Identification and mapping of \textit{Lr3} and a linked leaf rust resistance gene in durum wheat

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Abstract

Leaf rust, caused by \textit{Puccinia triticina}, is an important disease of durum wheat (\textit{Triticum turgidum} ssp. \textit{durum}) worldwide and can be controlled through the use of genetic resistance. Two leaf rust resistance genes in durum wheat lines, ‘Camayo’ and ‘Storlom’, were mapped to the long arm of chromosome 6B via amplified fragment length polymorphism (AFLP) analysis of bulked segregant populations. The leaf rust resistance gene in Storlom was identified to be \textit{Lr3} using a previously known co-segregating marker, \textit{Xmwg798}. We validated a sequence tagged site (STS) version of this marker and identified three AFLP markers that were associated with the resistance gene in Camayo. The lack of recombination between the two resistance genes present in Storlom and Camayo, and comparison of the phenotypic and molecular characteristics of Camayo and the common wheat (\textit{T. aestivum}) near-isogenic ‘Thatcher’ lines carrying \textit{Lr3a}, \textit{Lr3ka} and \textit{Lr3bg}, indicated that the resistance in Camayo is conferred by a previously unknown gene adjacent to the \textit{Lr3} locus. The two closely linked genes confer resistance to \textit{P. triticina} race BBG/BN prevalent on durum wheat in northwestern Mexico and should be deployed in combination with other resistance genes, to prolong their effectiveness.

Abbreviations: AFLP, amplified fragment length polymorphism; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; PCR, polymerase chain reaction; STS, sequence tagged site; SSR, simple sequence repeats.
Introduction

Leaf rust (caused by *Puccinia triticina*) has become one of the most important diseases of durum wheat (*Triticum turgidum ssp. durum*) in several countries. For example, the *P. triticina* race BBG/BN, first detected in Mexico in 2001, is virulent to the resistance gene(s) present in many commercial durum wheat cultivars from Mexico and other countries, and has caused significant economic losses in northwestern Mexico (Singh et al., 2004).

*P. triticina* races virulent on durum wheat are different from those affecting common wheat (Huerta-Espino and Roelfs, 1989; Singh, 1991; Ordonez et al., 2004; Goyeau et al., 2006), and the genes that confer resistance against these races are therefore expected to be different. Ordonez et al. (2004) found that durum leaf rust races in Mexico, the U.S. (California), France, Spain and Argentina, were similar in their avirulence/virulence pattern based on the common wheat (*T. aestivum*) differential sets for leaf rust used in North America (Long and Kolmer, 1989; Singh 1991). The similarity of the predominant Mexican durum wheat leaf rust race to those present in Spain (Martinez et al., 2005) and France (Goyeau et al., 2006) has also been confirmed. Durum wheat germplasm with resistance to race BBG/BN would therefore be useful in a wide range of production areas.

Despite the increasing problem of susceptibility of durum wheat to leaf rust, little is known on the occurrence or nature of resistance genes in durum wheat. Of the over 50 known designated leaf rust resistance genes, only *Lr14a* and *Lr23* originated from durum or emmer wheat and were characterized after their transference into common wheat (McIntosh et al., 1995). Marais et al. (2005) recently transferred another leaf rust resistance gene, designated as *Lr53*, from wild emmer (*T. turgidum ssp. dicoccoides*) to common wheat. The gene was located on chromosome 6BS using cytogenetic tools and then mapped using a chromosome 6S restriction fragment length polymorphism (RFLP) marker Xpsr167 (Marais et al., 2005). In contrast to hexaploid wheat, use of cytogenetic tools to map leaf rust resistance genes in durum wheat remained limited to two studies where either the Langdon durum D-genome disomic substitution lines (Hussein et al., 2005) or trisomics (Bhagwat et al., 2004) were used.

In the last decade molecular mapping has emerged as a powerful technique to find the chromosome location of genes that determine simply or quantitatively inherited traits (Gupta et al., 1999). Several leaf rust resistance genes in common wheat were mapped using these techniques (Friebe et al., 1992; Nelson et al., 1997; Brown-Guedira et al., 2003; William et al., 2003). Zhang et al. (2005) transferred into tetraploid wheat and molecularly characterized *Lr19* originating from *Lophopyrum ponticum* (*Agropyron elongatum*). This gene is effective against leaf rust races of durum wheat present in the U.S. (California) and in Mexico. Molecular tools can also aid in pyramiding effective leaf rust resistance genes present in durum wheat to prolong their life-span.

