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Development of a molecular marker for the adult plant leaf rust resistance gene *Lr35* in wheat

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Abstract The objective of this work was to develop a marker for the adult plant leaf rust resistance gene *Lr35*. The *Lr35* gene was originally introgressed into chromosome 2B from *Triticum speltoides*, a diploid relative of wheat. A segregating population of 96 F_2 plants derived from a cross between the resistant line Thatcher*Lr35* and the susceptible variety Frisal was analysed. Out of 80 RFLP probes previously mapped on wheat chromosome 2B, 51 detected a polymorphism between the parents of the cross. Three of them were completely linked with the resistance gene *Lr35*. The co-segregating probe BCD260 was converted into a PCR-based sequence-tagged-site (STS) marker. A set of 48 different breeding lines derived from several European breeding programs was tested with the STS marker. None of these lines has a donor for *Lr35* in its pedigree and all of them reacted negatively with the STS marker. As no leaf rust races virulent on *Lr35* have been found in different areas of the world, the STS marker for the *Lr35* resistance gene is of great value to support the introgression of this gene in combination with other leaf rust (*Lr*) genes into breeding material by marker-assisted selection.

Key words Leaf rust · Adult plant resistance · Sequence-tagged-site · *Triticum speltoides* · Wheat

Introduction

Leaf rust caused by *Puccinia recondita* f. sp. *tritici* is considered to be one of the most important fungal diseases of wheat. To-date, more than 40 leaf rust resistance genes have been characterized (Knott 1989; McIntosh et al. 1995). Most of them are effective from the seedling stage through the whole life of the plant, whereas a few of them are only effective at the adult stage. Resistance during this latter period is called adult plant resistance and was defined by Zadoks (1961) as a resistance that is only effective in the advanced plant growth stage but not at the seedling stage. An attack of the pathogen during heading (the adult stage) can cause severe grain yield losses due to reduced floret set (Roelfs et al. 1992). Therefore, resistance at the adult stage is of considerable economic significance in wheat breeding. Until now, seven genes which are only effective in the adult stage have been described (McIntosh et al. 1995). Although they are inherited in a monogenic fashion the type of resistance differs between these adult resistance genes. The resistance gene *Lr34* expresses resistance in a quantitative way (Drijepondt and Pretorius 1989; German and Kolmer 1992; Singh 1992). It causes an increased latency period and a decreased infection frequency and uredium size (Drijepondt and Pretorius 1989). In contrast, the resistance gene *Lr13* induces a hypersensitive reaction upon infection with an avirulent leaf rust race (McIntosh et al. 1995). So far, *Lr34* has been used in many breeding programs throughout the world, as it confers durable resistance in combination with other genes (German and Kolmer 1992). *Lr13* is probably one of the most widely distributed resistance genes worldwide (McIntosh et al. 1995) but it shows enhanced effectiveness only in combination with other resistance genes (Kolmer 1992). The combination of the adult plant resistance genes *Lr13* and *Lr34* appears to be the basis of most of the durable leaf rust resistance (Roelfs 1988). This example shows that a combination of adult plant resistance genes is an important factor for successful resistance breeding. *Lr35* also confers a hypersensitive reaction upon infection by an avirulent race (Kolmer 1997). In contrast to the resistance genes *Lr13* and *Lr34* which were derived from common wheat, the resistance gene *Lr35* was transferred by Kerber and

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Dyck (1990) from chromosome 2S of the diploid wild relative *Triticum speltoides* to chromosome 2B of hexaploid wheat. To our knowledge no virulent leaf rust races for *Lr35* have been found until know (Kerber and Dyck 1990; Kloppers and Pretorius 1995; Kolmer 1997; R.F. Park, personal communication; own data). The *Lr35* gene has not yet been used in modern varieties (McIntosh et al. 1995).

