RESEARCH

Chromosome Location, Linkage with Simple Sequence Repeat Markers, and Leaf Rust Resistance Conditioned by Gene *Lr63* in Wheat

J. A. Kolmer,* J. A. Anderson and J. M. Flor

ABSTRACT

RL6137, a 'Thatcher' backcross line of wheat (*Triticum aestivum* L.) has resistance to *Puccinia triticina* (causal agent of wheat leaf rust) derived from *T. monococcum*. To determine the chromosome location of this resistance, RL6137 was crossed with Thatcher and F_2 individual seedlings were tested for segregation of leaf rust resistance and segregation of polymorphic simple sequence repeat (SSR) markers. Leaf rust resistance genotypes of F_2 individuals were confirmed with segregation of F_3 families. The F_2 seedlings and F_3 families segregated for a single leaf rust resistance gene. The SSR markers *barc 57* and *barc 321* located on chromosome 3AS were tightly linked with the leaf rust resistance gene. The leaf rust resistance gene in RL6137 was designated as *Lr63*. This gene conditions low to intermediate infection types to most *P. triticina* isolates and can be used in wheat improvement programs to enhance leaf rust resistance.

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Abbreviations: IT, infection type; SSR, simple sequence repeat.

Leaf rust caused by *Puccinia triticina* Eriks. is a widespread and regularly occurring disease of wheat in the United States and worldwide. The *P. triticina* population in the United States is highly variable as more than 50 races are identified annually (Kolmer et al., 2008). Although more than 60 *Lr* genes have been designated in wheat (McIntosh et al., 2009), many of these genes no longer condition effective resistance since leaf rust races with virulence have increased in response to the release of wheat cultivars with race-specific resistance genes. To develop cultivars with high levels of effective resistance to leaf rust, it is necessary to add new genes that have not been previously exploited to wheat breeding germplasm.

Leaf rust resistance genes in wheat have been derived from common wheat, durum wheat (*T. turgidum)*, *T. tauschii*, *T. timopheevii*, *Aegilops umbellulata*, *Thinopyrum ponticum, Th. intermedium, Secale cereale, Ae. ventricosa, T. spelta, T. dicoccoides, Ae. kotschyi, Ae. sharonensis, Ae. peregrina, Ae. triuncialis, Ae. geniculata*, and *Ae. neglecta* (McIntosh et al., 2009). The Thatcher isogenic line RL6137 has leaf rust resistance derived from *T. monococcum* accession TMR5-J14-12-24 located on chromosome 3A (Valkoun and Kucerov, 1986; Valkoun et al., 1988; Dyck and Bartos, 1994). In previous research, RL6137 had low to intermediate infection types to the five *P. triticina* isolates that were tested and had a moderate level of field resistance to leaf rust (Dyck and Bartos, 1994). The objectives

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Table 1. Segregation for infection type† (IT) to leaf rust isolate BBBD in seedlings of Thatcher × RL6137 in F_2 individuals and F_3 families.

Generation	IT 2–2⁺	IT 2–3⁺	IT 3–3⁺	Expected ratio	χ^2	*P*‡
F_2	96		35	3:1	0.21	0.65
F_3	28	75	27	1:2:1	3.09	0.21

†Infections as described in Long and Kolmer (1989).

‡ Probability.

of this study were to further test the effectiveness of leaf rust resistance in RL6137 and to determine the location on chromosome 3A of this resistance.

MATERIALS AND METHODS

Seed of RL6137 (Thatcher*6/TMR5-J14-12-24) and the wheat cultivar Thatcher were planted in 15 cm diam. pots that were filled with steamed soil and placed in a growth cabinet at 18°C with a mixture of fluorescent and incandescent lighting with an intensity of 25 μmol s^{-1} m^{-2}. At the two leaf stage the plants were treated with Nutricot 13–13–13 NPK (Plantco Inc., Brampton, ON). At heading the Thatcher plants were emasculated and pollen shedding anthers from RL6137 were used to pollinate the Thatcher female parent. The F_1 seed was harvested and then planted in a growth cabinet to obtain F_2 seed.

