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Identification of microsatellites linked to \textit{Lr47}

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Abbreviations: PCR: polymerase chain reaction  
RFLP: restriction fragment length polymorphism

Leaf rust resistance gene \textit{Lr47} is located within an interstitial segment of \textit{Triticum speltoides} Taush. 7S chromosome translocated to the short arm of chromosome 7A of bread wheat. This gene is resistant against currently predominant races of leaf rust from Argentina. The objectives of this study were to identify microsatellites linked to this source of resistance as a potential tool to introgress this source of resistance. Isogenic lines with and without \textit{Lr47} developed from 10 cultivars/breeding lines as well as 10 microsatellites previously mapped in 7AS chromosome were used in this study. Microsatellite \textit{gwm 60} was the only marker that co-segregated completely linked to \textit{Lr47}. These data indicate that \textit{gwm 60} could be a valuable marker to introgress \textit{Lr47} in wheat germplasm.

Leaf rust (caused by \textit{Puccinia triticina} Erics.) is one of the mayor diseases of wheat worldwide. In South America, yield losses due to leaf rust occur annually throughout the hard red spring wheat regions of Argentina, Chile, Uruguay, Brazil and Paraguay. Incorporating genetic resistance to this pathogen into adapted germplasm is a mayor goal in wheat breeding programs, as using disease resistance genes minimizes the need for the application of costly fungicides, thus reducing environmental

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contamination risks and decreasing production costs. 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 a short number of high yielding, pure-line varieties in many parts of the world, including the South Cone, has reduced the wheat gene pool. In this context, genes from wild wheat relatives have contributed to increase the genetic diversity of hexaploid wheat in genes conferring resistance against leaf rust (Puccinia recondita Rob. ex Desm.), stem rust (Puccinia graminis Pers.), Hessian fly [Mayetiola destructor (Say)], Russian wheat aphid (Diuraphis noxia Mordvilko) between other pathogens (reviewed by McIntosh, 1991). At least 51 leaf rust resistance genes from wheat and wheat relatives have been catalogued (McIntosh et al. 2003) and molecular markers are available for many of them (Robert et al. 1999; Helguera et al. 2000; Helguera et al. 2003; Helguera et al. 2005; http://maswheat.ucdavis.edu/, verified August 20, 2005).

In some cases, resistance genes derived from wild relative translocations are not deployed in breeding programs because the linkage between targeted genes and undesirable genes on the alien segment usually result in yield and/or quality penalties. A good example is the Lr19 translocation including the undesirable linked yellow pigment gene (Prins et al. 2001; Zhang et al. 2005). Consequently, to be deployed in agriculture the alien chromosome segments should be as short as possible. The identification of alternative markers mapping in wild relatives translocations is a useful tool to generate shorter translocations carrying the desirable character without linked undesirable traits.

The leaf rust resistance gene Lr47 is located within a segment of Triticum speltaoides Taush. chromosome 7S transferred to the chromosome 7A of hexaploid wheat into an interstitial translocation 20-30 cm long (Dubcovsky et al. 1998). The 7AS-7S#1S-7AS.7AL T. speltaoides translocation was characterized using 14 RFLP markers previously mapped on the short arm of chromosome 7A" of T. monococcum (Dubcovsky et al. 1996) finding 7 RFLP loci that mapped into the translocation (Dubcovsky et al. 1998). RFLP markers are expensive and time consuming, therefore PCR based markers are an attractive alternative for breeding programs. Unfortunately, only PCR markers derived from the RFLP ABC465 (maps close to the last part of the 7AS-7S#1S-7AS.7AL translocation, close to the centromere) are available (Helguera et al. 2000).

An interesting source of PCR markers in wheat are the microsatellites (Tautz and Renz, 1984). These loci are amplified using primers (18-25 bp long) specific for sequences flanking hyper variable regions of tandem repeats of 2-4 base pairs. Several molecular maps including more than 320 microsatellites covering the whole wheat genome are available (Bryan et al. 1997; Roder et al. 1998; Paillard et al. 2003).

The aim of the present work was to characterize the 7AS-7S#1S-7AS.7AL T. speltaoides translocation using microsatellites previously mapped on 7A wheat chromosome to identify novel PCR markers linked to Lr47.

MATERIALS AND METHODS

Plant materials

One or more isogenic lines homozygous for Lr47 developed from 9 wheat cultivars and/or breeding lines from Argentina and USA were used in this study. The 7AS-7S#1S-7AS.7AL T. speltaoides translocation carrying Lr47 was introgressed in Prointa Puntal (hard red winter from Argentina), Prointa Oasis and Prointa Imperial (hard red springs from Argentina), Kern, Yecora Rojo and Express (hard red springs from USA), UC1037, UC1041 and RS15 (University of California breeding lines from USA), by marker assisted backcrossing (BC). Each cultivar/breeding line was crossed with Pavon-Lr47 (donor of the 7AS-7S#1S-7AS.7AL T. speltaoides translocation) and the nine F₁s were backcrossed with the respective parents. In each of the 4 BC generations (or 6 in Kern, Yecora Rojo, Express, UC1037, UC1041 and RS15) two individuals heterozygous for the 7AS-7S#1S-7AS.7AL T. speltaoides translocation were selected by marker assisted selection using Lr47 specific markers according to Helguera et al. (2000). Finally, BC₄ (or BC₅) plants heterozygous for 7AS-7S#1S-7AS.7AL translocation were self-pollinated and homozygous 7AS-7S#1S-7AS.7AL plants were selected from BC₄ F₂ (or BC₅ F₂) using codominant Lr47 markers (Helguera et al. 2000). These selected BC₄ F₂ plants are expected to be 96.8% (or...
more than 99% in Bc₂ F₂) identical to the recurrent parent and homozygous for the Lr47 gene. The nine recurrent parents, the breeding line Pavan-Lr47, Pavan without the 7AS-7S#1S-7AS.7AL translocation (hard red winter from CIMMYT) and Chinese Spring nullisomic – tetrasomic lines N7A N7B and N7D (Sears, 1954) were also used in this study. A diverse set of varieties from Argentina including Biointa 3001, Buck Pingo, ACA 302, Pointra Bon. Hurón, Klein Don Enrique, Baguette 10, Relmó Tijereta, ACA 223, Klein Estrella, Buck Biguá, Buck Mataco, Pointra Cinco Cerros and Klein Volcán was analyzed to validate gwm 60 as a marker for Lr47.