To maintain a wide spectrum of resistance against pathogens in cultivated wheat, the introgression of resistance genes derived from hexaploid wheat and its wild relatives is very valuable. The efficiency of introgression of alien genes to hexaploid wheat can be improved by the use of molecular markers (Asiedu et al. 1989). Molecular markers for translocated resistance genes have already been developed (Schachermayr et al. 1994, 1995; Autrique et al. 1995; Procunier et al. 1995; Dedryver et al. 1996; Naik et al. 1998). Morphological markers for *Lr13* and *Lr34* have also been described. A gene for hybrid necrosis (*Ne2^m*) was found to be linked to *Lr13* (Singh and Gupta 1991). However, *Ne2^m* can not be used for accurate detection of *Lr13* (Anand et al. 1991) in wheat breeding programs. A strong genetic association or pleiotropism with leaf tip necrosis was described as a morphological marker for *Lr34* (Singh 1992). To our knowledge no useful molecular markers for adult plant leaf rust resistance genes have been reported so far.

In the present study, we developed molecular markers based on RFLP and PCR technologies for the adult plant leaf rust resistance gene *Lr35*. These markers are completely linked with the *Lr35* resistance gene and will be a valuable tool to combine *Lr35* with other effective resistance genes and thus improve the durability of leaf rust resistance.

Materials and methods

Plant material

The resistant line R.L.6082 [Tc*6/R.L.5711, a near-isogenic line (NIL) of the spring wheat line Thatcher with the *Lr35* gene, developed by Dr. P. Dyck, Winnipeg, Canada] and the susceptible spring wheat variety Frisal (FAL-Reckenholz, Zürich, Switzerland) were crossed. 137 plants of the resulting F_2 progeny were tested for resistance under controlled conditions in a growth chamber. The linkage analysis was performed with a subset of 96 F_3 families also evaluated for resistance in the field. Forty eight European wheat and spelt breeding lines (described by Siedler et al. 1994) that do not contain the *Lr35* resistance gene were used to validate the specificity of the molecular marker for *Lr35*.

Artificial infection of adult plants under controlled conditions

Seeds were germinated on wet Whatman paper 3MM to ensure regular germination. Rooting seedlings were transferred into plastic tubes filled with sand. The plants were grown in a growth chamber under a strict hygienic regime to avoid contamination with other wheat pathogens. In the first week of growth, the light intensity was set to 150 µmol/m^2·s. Light intensity was then increased continuously in the following 2 weeks up to 450 µmol/m^2·s. The photoperiod was 16 h, with a day/night temperature of 19°C/15°C. Humidity was set permanently at 50%. 80 ml of a nutrient solution according to Hoagland (Jones 1982) were given twice daily.

F_2 individuals were artificially infected at the growth stage DC 51–55 (Zadoks et al. 1974) with leaf rust isolate 95502 avirulent for *Lr35* (kindly provided by Dr. R. Park, University of Sydney, Australia). Urediospores were sprayed as a suspension with mineral oil "Soltrol 170" (Philips Petroleum, Paris) on the entire plant. After inoculation, plants were kept at 16°C and 100% humidity for 24 h in the dark. Ten days after inoculation (growth conditions: photoperiod 16 h, 19°C/15°C day/night temperature, 90%, relative humidity) plants were scored for the infection type on the flag leaves. A scale ranging from 0 to 4 (Roelfs 1984) was used to describe the infection type (IT): ITs 0 (immune); (fleck), 1 (small uredinia with necrosis), and 2 (small uredinia with chlorosis) were considered as resistant, while ITs 3 (medium-size uredinia with or without chlorosis) and 4 (large uredinia without chlorosis) were considered as susceptible.

Scoring for leaf rust resistance in a field trial

A field trial was carried out in a field rust nursery (Haag, Switzerland) in 1998. Eleven seeds of each F_3 family chosen for linkage analysis were planted in 1.5-m rows. The field rust nursery was artificially inoculated with a mixture of 16 *P. recondita* f.sp. *tritici* races prevalent in Switzerland. The parents Th*Lr35* and Frisal were also evaluated for resistance in the rust nursery. Host response data were recorded three times on the flag leaf. The F_3 families were classified as homozygous-resistant, homozygous-susceptible or segregating (both resistant and susceptible individuals in a family) relative to Th*Lr35* and Frisal.