Approximately 140 F_2 seed were planted into 14 3.5 cm^2 square plastic pots, 10 seeds per pot, that were filled with vermiculite and placed on plastic trays, six pots per tray. When the primary leaves were fully expanded the F_2 plants, RL6137, and Thatcher were inoculated with race BBBD (Long and Kolmer, 1989) of *P. triticina*. For seedling inoculations approximately 1 mg of rust urediniospores were mixed with 0.2 mL of Soltol 170 oil (Phillips Petroleum, Borger, OK), and then spray inoculated onto plants using the equipment and methods previously described (Roelfs et al., 1992). After inoculation seedling plants were allowed to dry for 1 h and then placed in a mist chamber overnight at 18°C and 100% relative humidity. The seedlings were placed on a greenhouse bench after incubation. Seedlings were fertilized with a 20–20–20 NPK solution immediately after inoculation and at 14 d after planting. The infection types (ITs) on the primary leaves of individual plants were read at 10 to 12 d after inoculation. The ITs were classified using a 0 to 4 scale (Long and Kolmer, 1989). Infection types 0 (no visible sign of infection),; (hypersensitive flecks), 1 (small uredinia surrounded by necrosis) and 2 (small to moderate size uredinia surrounded by chlorosis) were considered as low (resistant) and IT from 3 (moderate size uredinia without necrosis or chlorosis) to 4 (large uredinia) were considered as high (susceptible). Mixtures of ITs were indicated by listing the most common IT first, followed by the less common ITs. Larger and smaller uredinia were indicated by "⁺" and "⁻", respectively. RL6137 was also inoculated as seedlings with 10 other *P. triticina* isolates.

After scoring for leaf rust IT, the F_2 plants were individually transplanted to 15 cm diam. pots in a greenhouse and grown to obtain F_3 families. Fifteen seed per F_3 family were planted in each 3.5 cm^2 pot and were inoculated with race BBBD when the primary leaves were fully expanded. The F_3 families were classified as homozygous resistant if all seedlings had low IT; segregating if seedlings varied for low and high IT; and homozygous susceptible if all seedlings had high IT. A χ^2 test (Steel and Torrie, 1980) was used to determine if the observed ratios of segregation for leaf rust resistance in the F_2 seedlings and F_3 families significantly deviated from the expected ratio.

In the field plot test, 50 seeds of RL6137 were planted in an inoculated rust nursery in 2 m rows spaced 30 cm apart between entries that were perpendicular to rows of a mixture of wheat cultivars Thatcher, Morocco (PI 278386), Max (CI 15093), and Little Club (CI 4066) that are susceptible to leaf rust. The spreader rows and entries were inoculated with a mixture of leaf rust races common to the north central United States (Kolmer et al., 2009). The plants were rated for leaf rust severity using the modified Cobb scale (Peterson et al., 1948). Leaf rust response in the adult plants was rated as previously described (Roelfs et al., 1992). The field plots were rated for leaf rust when the susceptible cultivar Thatcher had a leaf rust severity of 70 to 80% with a susceptible response.

Leaf segments 2 cm in length collected from the individual F_2 plants, Thatcher, and RL6137 were placed in 1.5 mL centrifuge tubes and snap frozen in liquid N. DNA was extracted from the leaf tissue of individual F_2 seedlings following the methods of Liu et al. (2006). Polymerase chain reaction (PCR) of the F_2 DNA was conducted in 10 μL reaction volumes each containing 3 μL of wheat DNA (30–45 ng total) with SSR primers. DNA of the F_2 plants was tested for segregation with SSR-*barc* primers *12, 34, 45, 51, 57, 86, 105, 179, 197, 284, 294, 310, 321, 1060, 1099*, and *1113*; the *gwm* primers *2, 4, 5, 30, 32, 133, 162, 218, 369, 480, 666*; the *gwmc* primers *11, 50, 96, 532, 640*; and *cfd 79*, which all map to chromosome 3A (Song et al., 2005). After an initial denaturing step for 3 min at 94°C, 35 cycles were conducted with 1 min at 94°C, 2 min at the annealing temperature of the individual primer pairs, followed by a separate final extension step of 10 min at 72°C. Polymerase chain reaction products were separated by polyacrylamide gels containing 32% (v/v) formamide (Litt et al., 1993). The gels were visualized by silver staining (Bassam et al., 1991). Linkage analysis of polymorphism generated by the SSRs and the segregation of leaf rust resistance in the F_2 plants of Thatcher × RL6137 was conducted with MAPMAKER for MacIntosh v 2.0 (Lander et al., 1987) at LOD 3.0 with the Haldane function. DNA of 21 spring wheat cultivars was haplotyped with three SSR markers linked to *Lr63*.