Herrera-Foessel et al. (2005) studied the genetic basis of resistance in the durum wheat lines ‘Camayo’ and ‘Storlom’ which are resistant to the Mexican durum
wheat *P. triticina* race BBG/BN. Under high leaf rust pressure in Mexico, Storlom was immune (0%) in field trials, while Camayo displayed up to 15% leaf rust severity according to the modified Cobb scale (Peterson et al., 1948) with a moderately resistant to resistant host response to infection. The populations used by Herrera-Foessel et al. (2005) were generated by crossing Storlom and Camayo with the susceptible cultivar ‘Atil C2000’ as well as by intercrossing the two resistant parents. Genetic studies conducted in the F₁, F₂ and F₃ generations indicated that both resistant parents carried a partially dominant leaf rust resistance gene and the allelic test in the F₂ generation indicated that the two resistance genes were linked in repulsion, because no completely susceptible plant was found. These two genes provide protection against *P. triticina* race BBG/BN predominant in northwestern Mexico as well as in other countries where durum wheat leaf rust races are similar.

The objective of our study was to determine the chromosomal location of the two leaf rust resistance genes identified by Herrera-Foessel et al. (2005) in the durum wheat lines Camayo and Storlom using molecular markers.

**Materials and methods**

*Plant materials*

The F₁ lines developed and characterized for leaf rust resistance by Herrera-Foessel et al. (2005) from the crosses Atil C2000 × Camayo (96 families) and Atil C2000 × Storlom (92 families), were used for bulked segregant analysis. For further analysis of the genetic linkage between the leaf rust resistance genes present in the two parents, 197 F₃ lines from Camayo × Storlom cross were generated by individually harvesting disease-free F₂ plants.

*Lr3* is a known leaf rust resistance locus on the long arm of chromosome 6B with three reported alleles; *Lr3a*, *Lr3bg* and *Lr3ka* (Haggag and Dyck, 1973; McIntosh et al., 1995). The ‘Thatcher’ near-isogenic common wheat lines RL6002, RL6007 and RL6042, which are known to carry *Lr3a*, *Lr3ka* and *Lr3bg*, respectively (Roelfs et al., 1992), and Thatcher (control), were included in greenhouse rust evaluations and molecular characterization for detecting if any of these alleles could be present in either Camayo or Storlom.

*Greenhouse and field studies*

The 197 F₃ families from the Storlom × Camayo cross were evaluated for leaf rust response in both field and greenhouse with *P. triticina* race BBG/BN. Approximately 60 plants per family were sown in the field in 0.8 m long paired rows on 80 cm wide raised beds. An artificial rust epidemic was initiated by inoculating spreader rows of the susceptible cultivar Atil C2000 sown as hills in the middle of a 0.4 m path on one side of the plot. Leaf rust reactions of F₃ lines were recorded when Atil C2000 displayed about 100% leaf rust severity based on the modified Cobb Scale (Peterson et al., 1948). Each F₃ family was evaluated for rust reaction and grouped into one of the following categories: 1) homozygous resistant
for the Camayo response (immune), 2) homozygous resistant for the Storlom response (about 15% rust severity and moderately resistant host reaction), 3) segregating for the response of the two parents, and 4) recombinants. The only recombinants that we could possibly identify phenotypically were homozygous susceptible families or families that segregated for susceptible plants.

Approximately 25 seeds of each of Storlom × Camayo F$_3$ families were evaluated as seedlings in the greenhouse. Attil C2000, Camayo, Storlom and the F$_1$ hybrids from the crosses Attil C2000 × Storlom, Attil C2000 × Camayo and Storlom × Camayo were also included. Ten days old seedlings at the 2-leaf stage were inoculated by spraying urediniospores of _P. triticina_ race BBG/BN suspended in non-phytotoxic mineral oil (Soltrol 170). Plants were then placed in a dew-chamber overnight before transferring them to the greenhouse at 18–25°C. Infection-type responses were recorded 10 days after inoculation using the 0-4 scale described in Roelfs et al. (1992). In this scale, infection-types ‘3’ and ‘4’ are categorized as a susceptible response and the remaining infection-types are considered as resistant.