DNA isolation and RFLP analysis

For DNA isolation, plant material from F_3 families consisting of 11 plants was harvested and pooled. DNA isolation and Southern blotting were performed as described by Graner et al. (1990). Genomic DNA of Frisal, Th*Lr35* and Thatcher was digested with five restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I).

Southern hybridization was performed with 80 RFLP probes previously mapped on chromosome 2B. The cDNA and genomic DNA probes used were derived from wheat (FBA, FBB, PSR, TAM and WG), *Triticum tauschii* (KSU), barley (BCD) and oat (CDO). The probes CDO370 and WG996 were described by Heun et al. (1991). The probes BCD260, BCD1119, CDO405 are described in the Graingene data base (http://wheat.pw.usda.gov). The probe MWG950 was described by Graner et al. (1991). The probe PSR540 was mapped by Devos et al. (1993). Dr. P. Leroy (INRA, Clermont-Ferrand and GIS Genoble Club, France) kindly provided the probes FBA199, FBA374, FBB4, FBB47 and FBB75. Probes KSUF11 and TAM18 were kindly provided by Dr. B.S. Gill (Kansas State University, USA) and Dr. G.E. Hart (Texas A&M University, USA), respectively.

Probe labelling with ^{32}P was performed with a labelling kit (Amersham, Switzerland) according to the manufacturer's instructions.

PCR-amplification of wheat genomic DNA with specific primers

The polymerase chain reaction (PCR) was performed in a 25-µl reaction volume. It contained 0.625 units of *Taq* DNA polymerase (Sigma, Switzerland), 1×PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.001% gelatin), 1 mM of each dNTP, 10 µM of primers and 50 ng of genomic DNA template. Amplification was performed in a PTC-200 thermocycler (MJ-Research, Bioconcept, Switzerland) as follows: after one cycle at 94°C for 3 min, the reaction was subjected to 30 cycles at 94°C for 45 s, 59°C for 45 s and 72°C for 1 min. The extension of the amplified product was achieved at 72°C for 5 min.

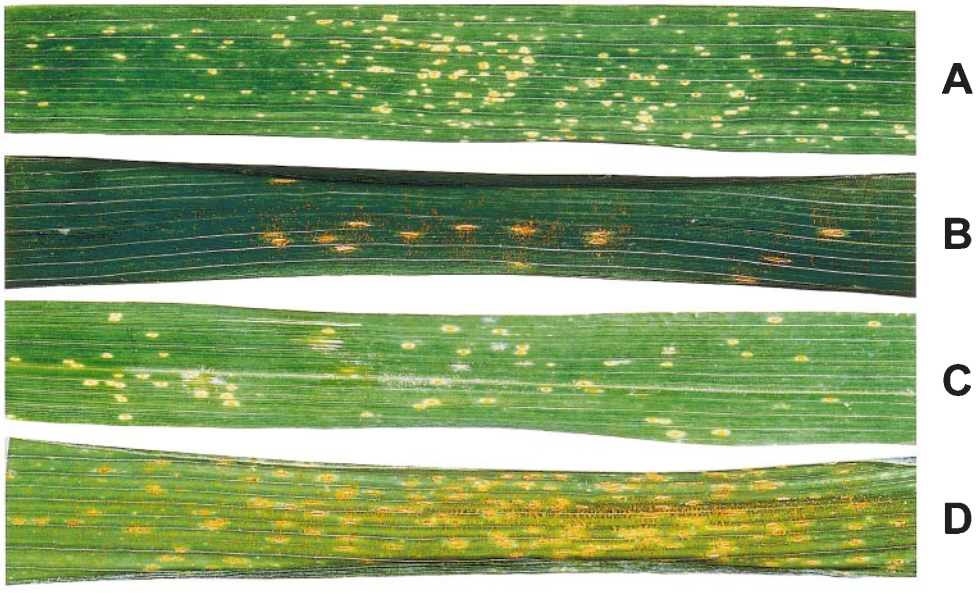

Fig. 1A–D Flag leaves of wheat grown in the growth chamber 10 days after artifical infection with the leaf rust isolate 95502. Resistant parent Th*Lr35* (**A**), susceptible parent Frisal (**B**), resistant F_2 individual (**C**) and susceptible F_2 individual (**D**)