RESULTS

In seedling tests, RL6137 had an IT of 2 to 2⁺ (medium to larger uredinia with chlorosis) to isolates BBBD, MBRJ, PNMQ, MCGJ, TNRJ, MCDS, TDBJ, MFBJ, and THBJ of *P. triticina*. Isolate KFBJ had an IT 3 on RL6137, and isolate TLGF had an IT 0; (very light hypersensitive flecks). In field plots at St. Paul, MN, RL6137 had leaf rust severity between 30 and 50% with a moderate resistance to moderate susceptible response.

The individual F_2 plants of Thatcher × RL6137 when tested with leaf rust race BBBD segregated in two IT classes of 2⁻ to 2⁺ and 3 to 3⁺ that fit a 3:1 ratio (Table 1, Fig. 1) that indicated the segregation of a single gene. The derived F_3 families segregated in a 1:2:1 ratio for families that were homozygous resistant for plants with IT 2⁻ to 2; families

that segregated for plants with IT 2 to 2^+ and plants that had IT 3 to 3^+; and families that were homozygous for susceptible plants with IT 3 to 3^+. The leaf rust resistant genotypes of the F_2 plants were determined based on the homozygosity or segregation of the respective F_3 families.

The primers *barc 57* and *barc 321* showed polymorphism between Thatcher and RL6137 and mapped at a distance of 2.9 cM to the leaf rust resistance in RL6137 on chromosome 3AS (Fig. 2). *Barc 310* also showed polymorphism between Thatcher and RL6137 and mapped 26 cM distal to *barc 321* and *barc 57* (data not shown). *Barc 284* was also polymorphic, but did not segregate with the leaf rust resistance.

Twenty-one spring wheat cultivars, Thatcher, and RL6137 were haplotyped with the three SSR markers linked to leaf rust resistance in RL6137. *Barc 321* produced a diagnostic amplicon of 191 bp with RL6137 and produced four different amplicons over 200 bp for all of the cultivars except for 'Ada' that had an amplicon of 198 bp (Fig. 3). *Barc57* and *Barc 310* did not amplify DNA fragments that were unique to RL6137 in this set of 23 wheat genotypes. Based on pedigree analysis and seedling IT to leaf rust (J. Kolmer, unpublished data, 2009), none of the 21 cultivars would have been expected to have *Lr63*.

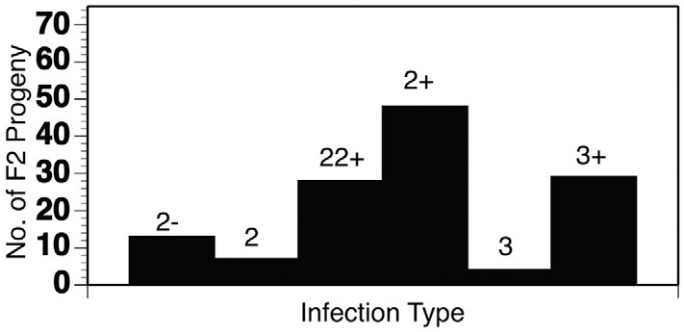

Figure 1. Distribution of leaf rust infection types in F_2 progeny of Thatcher × RL6137.

DISCUSSION

A single leaf rust resistance gene in RL6137 mapped to chromosome 3AS and was designated as *Lr63* (McIntosh et al., 2009). No other designated *Lr* genes have been mapped to chromosome 3AS. In field plots RL6137 had intermediate levels of resistance, however in combinations with other effective seedling or adult plant resistance genes *Lr63* can be used to develop wheat cultivars with highly effective leaf rust resistance. Combinations of seedling race specific genes and nonrace specific adult plant resistance genes in spring wheat cultivars (Kolmer and Oelke, 2006) have been proven to condition good levels of effective resistance over years, although none of the genes individually condition highly effective resistance.

