, individuals in which a single recombination event had occurred between wheat and the Sr22 introgression were identified in the mapping populations. In spite of reduced recombination between *T. boeoticum* and *T. aestivum* chromosomes, sufficient recombination events were found among 398 F3:4 lines derived from recombinant F2 progeny to recover multiple resistant individuals with smaller alien introgressions. Resistant lines were identified having less than 6% of the chromosome arm derived from *T. boeoticum*. These lines may provide a more agronomically desirable source of Sr22 that can be readily deployed in cultivars resistant to Ug99.
to provide stem rust resistance in many wheat growing areas of the world. Other broadly effective Sr genes transferred from wheat relatives, including Sr32, Sr39, and Sr40, remain undeployed in modern agriculture due to reduced agronomic performance associated with their presence on large alien chromosome introgressions (Singh et al., 2008).

The resistance gene Sr22 was introgressed into common wheat from wild and cultivated A-genome diploid wheats (The, 1973). It provides effective resistance to the variant stem rust races of the TTKS lineage (also referred to as Ug99) (Jin et al., 2007) that developed in Africa and now threaten world wheat production. The Sr22 gene was identified in the T. boeoticum sspp. aegilopoides (Link) Thell., accession G-21 (Gerechter-Amati et al., 1971) and the T. monococcum ssp. monococcum (Link) Thell. accession RL5244 (Kerber and Dyck, 1973). Introgressions from the two donors differ in the amount of alien chromatin present. The T. boeoticum introgression into the common wheat cultivar Steinwedel includes nearly the entire long arm and a portion of the short arm of chromosome 7A\(^{an}\). The T. monococcum introgression into Marquis consists of the distal region of 7A\(^{an}\)L (Kerber and Dyck, 1973; Paull et al., 1994). Khan et al. (2005), using simple sequence repeat (SSR) markers, genetically mapped Sr22 between Xfj2123 (proximal) and Xfj2019 (distal) with an interval distance of 11.2 cM, an interval previously estimated to be approximately 30 cM (Sourdille et al., 2005). The smaller genetic distance in the Sr22 mapping population was presumably the result of reduced recombination between the introgressed segment carrying the gene and wheat chromosome 7A.

Reduced recombination between chromosomes of the A genome of T. aestivum and the A\(^{an}\) genome of einkorn wheat relatives has been well documented (Dubcovsky et al., 1995; Luo et al., 2000). The Ph1 gene of hexaploid wheat restricts homoeologous pairing (Riley and Chapman, 1958), preventing recombination between homoeologous chromosomes. Consequently, introgressed chromosome segments from species that are not completely homologous may be inherited in large linkage blocks carrying substantial amounts of alien chromatin. Detrimental to agronomic performance have been reported in association with the Sr22 introgressions, including depression of yields and delayed heading date (Paull et al., 1994). In near isogenic lines (NILs) from different genetic backgrounds carrying Sr22, The et al. (1988) reported a mean yield that was not significantly lower than non-Sr22 controls. However, the results varied among genotypes, with some Sr22 NILs yielding as much as 10% less than non-Sr22 controls. Paull et al. (1994) reported that lines without the T. boeoticum introgression headed approximately 6 d earlier than lines heterozygous and homozygous for the introgression. In addition, reduced transmission of gametes carrying Sr22 has been observed for both the T. boeoticum and T. monococcum introgressions (The and McIntosh, 1975). Poor agronomic performance and reduced transmission of Sr22 have been attributed to linkage drag resulting from substantial chromatin derived from the A\(^{an}\)m genome relatives.

The associated negative effects on agronomic performance have hindered the use of Sr22 worldwide. However, deployment of this gene is of particular interest in contemporary wheat breeding because it confers resistance to Pgt race TTKSK and more virulent derivatives that have emerged from East Africa (Singh et al., 2008). Additionally, Sr22 confers resistance to all domestic Pgt races in the United States. In this study we report genetic linkage analysis of the Sr22 region in multiple populations and the identification of more tightly linked SSR marker loci for use in marker-assisted selection. In addition, recombinants have been identified that carry reduced amounts of T. boeoticum chromatin that may prove useful in the deployment of Sr22 in breeding populations.