Camayo, Storlom, near-isogenic Thatcher lines carrying _Lr3a_, _Lr3ka_ and _Lr3bg_, and the susceptible durum and common wheat checks, Attil C2000 and Thatcher, respectively, were compared for their seedling and adult plant rust reactions under three temperatures in the greenhouse. Seedlings (2-leaf stage) and adult plants (flag leaf stage) were inoculated with _P. triticina_ race BBG/BN following the same procedure as described earlier. After overnight incubation in a dew chamber at 18-20°C, plants were transferred to three greenhouses maintained at low, intermediate and high temperature regimes, respectively (Table 1). Approximately 20 seedlings and 8 adult plants per genotype were evaluated at each temperature. Infection-type responses according to Roelfs et al. (1992) were recorded for the second leaves (seedling stage) and flag leaves (adult plants) 11 and 13 days, respectively after inoculations.

_DNA isolation, bulked segregant analysis and AFLP assay_
Twenty seeds from each F$_3$ family of the entire Attil C2000 × Camayo (96 families), Attil C2000 × Storlom (92 families) and Camayo × Storlom (181 families where seed was available) crosses were planted in the greenhouse. Leaf tissues were harvested three weeks after planting, freeze-dried and used for DNA extraction according to a CTAB-based procedure (Hoisington et al., 1994).

For the bulked segregant analysis (Michelmore et al., 1991), DNA from each of 14 homozygous resistant and 14 homozygous susceptible F$_3$ families from Attil C2000 × Camayo and Attil C2000 × Storlom crosses were separately pooled to obtain one susceptible bulk and one resistant bulk for each population.

The Amplified fragment length polymorphism (AFLP) technique was used according to Vos et al. (1995) but with some modifications (Hoisington, et al., 1994; William et al., 2003). A pre-amplification step was carried out using _PstI/MseI_ restriction enzyme sites and a single selective nucleotide at the 3’-end of both the _PstI_ and _MseI_ primers. The sequence (5’- 3’) of the primers used for the pre-amplification step were (_MseI_) GAT GAG TCC TGA GTA AC/-G and (_PstI_)
GAC TGC GTA GGT GCA GA/-T. Ninety-six different AFLP primer enzyme combinations were applied to the resistant and susceptible bulks and their respective parents (Atit C2000, Camayo and Atit C2000, Storlom).

The AFLP bands were designated according to the nomenclature in KeyGene, http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html, e.g. band \textit{P33/M48}\textsubscript{352} indicates \textit{PstI} and \textit{MseI} primers 33 and 48, respectively and subscript is the estimated size in base pairs. The first set of 48 primer combinations involved four \textit{PstI}+\textit{NNN} primers, sequence is in brackets, (P33\textsubscript{[AAG]}, P36 \textsubscript{[ACC]}, P37\textsubscript{[ACG]}, P41\textsubscript{[AGG]}) with 12 \textit{MseI}+\textit{NNN} primers (M47\textsubscript{[CAA]}, M48\textsubscript{[CAC]}, M49\textsubscript{[CAG]}, M50\textsubscript{[CAT]}, M55\textsubscript{[CGA]}, M56\textsubscript{[CGC]}, M57\textsubscript{[CGG]}, M58\textsubscript{[CGT]}, M59\textsubscript{[CTA]}, M60\textsubscript{[CTC]}, M61\textsubscript{[CTG]}, M62\textsubscript{[CTT]}) and the second set of 48 primer combinations consisted of four different \textit{PstI}+\textit{NNN} primers (P81\textsubscript{[TAG]}, P87\textsubscript{[TGA]}, P88\textsubscript{[TGC]}, P89\textsubscript{[TGG]}) with 12 additional \textit{PstI}+\textit{NNN} primers (M63\textsubscript{[GAA]}, M64\textsubscript{[GAC]}, M65\textsubscript{[GAG]}, M66\textsubscript{[GAT]}, M67\textsubscript{[GCC]}, M68\textsubscript{[GCG]}, M69\textsubscript{[GCT]}, M70\textsubscript{[GTC]}, M75\textsubscript{[GTA]}, M76\textsubscript{[GTC]}, M77\textsubscript{[GTG]}, M78\textsubscript{[GTT]}).