None of the other designated *Lr* genes in wheat have been derived from *T. monococcum*. However, Hussien et al. (1998) mapped leaf rust resistance genes in winter wheat lines on chromosomes 1A, 5A, and 6A that were derived from *T. monococcum* ssp. *monococcum* or from ssp. *boeticum*. Sokiewicz et al. (2008) mapped a leaf rust resistance gene in triticale on chromosome 2A that was derived from *T. monococcum*. A

Figure 2. Linkage on chromosome 3AS of simple sequence repeat markers *barc57* and *barc321* with the leaf rust resistance gene *Lr63* in Thatcher wheat line RL6137.

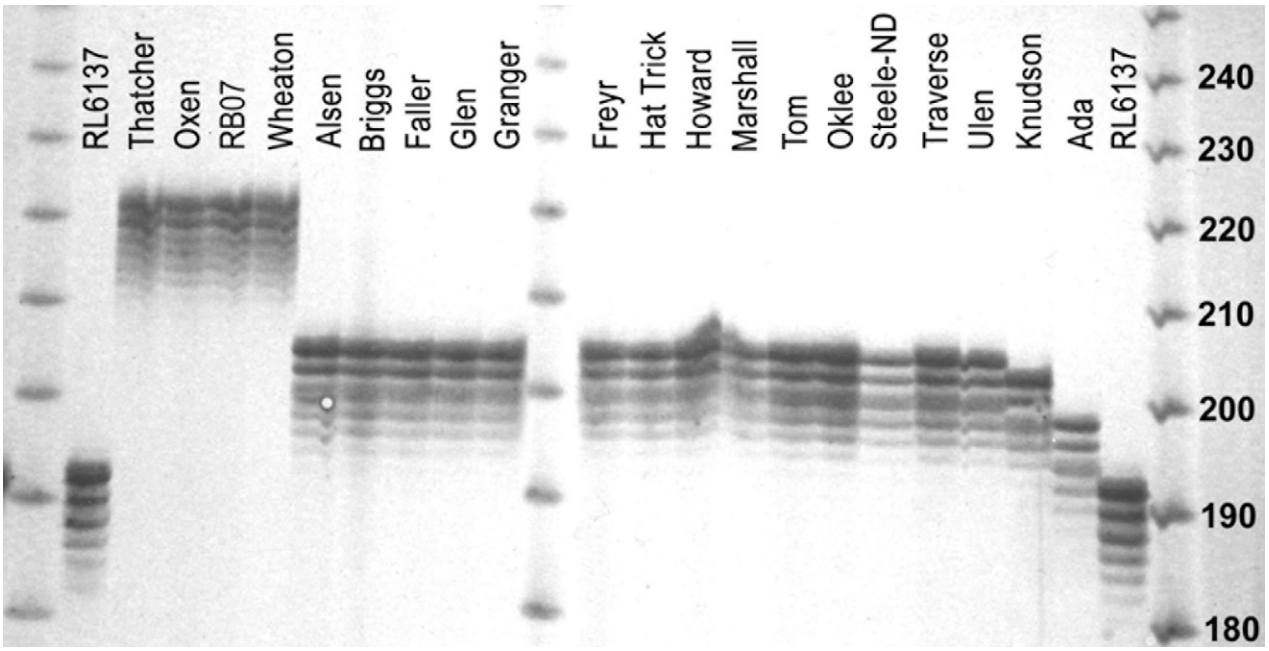

Figure 3. Polymerase chain reaction products amplified by *barc321* with 21 spring wheat cultivars and RL6137.

winter wheat line with resistance to powdery mildew [*Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* E. Marchal] derived from *T. monococcum* subsp. *aegilopoides* (Shi et al., 1998) also had resistance to leaf rust that segregated as a single gene in F_2 individuals and in F_3 families after three backcrosses to Thatcher wheat (J. Kolmer, unpublished data, 2009). *Triticum monococcum* has not been widely used as a source of leaf rust resistance and other distinct leaf rust resistance genes may yet be derived from this species. Small amounts of RL6137 seed are available by request from the senior author.

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