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Identification of microsatellites linked to *Lr47*

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Keywords: *gwm 60*, leaf rust, marker assisted selection, *Puccinia triticina* Ericks., *Triticum aestivum*.

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

Leaf rust resistance gene *Lr47* is located within a interstitial segment of *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 *Lr47* developed from 10 cultivars/breeding lines as well as 10 microsatellites previously mapped in 7AS chromosome were used in this study. Microsatellite *gwm 60* was the only marker that co-segregated completely linked to *Lr47*. These data

indicate that *gwm 60* could be a valuable marker to introgress *Lr47* in wheat germplasm.

Leaf rust (caused by *Puccinia triticina* Ericks.) 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.

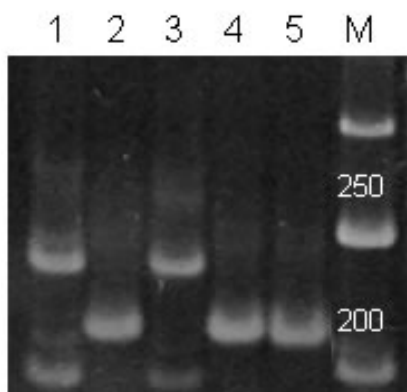


Figure 1. PCR amplification products using *Barc 70* microsatellite. Genomic DNAs were extracted from: Prointa Puntal (1), Prointa Puntal *Lr47-1* (2), Prointa Puntal *Lr47-2* (3), Pavon (4) and Pavon 7AS-7S#1S-7AS.7AL(5). M is a DNA size standard (Biodynamics).

Triticum speltoides Taush ($2n=14$, S genome) is an attractive source of high levels of resistance to leaf, stem and stripe rust of wheat (Dvorak, 1977), and leaf rust resistance genes *Lr28*, *Lr35*, *Lr36*, *Lr47* and *Lr51* were derived from this species (McIntosh et al. 2003; Helguera et

al. 2005), as different *T. speltoides* chromosomes translocated to different wheat chromosomes.

The leaf rust resistance gene *Lr47* is located within a segment of *Triticum speltoides* Taush. chromosome 7S transferred to the chromosome 7A of hexaploid wheat into a interstitial translocation 20-30 cM long (Dubcovsky et al. 1998). The 7AS-7S#1S-7AS.7AL *T. speltoides* translocation was characterized using 14 RFLP markers previously mapped on the short arm of chromosome 7A^m 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. speltoides* 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. speltoides* 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. speltoides* 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 translocation were selected by marker assisted selection using *Lr47* specific markers according to Helguera et al. (2000). Finally, BC₄ (or BC₆) 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 BC₆ 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 BC₆ F₂) identical to the recurrent parent and homozygous for the *Lr47* gene. The nine recurrent parents, the breeding line Pavon-*Lr47*, Pavon 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, Prointa Bon. Hurón, Klein Don Enrique, Baguette 10, Relmó Tijereta, ACA 223, Klein Estrella, Buck Biguá, Buck Mataco, Prointa 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 Prointa Puntal, Prointa Oasis and Prointa 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 *wms 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 94°C, 45 sec between 55 to 65°C depending on the primer combination and 1 min at 72°C were performed, followed by an elongation step of 10 min at 72°C. 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. Prointa Puntal, Prointa Oasis and Prointa Imperial improved the resistant to local leaf rust isolates relative to the recurrent parents. In all tested years, isolines carrying *Lr47* showed resistance

against local leaf rust races while recurrent parents showed moderately susceptible or susceptible responses. The host responses observed in isolines carrying *Lr47* agree with previous data obtained from Pavon-*Lr47* showing resistance against nine leaf rust races (Dubcovsky et al. 1998). This novel source of leaf rust resistance has not been used in commercial wheat cultivars from South America. The good performance of *Lr47* against local strains of leaf rust isolates suggests that this gene would be a useful source of resistance against this pathogen in wheat.

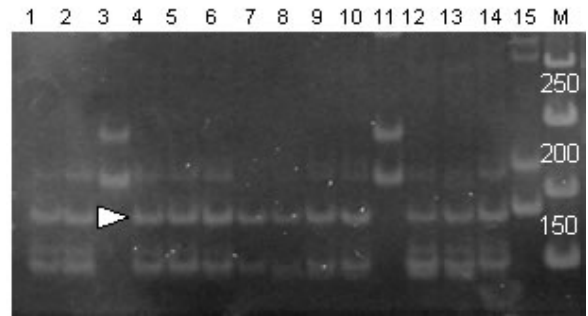


Figure 2. PCR amplification products using *gwm 60* microsatellite. Genomic DNAs were extracted from isolines of: Prointa Puntal-*Lr47* (lanes 1-2), Prointa Oasis-*Lr47* (4 to 7), Pavon-*Lr47* (8 to 10), Kern-*Lr47* (12-13) Prointa Puntal without *Lr47* (3), Pavon (11) and Kern (15). M is a DNA size standard (Biodynamics). A white arrowhead indicates the position of the PCR fragment associated with *Lr47*.

Molecular characterization of the 7AS-7S#1S-7AS.7AL *T. speltoides* translocation using microsatellites

Based on electrophoretic conditions used in this study, four of the ten tested microsatellites (*gwm 635*, *wmc 17*, *wms 666*, *wmc 83*) were monomorphic, considering Pavon, Pavon-*Lr47* and the isolines with/without *Lr47* from the 9 cultivars/breeding lines used in this study, so no additional information was obtained from them.

Another group of microsatellites (*Barc 70*, *wmc 168*, *Barc 174*, *wmc 497*, *Barc 154*), was monomorphic for Pavon and Pavon-*Lr47* but polymorphic when isolines with and without *Lr47* were considered in the analysis, suggesting that this group of markers have a physical position outside the 7AS-7S#1S-7AS.7AL translocation. This hypothesis could be corroborated in microsatellite *Barc 70*. Using this marker, Pavon and Pavon-*Lr47* amplified a fragment of approximately 220 bp (lanes 4 and 5 in Figure 1), the cultivar Prointa Puntal showed a approximately 240 bp fragment (lane 1) and isolines Prointa Puntal *Lr47*-1 and Prointa Puntal *Lr47*-2 showed fragments of 220 and 240 bp (lanes 2 and 3, Figure 1).

The most probable explanation of this result is the occurrence of a crossing over between *Lr47* and *Barc 70* in Prointa 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 Pavon and Pavon-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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