One of the three selective bases for the \textit{PstI} restriction enzyme was end-labeled with digoxigenin (Operon Technologies, Alameda, CA, USA). Bands were detected using a chemiluminescent system, which included the transference of the amplification product to a nylon membrane, washing the membrane with anti-dig/alkaline phosphatase and CSPD, and developing X-ray films (Hoisington et al., 1994). The size of the marker was estimated by comparing the position of the AFLP bands with a dig-labeled size marker that was run in the same gel. When polymorphisms were identified between the bulks and the parents, the individual families that made the bulks (14 homozygous resistant and 14 homozygous susceptible) were screened with the same AFLP primer combinations. If linkage was found between the AFLP marker and the resistance gene, the entire \textit{F}\textsubscript{2} population from each respective cross, including the segregating families that originated from heterozygous \textit{F}\textsubscript{2} plants, was used for establishing the linkage between the AFLP marker and the resistance gene.

\textit{Partial linkage analysis}

The AFLP primers that produced polymorphic bands and that were linked to resistance genes either in Camayo or Storlom were screened for polymorphisms in six sets of common wheat (\textit{T. aestivum}) parents; Opata M85, Synthetic W-7984 (Altar C84/Aegilops squarrosa) (Roeder et al., 1998), Oligoculm, Fukuho-komugi (Suenaga et al., 2004), Frontana and Inia 66 (unpublished CIMMYT data). These common wheat parents have been used in developing either recombinant inbred lines or double haploids for the genotyping and construction of linkage maps with RFLP, RAPD and SSR markers. The scoring data for the genetic map of the population Opata M85 × Synthetic W-7984 (also referred to as the international Triticeae mapping initiative, ITMI, population) is publicly available in GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml).

Whenever the identified AFLP bands were polymorphic in any of the common wheat parents, segregation of the same band was investigated in available progenies.
of the respective common wheat mapping population. The number of lines evaluated varied from 96 to 114 depending on the population. The segregation pattern of the AFLP band in the common wheat populations was used for the linkage analysis in MAPMAKER Version 3.0 at minimum 3.0 log of odds (Lander et al., 1987).

**SSR and STS markers**

Thirty-three SSR markers, chosen for even distribution on chromosome 6B according to Somers et al. (2004) and sequence-tagged site (STS) primer Xmwg798 (Kunzel et al., 2000), were also used for screening Camayo and Storlom and their respective bulks. The PCR reactions were performed according to Hoisington et al. (1994). The PCR program used depended on the optimal annealing temperature for each primer. The PCR products were separated on 3% agarose gels with 2/3 metaPhor agarose® and 8% acrylamide (29:1) gels, and detected with ethidium bromide for agarose gels and silver staining for acrylamide gels.

The *Lr3* locus is positioned on the long arm of chromosome 6B (McIntosh et al., 1995). Sacco et al. (1998) identified an RFLP marker *Xmwg798* that co-segregated with the allele *Lr3a* in the common wheat cultivar Sinvalochro MA. This RFLP marker has been converted to a PCR-based STS marker (Kunzel et al., 2000). The sequence of *Xmwg798* is available at GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml). We tested the STS marker *Xmwg798* in Atil C2000, Camayo, Storlom, and in the F₃ populations of Atil C2000 × Storlom and Camayo × Storlom crosses.

**Statistical analysis**

The chi-squared analysis ($\chi^2$) was conducted for testing the hypothesis that the observed segregation ratio of markers in the populations did not deviate significantly from expected ratio at the 5% probability level. The same test was also used for comparing the phenotypic responses of F₃ families in the Camayo × Storlom cross (Table 2).

**Results**

**Chromosomal location of resistance genes in Camayo and Storlom**

Of the 96 different AFLP primer combinations used on both Camayo and Storlom mapping populations, only three bands—*P33/M48, P81/M66, P87/M70*—were associated with the resistance gene in Camayo but a marker associated to the resistance gene in Storlom could not be found. Only one of these polymorphic markers—*P87/M70*—could be mapped to chromosome 6BL in the Opata M85/Synthetic W-7984 (ITMI) reference mapping population (Figure 1). A total of 114 recombinant inbred lines from the ITMI population were used for the linkage analysis. The segregation status of the marker conformed to a 1:1 ratio in the $\chi^2$-test as expected in a recombinant inbred population.
Three AFLP markers, \( P33/M48_{352} \), \( P81/M66_{165} \), and \( P87/M70_{128} \), were associated with the resistance gene in Atil C2000 × Camayo population with a distance of 2.1, 3.2 and 5.5 cM, respectively (Figure 2). The resistance gene was located between the markers \( P33/M48_{352} \) and \( P81/M66_{165} \). Segregation of the three AFLP bands associated with the resistance gene showed a good fit to a 3:1 ratio expected for a dominant marker in the F\(_3\) generation.