Conversion of an RFLP probe to a STS marker

A 0.9-kb *Bam*HI/*Hin*dIII fragment of the probe BCD260 was subcloned. The resulting clone BCD260/0.9 was used as a RFLP probe in mapping analysis. This fragment was sequenced and the primers BCD260F1 (5′ GAA GTT AAA GAG GTC TTG AC 3′) and BCD260R2 (5′ GAA GTA GTC CGC TAC CAC AG 3′) were designed at each end of the cloned fragment (Microsynth, Switzerland). They were used for PCR-amplification of genomic DNA isolated from Th*Lr35*, Frisal and the F_2 population. A CAPS (Cleaved Amplified Polymorphic Sequence) marker was developed by digesting the amplified fragments with the restriction enzyme *Dde*I (4-bp recognition site, CTNAG). Ten units of restriction enzyme were added directly to the reaction after amplification. After 2 h of digestion at 37°C, the fragments were separated on a 2% agarose gel.

The PCR fragments amplified between BCD260F1 and BCD260R2 (1.5 kb) were subcloned into the "pGEM-T Easy" vector (Promega, Switzerland) and sequenced. The specific STS primer 35R2 (5′ TTT TGA GAA TCA GTC ATC AC 3′) was designed in the insertion found in the Th*Lr35* allele. Visualization of the PCR product amplified by the primer combination BCD260F1 and 35R2 was done on a 1% agarose gel stained with ethidium bromide.

Linkage analysis

Linkage estimation was based on the recombination frequency r (defined as the summed frequency of recombination types among the total progeny). The recombination frequency was transformed to map units [centi Morgans (cM)] without using the Kosambi function. The recombination frequency was so low that we estimated the probability of double crossing-over as zero and the recombination frequencies as additive.

Results

Segregation analysis of the F_2 population derived from the cross Th*Lr35*×Frisal

To map the *Lr35* resistance gene, 137 F_2 individuals derived from a cross between the line Th*Lr35* and the variety Frisal were scored for resistance. Evaluation of resistance was done after artificial infection at the adult stage under controlled conditions in a growth chamber. The infection type of the F_2 plants was either 0–1 (immune–small uredinia) like the resistant parent Th*Lr35* or 4 (large uredinia without chlorosis) like the susceptible parent Frisal (Fig. 1). Intermediate reaction types of heterozygous plants could not be distinguished (data not shown). To confirm the results obtained with the F_2 plants, additional resistance tests were performed in the F_3 and F_4 generations. Out of 137 F_2 individuals, 103 showed a resistant reaction whereas 34 were susceptible. The 3:1 segregation (χ^2=0.0024) for a monogenically inherited gene confirmed the dominant action of the *Lr35* resistance gene.

A subset of 96 F_3 families (74 resistant plants, 22 susceptible plants) was randomly chosen and scored for susceptibility or resistance in a field trial. The progeny of the susceptible F_2 plants were homozygous-susceptible in the field, whereas the progeny of the resistant F_2 plants were either homozygous-resistant or segregating. Ninety F_3 families showed the same infection type as the F_2 individuals in the growth chamber. For six F_3 families no phenotypic data were obtained in the field. These results demonstrated that the phenotypic data generated in the growth chamber are identical to those obtained under field conditions. Genetic mapping was performed in this subpopulation as heterozygous F_2 individuals could be distinguished from the homozygous-resistant plants by segregation in the F_3 families.