**MATERIALS AND METHODS**

**Plant Materials**

For linkage analysis of Sr22, two mapping populations were developed. The resistant parent for both crosses was the germplasm Sr22Tb with the pedigree Steinwedel*2*/Speltm*2*/T. boeoticum G-21. Seed of this Steinwedel selection that has the 7A\(^{an}\)m chromosomal translocation from T. boeoticum carrying the stem rust resistance gene Sr22 (The, 1973) was provided by Dr. Yue Jin, USDA-ARS Cereal Disease Lab, St. Paul, MN. Two Steinwedel accessions, PI 41081 and PI 27018, obtained from the USDA National Small Grains Collection were used to compare the haplotypes of SSR loci linked to Sr22 between the germplasm Sr22Tb and the original cultivar Steinwedel.

A population of 138 F\(_2\)\(_3\) lines, referred to as U5615-F\(_2\)\(_3\)\(_3\), was developed from the cross of Sr22Tb with the hard winter wheat cultivar 2174 (PI 602595). An additional population of 139 F\(_2\)\(_3\) lines, referred to as U5616-F\(_2\)\(_3\)\(_3\), was developed from a cross between Sr22Tb and the hard winter wheat cultivar Lakin (PI 617032). From these populations, three F\(_2\)\(_3\) families having recombination between distal marker Xfj2019 and proximal marker Xbarc121 in the Sr22 region were selected for development of additional mapping populations and identification of recombinants having smaller introgression segments. Linkage analysis was performed using SSR loci segregating in F\(_2\)\(_3\) populations developed from the F\(_2\) plants U5615-72, U5615-98, and U5616-20. From the recombinant plant U5615-72 that was homozygous for alleles from parent line 2174 at the distal Xfj2019 locus and heterozygous for Sr22, 104 F\(_3\)\(_4\) lines were developed (population U5615-72-F\(_3\)\(_4\)). From the U5615-98 recombinant that is homozygous for alleles from parent line 2174 at the proximal Xbarc121 locus and heterozygous for Sr22, 140 F\(_3\)\(_4\) lines were developed (population U5615-98-F\(_3\)\(_4\)). A population of 152 F\(_3\)\(_4\) lines was derived from the recombinant F\(_2\) plant U5616-20 that was homozygous for Lakin alleles at the Xbarc121 locus and heterozygous for Sr22 (population U5616-20-F\(_3\)\(_4\)).

The chromosomal assignment of the SSR loci to the long arm of chromosome 7A was done using Chinese Spring nullisomic–tetrasmic and ditelosomic stocks (Sears, 1954; Sears and Sears, 1978) for this chromosome. Chromosome physical mapping of SSR loci was done using Chinese Spring chromosomal
14 d after inoculation. Rust evaluations were replicated so that a total of at least 20 seedlings from each line were evaluated.

Stem rust reaction of parent lines and selected recombinants having reduced T. boeoticum chromatin were determined using eight races of P. graminis f. sp. tritici, including races of the TTKS lineage at USDA-ARS Cereal Disease Laboratory (St. Paul, MN). Inoculation and disease assessment procedures were reported previously (Jin et al., 2007). Virulence/avirulence formulas of the tested races are given in Table 1.

Molecular Marker Analyses
Genomic DNA was extracted from F₂ plants and from a bulk of 10 plants of each F₃:4 line. DNA isolations were done according to a protocol modified from Pallotta et al. (2003) or using a QIAGEN DNeasy 96 Plant kit (QIAGEN, Valencia, CA) according to the manufacturer’s instructions.

Parent lines 2174, Lakin, Sr22Tb, and Steinwedel were screened for polymorphism with 42 SSR markers previously mapped to the long arm of chromosome 7A including: barc29, barc49, barc108, barc121, barc192, barc195, barc221, cfa2019, cfa2040, cfa2123, cfa2257, cfd20, cfd68, cfd193, gwm4, gwm10, gwm63, gwm260, gwm276, gwm282, gwm332, gwm344, gwm473, gwm532, gwm573, psp3094, wmc17, wmc96, wmc116, wmc139, wmc107, wmc139, wmc273, wmc346, wmc426, wmc488, wmc525, wmc607, wmc633, wmc790, and deletion stocks of 7AL. The lines used in deletion mapping include 7AL-15 (0.99), 7AL-9 (0.89), 7AL-13 (0.83), 7AL-8 (0.83), 7AL-21 (0.74), 7AL-10 (0.49), and 7AL-1 (0.39) (Endo and Gill, 1996) (Fig. 1a). All aneuploid and deletion stocks were obtained from the Wheat Genetics and Genomics Resource Center at Kansas State University, Manhattan, KS.