Atil C2000, Camayo and Storlom, and the two F\(_1\) populations derived from Atil C2000 × Camayo and Atil C2000 × Storlom crosses were also tested with SSR markers located on chromosome 6B. Seven SSR markers (\( Xwms107 \), \( Xwms613 \), \( Xwmc105 \), \( Xwmc179 \), \( Xwmc419 \), \( Xbarc24 \), \( Xbarc127 \)) were polymorphic either in Atil C2000 and Camayo, or Atil C2000 and Storlom, or both, but none of the markers was successful in establishing linkage with the resistance gene.

**Identification of the resistance gene in Storlom**

The STS version of the marker \( Xmwg798 \), reported to co-segregate with \( Lr3a \) in the common wheat cultivar Sinvalcho MA (Sacco et al., 1998), was present in Storlom but absent in Camayo and the susceptible parent Atil C2000. Co-segregation of dominant marker \( Xmwg798 \) with resistance in the Atil C2000 × Storlom population of 92 F\(_3\) families indicated that the resistance gene present in Storlom is \( Lr3a \). The segregation of \( Xmwg798 \) in the F\(_3\) population conformed to the expected 3:1 ratio.

**Linkage of resistance genes in Camayo and Storlom**

In the field evaluation of the F\(_3\) population from the Camayo × Storlom cross, the families either displayed resistance responses similar to one of the parents or a combination of both (data not shown). No homozygous susceptible family or a family segregating for susceptible plants was found. The absence of susceptible plants (recombinants) among more than 5,000 plants evaluated in the 115 F\(_3\) families, derived from heterozygous F\(_2\) plants, indicates that the resistance genes in the two parents is most likely allelic or very closely linked.

The leaf rust infection-type responses of F\(_3\) families in the greenhouse were either homozygous ‘0;’ and ‘1;’ or segregating for ‘0;’ and ‘1;’ (Table 2). A few families had plants with higher responses than either of the parents (‘X+’), but no completely susceptible recombinant plant was observed. In the same greenhouse test the infection-type responses of Camayo and Storlom were ‘1;’ and ‘0;’, respectively. The responses of F\(_1\) plants from the Atil C2000 × Storlom and Atil C2000 × Camayo crosses were ‘1;’ and ‘X’, respectively, whereas the F\(_1\) plants of the Camayo × Storlom cross displayed infection-type ‘0;’.

Screening for the three AFLP markers, \( P33/M48_{352} \), \( P81/M66_{165} \), and \( P87/M70_{128} \), and the STS marker \( Xmwg798 \) was done on the 181 F\(_3\) families of the Camayo × Storlom population to establish the linkage between the genes present in the two parents. The AFLP markers acted in repulsion with respect to the STS marker. The \( Xmwg798 \) marker showed co-segregation with the resistance in
Storlom and was always absent in the families displaying responses of Camayo (Figure 3). The genetic distance between the AFLP markers linked to the resistance gene in Camayo and the resistance gene in Storlom was 1.2, 5.0 and 8.1 cM for \( P33/M48_{352} \), \( P87/M70_{128} \) and \( P81/M66_{165} \), respectively. All the markers showed a good fit to the expected 3:1 ratio based on the \( \chi^2 \)-test in the entire F\(_3\) population of the Camayo × Storlom cross.

**Molecular characterization of Lr3 alleles**

The STS marker \( Xmwg798 \) was present in the near-isogenic lines carrying \( Lr3a \), \( Lr3ka \) and \( Lr3bg \), and was absent in the Thatcher control (Figure 4). The three \( Lr3 \) near-isogenic lines and the control were also evaluated with the three AFLP markers linked to the gene in Camayo. Markers \( P33/M48_{352} \) and \( P81/M66_{165} \) were present in the three near-isogenic lines, as well as Thatcher, whereas \( P87/M70_{128} \) was absent in all of them.