Genetic mapping of the *Lr35* gene

Eighty RFLP probes which were previously shown to be located on chromosome 2B were chosen for hybridization. Out of this set, 51 probes showed polymorphism between the two parents Th*Lr35* and Frisal. Fourteen

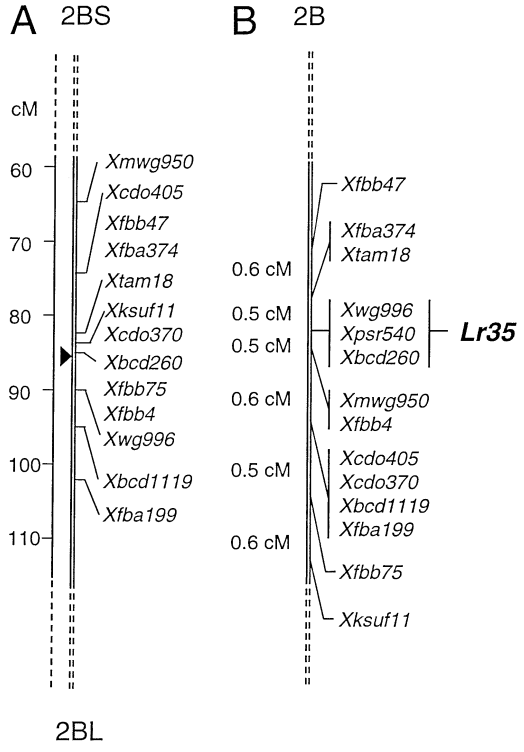

Fig. 2 Genetic map of chromosome 2B in a cross between Synthetic×Opata (**A**) and a cross between Th*Lr35*×Frisal (**B**). The *arrowhead* indicates the centromere of chromosome 2B of Synthetic×Opata. The orientation of chromosome 2B in the cross Th*Lr35*×Frisal is not known

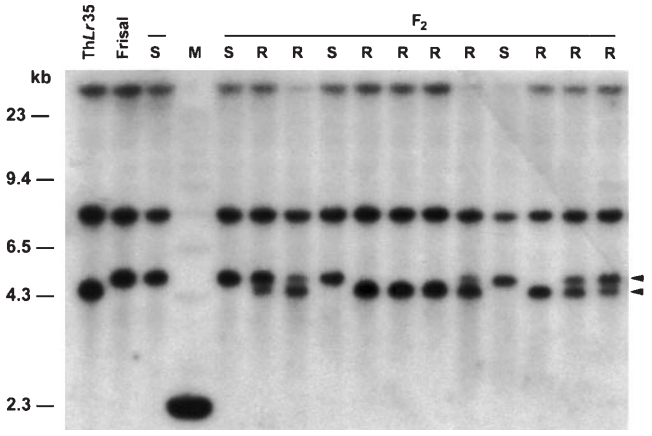

Fig. 3 Southern-hybridization pattern of *Xba*I-digested genomic DNA with the probe BCD260/0.9. DNA was extracted from Th*Lr35*, Frisal and the pooled F_3 progeny of 13 F_2 indivicuals from the segregating mapping population (*R* resistant phenotype, *S* susceptible phenotype). The molecular-weight marker *M* is λ DNA digested with *Hin*dIII. The *arrowheads* indicate the polymorphic bands of 4.3 kb and 4.5 kb from the resistant and the susceptible alleles, respectively