Stem Rust Evaluations
Puccinia graminis f. sp. tritici, race RKQQC (virulence/avirulence formula: Sr5, 6, 7b, 8a, 9a, 9b, 9d, 9g, 21, 36, McN/Sr9e, 10, 11, 13+17, 24, 30, 31, 38, Tmp [based on revised nomenclature from Jin et al., 2008]) is avirulent to Sr22 and was used for phenotypic analysis of mapping populations. At least 10 seedlings of all F₂:3 and F₃:4 lines and the resistant and susceptible parents were grown in 10 by 10 cm square pots in Metro-Mix 200 vermiculite–peat–perlite medium (Hummert, Inc., Earth City, MO) in a greenhouse. Urediniospores were removed from liquid nitrogen storage and heat-shocked in a 42°C water bath for 5 min. Spores were suspended in Soltrol 170 isoparaffin oil (Chevron Phillips Chemical Company LP, The Woodlands, TX) and sprayed onto two- to three-leaf stage seedlings. Inoculated plants were incubated in a dew chamber at 24 ± 1°C, 100% relative humidity for 16 h and then grown in a greenhouse at 21 ± 4°C with 16 h light/8 h dark cycle. Infection types (ITs) described by Stakman et al. (1962) were assessed 14 d after inoculation. Rust evaluations were replicated so that a total of at least 20 seedlings from each line were evaluated.

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wmc809. Markers amplifying polymorphic fragments were run on aneuploid and deletion stocks to validate their chromosomal location and then evaluated on mapping populations. Thirteen markers amplifying polymorphic fragments between Sr22Tb and both hard winter wheat parents and assigned to chromosome 7A were used for linkage analysis of segregating populations.

The polymerase chain reaction (PCR) master mix for SSR primers consisted of 2 μL of 20 ng μL⁻¹ genomic DNA template, 0.40 μL of a 10 μM mixture of forward and reverse primers, 0.18 μL (0.9 U) of Taq polymerase, 1.20 μL of 10× buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, pH 8.3), 0.96 μL of a 100 μM mixture of dNTPs, and 7.26 μL of water, bringing the total reaction volume to 12 μL. A touchdown profile was used that consisted of an initial denaturation at 95°C followed by 15 cycles of 95°C (45 s), 65°C (45 s) decreasing by 1°C each cycle, and 72°C (60 s), followed by 25 cycles of 50°C annealing temperature. The cycling conditions for markers requiring specific annealing temperatures included an initial denaturation of 95°C followed by 35 cycles of 95°C (45 s), 49°C or 60°C (45 s), and 72°C (60 s), followed by a final extension at 72°C (4 min). The forward primers for all SSR markers were 5'-modified to include the fluorescent dye 6-FAM. Amplifications were performed using an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany). Sizing of PCR products was performed by capillary electrophoresis using a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Analysis of PCR fragments was performed using GeneMarker 1.60 software (SoftGenetics, State College, PA). Genetic linkage analysis was performed using MAPMAKER v 3.0 (Lander et al., 1987). Marker order was established using multipoint analysis and the Haldane centimorgan function with a minimum LOD of 3.0. Segregation of marker loci and resistance reaction was evaluated using a χ² goodness-of-fit test. Differences in recombination frequency between populations were determined according to Allard (1956).

RESULTS AND DISCUSSION

Phenotypic Evaluation

Germplasm Sr22Tb exhibited ITs of 2− or 2 when inoculated with diverse races of Pgt, including races TTKSK and TTKST (Table 2). Susceptible reactions (IT = 4) were observed on parent line 2174 when inoculated with six of the eight races of Pgt. Resistant reactions were observed when cultivar 2174 was inoculated with isolates MCCFC and RKQQC, indicating the presence of a race-specific stem rust resistance gene in this parent. The infection type was variable among different inoculation experiments when 2174 was challenged by RKQQC, suggesting the presence of an environmentally sensitive resistance gene(s) in this line (data not shown). The parent line Lakin was consistently susceptible to all isolates.