**Infection-type responses of near-isogenic Thatcher lines with Lr3 alleles and durum wheat lines**

Storlom displayed infection-type ‘;’ in seedlings and ‘0’ in adult plants (Table 1). These were slightly lower infection-types than the testers for \( Lr3a \) and \( Lr3bg \) that ranged between ‘;’ and ‘;1;’ in seedlings and ‘0’ and ‘;’ in adult plants. Camayo had higher infection-type responses than the three \( Lr3 \) testers, particularly in adult plants and at intermediate and high temperatures, indicating that the resistance gene in Camayo could be different from any of the three \( Lr3 \) alleles. Moreover, the resistance in Camayo appeared to be better expressed at lower temperatures. This was not observed for any of the \( Lr3 \)-isogenic lines. The susceptible durum wheat, Atil C2000, and common wheat, Thatcher, both displayed the highly susceptible infection-type ‘4’.

**Discussion**

Using partial linkage mapping and bulked segregant analysis, we have established the genomic position to chromosome arm 6BL of two very closely linked partially dominant leaf rust resistance genes in the durum wheat genotypes Camayo and Storlom. The gene in Storlom was located at the \( Lr3 \) locus by using the marker \( Xmwg798 \) that was previously reported to co-segregate with \( Lr3a \) in the common wheat cultivar Sinvalocho MA (Sacco et al., 1998). Sacco et al. (1998) identified the RFLP marker \( Xmwg798 \) whereas our study used the same marker, converted to a PCR-based STS marker (Kunzel et al., 2000). No recombination was observed between the STS marker and the resistance in Storlom, indicating that the marker was reliable for the assay of the resistance gene.

Three AFLP markers were identified to be linked with leaf rust resistance genes in Camayo, with the nearest marker located at a distance of 2.1 cM to \( P33/M48_{352} \) (Figure 2). The linkage between \( Lr3a \) in Storlom and the gene in Camayo was studied in the Camayo × Storlom F\(_3\) population in the greenhouse and field, and by screening with the three AFLP markers and the STS marker in the Camayo ×
Storlom F$_3$ population. The absence of susceptible families, or families segregating with susceptible plants, in the field and greenhouse screenings, indicated the lack of recombination between the resistance genes identified in Camayo and Storlom. The three AFLP markers showed close association with the STS marker Xmwg798 with recombination distances ranging between 1.2 and 8.1 cM (Figure 3). This indicates that the gene in Camayo is either a different allele at the Lr3 locus or, more probably, very closely linked.

Herrera-Foessel et al. (2005) estimated a recombination frequency of 20% between the leaf rust resistance genes identified in Camayo and Storlom. This estimate was based on leaf rust evaluations of the F$_2$ population of the Camayo × Storlom cross. In the study presented here, further investigation using F$_2$-derived F$_3$ families and molecular markers led to a much closer distance between the two genes in Camayo and Storlom. The gene/allele in Camayo seems to be temperature-sensitive and the background genotype could also have an important influence. These factors could explain why some F$_2$ plants had higher responses than the F$_1$ plants from the same cross (Herrera-Foessel et al., 2005) and why few plants in the F$_3$ in our study had higher infection-type responses (up to ‘X+’ according to the 0-4 infection-type scale).

In our study, all tested SSR markers located in chromosome 6B according to Somers et al. (2004) failed in establishing linkage with any of the two genes in Storlom or Camayo. The Lr3 locus is positioned in an area with low density of available SSR markers. None of the SSR markers tested was positioned within the linkage group that was generated in the ITMI genetic map when analyzing the AFLP marker P87/M70$_{128}$ (Figure 1). Similar results were found by Khan et al. (2005) who failed to show any association to Lr3a present in the common wheat cultivar ‘Yarralinka’ when testing eight SSR markers specific to chromosome 6B, but instead identified an inter-simple sequence repeat (ISSR) marker, UBC840$_{540}$, at a distance of 6.0 cM.

Although Singh et al. (1992) postulated the presence of Lr3a together with an unknown gene in one of the fifty durum wheat lines maintained at Punjab Agricultural University, India, our study represents the first molecular confirmation of this gene in durum wheat. Storlom and Camayo are resistant to all Mexican races of P. triticina (data not presented) and they most likely carry the unidentified resistance gene present in Altar C84 and Atil C2000 that became ineffective with race BBG/BN (Singh et al., 2004). We therefore could not determine the race-specificity of the resistance genes present in Storlom and Camayo with other Lr3a, Lr3ka and Lr3bg avirulent or virulent races.