Tests for resistance to leaf rust

Isolines carrying Lr47 from Pointra Puntal, Pointra Oasis and Pointra Imperial were grown in Marcos Juárez (Argentina) as part of the National Institute of Agricultural Technology (INTA) Wheat Breeding Program during years 2000, 2001, 2002 and 2003 to evaluate leaf rust resistance against local races of the pathogen. In the field experiment, recurrent parents (highly susceptible against local leaf rust races) were also included as controls.

DNA extraction and PCR markers

Genomic DNA was extracted from fresh leaves of single plants using a fast, small-scale DNA isolation procedure based on Wining and Langridge (1991).

A total of 10 wheat microsatellites previously mapped on 7A chromosome were included in this study: gwm 60, gwm 635 and wmc 666 from Röder et al. (1998); wmc 168, wmc 497, wmc 83 and wmc 17 from the Wheat Microsatellite Consortium (Gupta et al. 2002); Barc 70, Barc 174, and Barc 154 (Somers et al. 2004).

The PCR reactions were performed in 25 µl aliquots in a PTC-100 (MJ Research) thermal cycler. The reaction buffer contained 100 ng of template DNA, 1X Taq polymerase buffer (Promega Corp. Madison, WI), 1.0 U Taq (Promega), 0.2 mM of each deoxynucleotide, 0.2 µM of each primer and 1.5 mM of MgCl₂. Forty cycles of 45 sec at 94ºC, 45 sec between 55 to 65ºC depending on the primer combination and 1 min at 72ºC were performed, followed by an elongation step of 10 min at 72ºC. PCR reactions (10 µl each) were run on 8% polyacrylamide gels 15 cm long (Ruby SE600, Amersham BioSciences, CA), stained with ethidium bromide [0.5 g/L] and visualized with UV.

RESULTS AND DISCUSSION

Infection types to Puccinia triticina

Field tests carried out in Marcos Juárez (Argentina) during years 2002, 2003 and 2004, showed that the presence of Lr47 in BC₂ F₂ isolines from cvs. Pointra Puntal, Pointra Oasis and Pointra Imperial improved the resistant to local leaf rust isolates relative to the recurrent parents. In all tested years, isolines carrying Lr47 showed moderate to resistant responses with the exception of 2002, when the pathogen spread later in the season and bacterial leaf blight was observed, which caused a slight decline in the yields. However, in all other years, the isolines with Lr47 showed significantly higher resistance, with an average of more than 99% in Bc₂ F₂.

The most probable explanation of this result is the occurrence of a crossing over between Lr47 and Barc 70 in Pointra Puntal Lr47-2. This can be possible only if Barc 70 is outside of the 7AS-7S#1S-7AS.7AL translocation, as the
recombination between 7A and 7S chromosomes is prevented in presence of the Ph1 gene (Helguera et al. 2000). Similar data was obtained from microsatellites wmc 168, Barc 174, wmc 497, Barc 154.

The only marker that showed clear polymorphisms between Pavin and Pavin-Lr47 was gwm 60 (lane 11 and lanes 8-10, respectively in Figure 2), suggesting that it maps into the 7AS-7S#1S-7AS.7AL translocation. Nulli-tetrasomic analysis confirmed that the polymorphic fragment amplified by gwm 60 belongs to 7A chromosome (data not shown). When gwm 60 was evaluated in isolines with and without Lr47, a fragment of approximately 180 bp was observed only in isolines carrying Lr47 (Figure 2). In isolines without Lr47 PCR fragments of size different from 180 bp were amplified in all cases (lanes 3, 11 and 15 in Figure 2).

The scoring obtained with gwm 60 considering at least 30 isolines with and without Lr47 used in this study is identical to the scoring obtained using Lr47 markers developed by Helguera et al. (2000) and used in the backcrossing program that generated the isolines, this data demonstrates that gwm 60 is a good alternative marker to introgress Lr47 and a useful tool for the future dissection of this T. speltoides chromosome segment. To validate this marker in a larger set of wheat cultivars, 13 wheats from Argentina were evaluated (see Plant Materials section) and none of the tested cultivars amplified the 180 bp fragment associated with 7AS-7S#1S-7AS.7AL translocation.

The Xgwm60 locus has been mapped in chromosome 7A between loci Xabc158 and Xcdo475 (Röder et al. 1998), and Xcdo475 is at least 16-cM away from Xabc465 (Dubcovsky et al. 1998), which is the RFLP locus previously converted to a PCR marker for Lr47 (Helguera et al. 2000). The fact that loci Xgwm60 and Xabc465 are not so closely linked would be helpful to select shorter 7S chromosome segments carrying Lr47 through a second round of homologous recombination. Preliminary results suggest that the T. speltoides chromosome segment has a negative effect on flour yield, and therefore it will be desirable to separate this negative effect from Lr47. The gwm 60 marker reported here will be a useful tool to achieve this objective.

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REFERENCES