In each segregating population, individuals homozygous or heterozygous for Sr22 exhibited a low IT of 1− to 2 on challenge with Pgt race RKQQC. Uredinia were typically small and surrounded by chlorosis rather than necrosis. Individuals in the populations with an IT of 2+ or greater were considered susceptible (sr22sr22).

Analysis of segregation ratios indicated that resistance to stem rust was due to a single dominant gene in both populations (Table 3). Although reduced transmission of Sr22 had been previously reported by Paull et al. (1994), there was no significant deviation from the 1:2:1 segregation ratio expected for a single dominant gene. Encouragingly, the variable seedling resistance to RKQQC in 2174 did not interfere with our ability to score Sr22 in F₃ families.

Genetic and Physical Mapping of the Sr22Tb Introgression

Of the 42 SSR loci evaluated, 13 were polymorphic between Sr22Tb and the hard winter wheat parents 2174 and Lakin. Linkage analysis of these makers on the U5615-F₂:₃ and the U5616-F₂:₃ populations placed the markers onto two linkage groups. Nine of the SSR markers were placed onto a linkage group with Sr22. These loci included Xbarc121, Xcsa2019, Xcsa2123, Xgwm260, Xgwm332, Xpsp3094, Xwmc607, Xwmc633, and Xwmc790 (Fig. 1). Four other loci, Xcsa2257, Xgwm344, Xwmc346, and Xwmc525, formed a second unlinked group greater than 60 cM distal to Sr22 (LOD > 3; map not shown). These four markers have previously been located terminally on 7AL (Miranda et al., 2007; Perugini et al., 2007).

The orders of markers in the Sr22 linkage group agree in both populations (Fig. 1). The nine linked markers covered a region of 5.6 cM in the U5615-F₂:₃ population, compared to a distance of 12.6 cM in the U5616-F₂:₃ population. Thus, there was a twofold reduction in recombination in the 2174/Sr22Tb population compared with the population derived from the cross Lakin/Sr22Tb (P < 0.01). In
Table 2. Infection types of parent lines 2174, Lakin, Sr22Tb, and selected recombinants when inoculated with eight different races of _Puccinia graminis_ f. sp. _tritici_.

<table>
<thead>
<tr>
<th>Line</th>
<th>TTKSK (04KEN156/04)</th>
<th>TTKST (06KEN19-V-3)</th>
<th>TPMKC (74MN1409)</th>
<th>TTTTF (01MN84A-1-2)</th>
<th>RKQOC (99K7S6A)</th>
<th>QFCSC (06N7D6C)</th>
<th>QTHJC (75N7D7C)</th>
<th>MCCFC (59K5S19)</th>
</tr>
</thead>
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<tr>
<td>Lakin</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Sr22TB</td>
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<td>2−</td>
<td>2</td>
<td>2−</td>
<td>2−^5</td>
<td>2−</td>
<td>2−</td>
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</tr>
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<td>2174</td>
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<td>4</td>
<td>4</td>
<td>3/13−</td>
<td>4</td>
<td>4</td>
<td>2−</td>
</tr>
<tr>
<td>U5615-98-120^2</td>
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<td>2−/4</td>
<td>2/4</td>
<td>2</td>
<td>2−/3</td>
<td>2−</td>
<td>2/4</td>
<td>2−</td>
</tr>
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<td>U5615-98-136</td>
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<td>2−</td>
<td>2</td>
<td>2</td>
<td>1/2−</td>
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<td>2−</td>
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<td>2/4</td>
<td>2/4</td>
<td>2</td>
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<td>2−/4</td>
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<td>2−/4</td>
<td>2−/4</td>
<td>2</td>
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<td>U5616-20-154-7</td>
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<td>2−</td>
<td>2</td>
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<td>2</td>
<td>2</td>
<td>2−</td>
</tr>
<tr>
<td>U5616-20-9-9-15</td>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2−</td>
</tr>
</tbody>
</table>

^1 Race isolates given in parentheses.

^2 Line is heterozygous at marker loci linked to Sr22.

^3 Two or more infection types present on the same plant, with the predominant type given first.