The RFLP marker Xmwg798 was previously mapped in a durum wheat population. Du and Hart (1998) used the T. turgidum ssp. durum cultivar Langdon - T. turgidum ssp. dicoccoides chromosome 6A and 6B recombinant substitution lines, and a F$_2$ population derived from a Langdon - T. turgidum ssp. dicoccoides disomic chromosome 6A substitution line × Langdon cross to construct extended 6A and 6B linkage maps. The RFLP marker Xmwg798 was mapped in the long arm of both chromosomes. Even if this marker has been mapped in two different
chromosomes, in the durum wheat populations used in our study (F$_1$ Atil C2000 × Storlom, F$_1$ Camayo × Storlom) and in the three Lr3 near-isogenic common wheat lines, the STS marker Xmwg798 was useful in detecting the Lr3 locus present only on chromosome 6BL.

The three known alleles at the Lr3 locus are differentiated by their different responses to a collection of common wheat P. triticina races. Lr3a, commonly referred as Lr3, is known to be present in Democrat, Mediterranean, as well as in other common wheat cultivars such as Sinvalocho MA (Haggag and Dyck, 1973; McIntosh et al., 1995). Allele Lr3bg was originally identified in the common wheat cultivar Bage, and Lr3ka in the common wheat Klein Aniversario (Haggag and Dyck, 1973; McIntosh et al., 1995). We determined the presence of the STS marker Xmwg798 in the three near-isogenic common wheat lines—Lr3a, Lr3bg and Lr3ka—and its absence in the Thatcher control, indicating that this marker is locus-specific rather than allele-specific. The three known Lr3 alleles therefore cannot be differentiated using Xmwg798. Based on our results it cannot therefore be excluded that the gene in Storlom is Lr3bg rather than Lr3a. The presence of Lr3ka in Storlom is doubtful because the seedling and adult plant infection-type response of the near-isogenic common wheat line carrying Lr3ka is very different from the response of Storlom (Table 2).

The Lr3 allele present in Storlom could have been transferred from hexaploid wheat, as Storlom is a backcross derivative from a cross between the common wheat cultivar Sitella and the durum wheat line Musk. Musk is a cross between Altar C84 (susceptible) and the common wheat Alondra.

The resistance allele in Camayo is most likely derived from an Ethiopian landrace which is one of the parents. The second parent, Altar C84, is susceptible to the Puccinia triticina race BBG/BN (Herrera-Foessel et al. 2005; Singh et al., 2004). With race BBG/BN, Camayo had higher seedling and adult plant infection-type responses than those displayed by the near-isogenic common wheat lines carrying any of the three known alleles at Lr3 locus (Table 2). The resistance gene in Camayo was better expressed at lower temperatures, which was not the case for any of the three Lr3 alleles in our studies. In other studies (Dyck and Johnson, 1983), the resistance of the three known Lr3 alleles has been found to be less expressed at low temperatures when P. triticina isolates from common wheat that give intermediate infection-type responses, were used. Camayo did not have the Lr3 locus-specific STS marker Xmwg798, which was present in Storlom and in Thatcher near-isogenic tester lines for the three known Lr3 alleles. We therefore suggest that the resistance in Camayo must be conferred by a different gene located near the Lr3 locus rather than by another allele at the same locus.

Only a few of the more than 50 leaf rust resistance genes that have been designated in common wheat are known to have originated from durum wheat, and not many leaf rust resistance genes in durum wheat have been identified. The potential value of deploying Lr3a/Lr3bg in common wheat is considered low, since virulence in common wheat leaf rust races to these alleles is frequent (McIntosh et al., 1995). The deployment of Lr3a/Lr3bg in common wheat resulted in a rapid
buildup of virulence in common wheat specific races. The durum wheat leaf rust race, BBG/BN, is however avirulent to most of the known designated leaf rust resistance genes previously identified in common wheat, including the three Lr3 alleles. The potential value of many of these known common wheat genes could therefore be considered high when used in durum wheat in Mexico and in other countries with similar durum wheat leaf rust populations. In order to enhance the longevity of these genes in durum wheat they should be deployed in combination with other effective leaf rust resistance genes.

Acknowledgements

We are grateful for financial support from the Swedish Agency for Research Cooperation with Developing Countries (SIDA-SAREC) and CIMMYT and we acknowledge Virginia Garcia for technical support in the molecular laboratory. We also thank Daisy Ouyta for editing the manuscript.