^4 Two scores separated by a slash indicates heterogeneity in infection type, with the predominant phenotype given first.

Both populations markers flanking Sr22 were identified. In U5615–F2:3, Sr22 was flanked proximally by _Xgwm260_ at a distance of 0.6 cM and distally at 0.4 cM by cosegregating loci _Xwmc633_, _Xgwm332_, and _Xwmc790_. In the U5616–F2:3 population, proximal marker locus _Xgwa2123_ was 4.2 cM from Sr22 and the cosegregating loci _Xwmc633_, _Xgwm332_, and _Xwmc790_ were 1.8 cM distal.

Nullisomic and ditelsomic analysis of SSR loci placed _Xbarc121_, _Xafa2019_, _Xafa2123_, _Xgwm332_, _Xpsp3094_, _Xwmc607_, _Xwmc633_, and _Xwmc790_ on the long arm of chromosome 7A and _Xgwm260_ on the short arm. Marker _Xwmc607_ was located on the proximal region between the centromere and the 7AL-1 breakpoint. The _Xbarc121_ locus is located between deletion breakpoints 7AL-10 (fraction length [FL] = 0.49) and 7AL-21 (FL = 0.74) and the _Xafa2123_ locus maps in the submicroscopic region between deletion breakpoints 7AL-8 and 7AL-13, both of which have FL = 0.83. The loci distal to Sr22, _Xwmc633_, _Xgwm332_, _Xwmc790_, _Xpsp3094_, and _Xafa2019_ are located between the breakpoints 7AL-9 (FL = 0.89) and 7AL-15 (FL = 0.99) (Fig. 1a). These data place Sr22 in the deletion interval between the 0.83 and 0.99 breakpoints.

Six SSR loci in the linkage group with Sr22 including _Xwmc607_, _Xbarc121_, _Xwmc633_, _Xgwm332_, _Xwmc790_, and _Xafa2019_ were evaluated and determined to be polymorphic between Steinwedel and the donor line Sr22Tb (Table 4). These data, combined with our physical mapping of the markers, confirm the result of Paull et al. (1994) that the transfer from _T. boeoticum_ in Sr22Tb involves at least 89% of the long arm of chromosome 7A as well as a portion of the short arm.

Although more recombination was observed in the U5616–F2:3 population than the U5615–F2:3 population, a comparison of the physical location of markers and genetic distances indicate low levels of recombination in both populations, particularly in the proximal portion of the chromosome. Only 3.5 and 5.6 cM of genetic distance were observed in U5615–F2:3 and U5616–F2:3 populations, respectively, between markers _Xgwm260_ and _Xafa2123_ that span 83% of the long arm of chromosome 7A plus a portion of the short arm. In the region distal to Sr22, 1.1 and 1.0 cM of genetic distance was observed between SSR markers located in the deletion interval between the breakpoints for 7AL-9 (FL = 0.89) and 7AL-15 (FL = 0.99).

**Linkage Analysis of F3:4 Recombinant Populations**

To develop lines having a smaller amount of _T. boeoticum_–derived chromatin, additional populations of F3:4 families were developed from three recombinant F2 individuals (U5615-72, U5615-98, and U5616-20; Fig. 2) Linkage analysis of 89 F3:4 recombinants identified five marker loci that cosegregated with Sr22 (Table 3). Segregation of Sr22 in F2:3 and F3:4 populations, including number of lines (n), the observed and expected genotypic frequencies, and χ² and P values for fit to the 1:2:1 segregation expected for a single dominant gene.

Table 3. Segregation of Sr22 in F2:3 and F3:4 populations, including number of lines (n), the observed and expected genotypic frequencies, and χ² and P values for fit to the 1:2:1 segregation expected for a single dominant gene.
analyses of the F$_{3:4}$ populations were performed for loci where the U5615-72, U5615-98, and U5616-20 individuals were heterozygous. In all three F$_{3:4}$ populations, marker orders were consistent with the orders observed in the F$_{2:3}$ populations (Fig. 1). In all populations, Xwmc633 was closest to Sr22 with genetic distances ranging from 1.3 to 2.1 cM distal.

The U5615-72-F$_{3:4}$ population consisted of 104 lines that segregated for Sr22, proximal loci Xbarc121, Xcfa2123, Xwmc607, and Xgwm260 as well as distal loci Xgwm332, Xpsp3094, Xwmc633, and Xwmc790. Recombination occurred between the Xcfa2019 locus and Sr22 in the previous generation; thus, the U5615-72-F$_{3:4}$ population was fixed for alleles from parent line 2174 in the region of 7AL distal to the Xcfa2019 locus (Fig. 2). In the related U5615-98 F$_{3:4}$, 140 lines segregated for Sr22 and distal loci Xcfa2019, Xgwm332, Xpsp3094, Xwmc633, and Xwmc790. This population was fixed for alleles from parent line 2174 at the SSR loci proximal to Sr22 (Fig. 2). In both cases, recombination levels were greater ($P < 0.01$) than observed in the original U5615-F$_{2:3}$ and were similar to the distances observed for the U5616-F$_{2:3}$ and U5616-20 F$_{3:4}$ populations.

### Identification of Recombinants

Several recombinant genotypes were identified that carry reduced levels of *T. boeoticum* chromatin associated with Sr22. In the U5615-98-F$_{3:4}$ population that was fixed for table 4. Simple sequence repeat markers used for linkage analysis of Sr22, parent allele sizes in base pairs.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Steinwedel</th>
<th>Sr22Tb</th>
<th>2174</th>
<th>Lakin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xgwm260</td>
<td>—†</td>
<td>153</td>
<td>163</td>
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</tr>
<tr>
<td>Xwmc607</td>
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<td>89</td>
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<td>Xbarc121</td>
<td>234</td>
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<td>241</td>
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<tr>
<td>Xcfa2019</td>
<td>237</td>
<td>238</td>
<td>217</td>
<td>217</td>
</tr>
</tbody>
</table>

†Unavailable data.

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The U5615-72-F$_{3:4}$ population consisted of 104 lines that segregated for Sr22, proximal loci Xbarc121, Xcfa2123, Xwmc607, and Xgwm260 as well as distal loci Xgwm332, Xpsp3094, Xwmc633, and Xwmc790. Recombination occurred between the Xcfa2019 locus and Sr22 in the previous generation; thus, the U5615-72-F$_{3:4}$ population was fixed for alleles from parent line 2174 in the region of 7AL distal to the Xcfa2019 locus (Fig. 2). In the related U5615-98 F$_{3:4}$, 140 lines segregated for Sr22 and distal loci Xcfa2019, Xgwm332, Xpsp3094, Xwmc633, and Xwmc790. This population was fixed for alleles from parent line 2174 at the SSR loci proximal to Sr22 (Fig. 2). In both cases, recombination levels were greater ($P < 0.01$) than observed in the original U5615-F$_{2:3}$ and were similar to the distances observed for the U5616-F$_{2:3}$ and U5616-20 F$_{3:4}$ populations.

### Identification of Recombinants

Several recombinant genotypes were identified that carry reduced levels of *T. boeoticum* chromatin associated with Sr22. In the U5615-98-F$_{3:4}$ population that was fixed for...
alleles from parent line 2174 at marker loci proximal to Sr22, seven genotypes were identified that are resistant to stem rust and in which recombination had occurred between Sr22 and the most distal marker Xca2019. Three lines fixed for T. boeoticum alleles at distal loci Xwmc633, Xgwm332, Xwmc790, and Xpsp3094 were homozygous resistant to race RQQQC, of which recombinant U5615-98-136 is shown as example in Fig. 2. Two of these recombinants, U5615-98-136 and U5615-98-48, were evaluated for reaction to nine additional races of stem rust, including TTKSK and TTKST, and exhibited widely effective resistance to all races (Table 2). Four recombinants were heterozygous for Sr22, Xwmc633, Xgwm332, Xwmc790, and Xpsp3094, and homozygous for alleles from parent line 2174 at Xca2019. Two of these lines (U5615-98-120 and U5615-98-144) were tested and segregated for resistance to diverse races of stem rust (Table 2). These populations can be used to identify new recombinants with further reduced alien chromatin in subsequent generations of inbreeding.

In the U5616-20 population, three genotypes were identified as segregating for resistance to stem rust race RQQQC and homozygous for Lakin alleles at flanking markers Xca2123 and Xwmc633, of which recombinant U5616-20-154 is shown as an example in Fig. 2. Plants were self-pollinated and five lines homozygous resistant to all tested races of stem rust were identified (Table 2).