References


Table 1. Infection-type responses in seedlings (2-leaf stage) and adult plants (flag leaf stage) for two resistant durum wheat lines, three Lr3 alleles carrying near-isogenic ‘Thatcher’ lines and the susceptible checks when inoculated with P. triticina race BBG/BN and incubated at three temperature regimes.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Low temp. ‡</th>
<th>Intermediate temp. §</th>
<th>High temp. ¶</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seedling</td>
<td>Adult</td>
<td>Seedling</td>
</tr>
<tr>
<td>Attil C2000</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Camayo</td>
<td>1-</td>
<td>1</td>
<td>23C X-</td>
</tr>
<tr>
<td>Storlom</td>
<td>;</td>
<td>;</td>
<td>0</td>
</tr>
<tr>
<td>Thatcher</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>RL6002 (Lr3a)</td>
<td>1-</td>
<td>0;</td>
<td>;</td>
</tr>
<tr>
<td>RL6007 (Lr3ka)</td>
<td>1-</td>
<td>1=</td>
<td>1-</td>
</tr>
<tr>
<td>RL6042 (Lr3bg)</td>
<td>1-</td>
<td>;</td>
<td>;</td>
</tr>
</tbody>
</table>

† Infection-types are based on a 0-to-4 scale (Roelfs et al., 1992), where '0' = no uredinia or other macroscopic signs of infection; ';' = no uredinia, but hypersensitive necrotic or chlorotic flecks of varying sizes present; '1' = small uredinia surrounded by necrosis; '2' = small to medium uredinia surrounded by green islands; 'X' = random distribution of variable-sized uredinia on single leaf with a pure culture; '3' and '4' = medium and large uredinia, respectively, without chlorosis or necrosis; '+' and ‘−’ or ‘=’ = uredinia somewhat larger and smaller, respectively, than normal for infection-type; ‘C’ = more chlorosis than normal for the infection-type. More than one designation represents a range of infection-types.

‡ 14-19°C and 15-20°C for seedling and adult plant tests, respectively.
§ 15-24°C and 16-24°C for seedling and adult plant tests, respectively.
¶ 18-36°C and 18-40°C for seedling and adult plant tests, respectively.
Table 2. Greenhouse infection-type responses of F₃ families from the Camayo × Storlom cross to P. triticina race BBG/BN and P-value of χ² test to verify the 1:2:1 segregation ratio.

<table>
<thead>
<tr>
<th>Infection-type response†</th>
<th>Number of F₃ families</th>
<th>Observed</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>;1 (Camayo phenotype)</td>
<td>37</td>
<td>43.75</td>
<td></td>
</tr>
<tr>
<td>0; (Storlom phenotype)</td>
<td>39</td>
<td>43.75</td>
<td></td>
</tr>
<tr>
<td>0; to ;1 (few X)</td>
<td>99</td>
<td>87.5</td>
<td></td>
</tr>
<tr>
<td>Total families</td>
<td>175</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value of χ² test</td>
<td></td>
<td>0.22</td>
<td></td>
</tr>
</tbody>
</table>

† Infection-type responses follow a 0-4 Scale (Roelfs et al., 1992) where 0; = no uredinia, but some hypersensitive necrotic or chlorotic flecks present. ;1 = hypersensitive necrotic or chlorotic flecks with small uredinia. X = random distribution of variable-sized uredinia on single leaf with a pure culture.
Figure 1. Linkage group of AFLP marker P87/M70\textsubscript{128} associated with the leaf rust resistance gene in durum wheat Camayo according to the ITMI genetic map (distances are displayed in cM).
Figure 2. Distance in cM between three AFLP markers and the leaf rust resistance gene (Rgene) using the entire population of 96 F3 families from the Atil C2000 × Camayo cross.
Figure 3. Distance in cM between three AFLP markers linked to the leaf rust resistance gene in Camayo (RgeneStorlom), and the STS marker Xmwg798 linked to the gene in Storlom (RgeneCamayo), using the molecular and phenotypic responses of 197 F$_3$ families from the Camayo × Storlom cross. The two leaf rust resistance genes, in Camayo and in Storlom, respectively, are linked in repulsion.
Figure 4. Characterization of the STS marker Xmwg798 in Thatcher control, ThatcherLr3a, ThatcherLr3ka, ThatcherLr3bg, Atil C2000, Camayo and Storlom.