CONCLUSION

Although the stem rust resistance gene Sr22 provides resistance to all known races of Pgt, it has not been widely used in agriculture. This is likely due to detrimental effects associated with introgression from the A\textsuperscript{m} genome diploid donor species, T. boeoticum. Our data support the hypothesis of Paull et al. (1994) that the proximal regions of both the short and long arm of chromosome 7A are comprised of T. boeoticum chromatin in lines having Sr22 derived from Sr22Tb. Paull et al. (1994) reported that the restriction fragment length polymorphism locus polymorphic between T. boeoticum and hexaploid wheat in the distal region of 7AL, Xpsr119, was not transmitted from T. boeoticum. They speculated that the terminal region of 7AL in Sr22 hexaploid lines is likely of T. aestivum origin while the proximal region was derived from T. boeoticum. The 13 SSR loci mapped in this study segregated into two unlinked groups, although all markers except Xgwm260 were located on the long arm of 7A. Four of the SSR loci that are located in the most distal region of 7AL were not linked to Sr22 and underwent much greater levels of recombination than observed in the Sr22 region (data not shown). These data agree with the hypothesis that the terminal region of the long arm of 7A in the Sr22Tb donor parent is of T. aestivum origin.

Low levels of recombination were observed in the F\textsubscript{2:3} populations when compared with previous intraspecific SSR maps of chromosome 7A. Somers et al. (2004) reported the distance between Xgwm260 and Xca2019 to be 37 cM. The same interval in our study represented 5.6 cM in the U5615-F\textsubscript{2:3} population and 12.6 cM in the U5616-F\textsubscript{2:3} population. Although both populations exhibited suppressed recombination, greater recombination was observed in the F\textsubscript{2:3} population from the cross Lakin/Sr22Tb than the 2174/Sr22Tb. However, similar levels of recombination in the region were observed in the F\textsubscript{1:4} populations from both crosses, indicating that the observed reduction in the original 2174/Sr22Tb F\textsubscript{2:3} may have been specific to that population.

The and McIntosh (1975) reported reduced transmission of Sr22 in pooled populations of lines with Sr22 from both T. monococcum and T. boeoticum. In this study, segregation for 7AL alleles including Sr22 did not deviate significantly from the expected 1:2:1 ratio in all populations.

Previously, the SSR loci Xca2123 and Xca2019 were identified as flanking markers for Sr22 (Khan et al., 2005). These same markers were polymorphic in both of our populations and were found to flank Sr22. Reduced levels of recombination associated with Sr22 were identified by Khan et al. (2005) in a single F\textsubscript{1:4}–derived, single seed descent population, on which mapping was done only on homozygous resistant and susceptible lines. In the present study, all individuals in multiple populations were genotyped with codominant markers allowing for more accurate estimates of genetic distances. Mapping in the F\textsubscript{1:4} allowed for the identification of critical recombinants with reduced T. boeoticum chromatin. From these recombinants, additional mapping populations were made and recombinants with even further reduced alien chromatin were identified.

The recombinant lines carrying reduced T. boeoticum chromatin identified in this study will be useful in breeding programs. Using these lines as donor parents should help to ameliorate the negative effects associated with Sr22. These recombinant lines are being backcrossed to hard and soft winter wheat cultivars to produce populations and NILs for which the effects of the introgression on agronomic performance can be evaluated. The marker loci documented in this study will prove useful in the marker-assisted selection of Sr22. The most closely linked marker locus to Sr22 across all populations was the distal locus Xwmc633, while the proximal flanking marker Xca2123 could also be used but greater recombination between this locus and Sr22 was observed. Markers identified in this study can be used for selection among the progeny of the recombinant lines from the U5615-98 population to further reduce the size of the T. boeoticum chromosome segment. Homozygous resistant F\textsubscript{1:5} progeny have been identified from each of the U5616-20–derived recombinant plants. The use of marker-assisted selection in progeny from crosses with the U5616-20 recombinants will require the development of markers more closely linked to Sr22. Recombinants with reduced T. boeoticum chromatin are available on request for development of wheat cultivars having resistance to stem rust.
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References