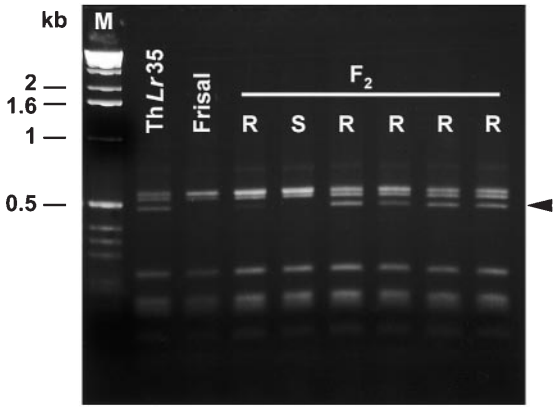

Fig. 4 A CAPS marker for the *Lr35* resistance gene. PCR was performed on genomic DNA of Th*Lr35*, Frisal, five resistant (*R*) and one susceptible (*S*) F_2 individuals with the primers BCD260F1 and BCD260R2 followed by digestion with *Dde*I. The *arrowhead* shows the additional fragment of 450 bp found in the resistant parent and the resistant F_2 individuals. The fragments were separated on an agarose gel

probes which gave a clear hybridization pattern, and were inherited as co-dominant markers, were mapped in a segregating population consisting of 90 F_3 families grown in the field. The 14 co-dominant RFLP probes formed one linkage group. Three probes, BCD260/0.9, WG996 and PSR540, showed complete linkage to the *Lr35* resistance gene in the mapping population (Fig. 2). The probes FBB4 and MWG950 were located at 0.5 cM from the gene *Lr35*, whereas the probes FBA374 and TAM18 mapped at 0.5 cM on the opposite side of the resistance gene. The probes BCD1119, CDO370, CDO405 and FBA199 co-segregated at 1.1 cM from the resistance gene, while the probe FBB47 was located on the other side of the *Lr35* gene at 1.1 cM. Thirteen of the probes used have already been mapped in the cross Synthetic×Opata (http://wheat.pw.usda.gov/ggpages/linemaps/Wheat/SxO_2.html) and covered a distance of approximately 39 cM (Fig. 2). In the cross Th*Lr35*×Frisal, the genetic region which was covered on chromosome 2B by these markers spanned only 3.3 cM, suggesting that the recombination frequency was reduced.

Conversion of the RFLP marker BCD260 into a sequence-tagged-site (STS)

Hybridisation with the probe BCD 260/0.9 resulted in a simple hybridization pattern with only three fragments in each parental line (Fig. 3). The polymorphic fragments of 4.3 kb and 4.5 kb in Th*Lr35* and Frisal, respectively, showed complete linkage with the *Lr35* resistance gene.

The probe BCD 260/0.9 was sequenced. A primer was designed at each end of the fragment (BCD260F1/BCD260R2). PCR-amplification with these primers on genomic DNA of both parents, Th*Lr35* and Frisal, resulted in non-polymorphic amplification products of 1.5 kb. The larger size of the fragment amplified from wheat genomic DNA is due to the presence of an intron of 900 bp compared to the barley cDNA BCD 260/0.9 (data not shown). Digestion of the amplification products with the restriction enzyme *Dde*I resulted in one additional fragment of 450 bp in Th*Lr35* (Fig. 4).

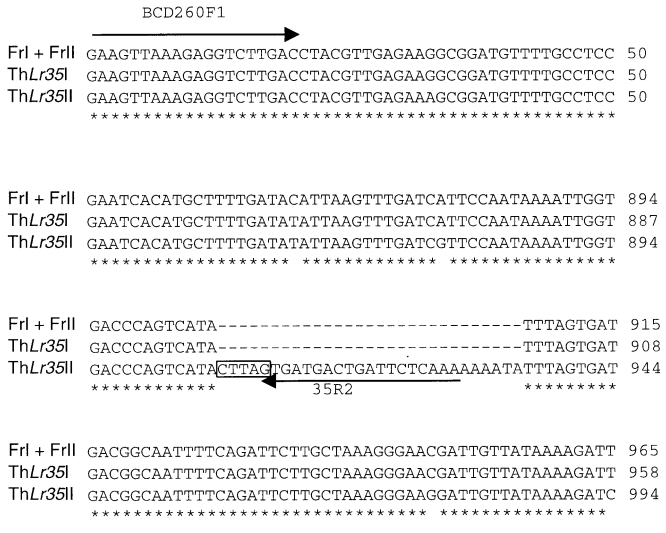

Fig. 5 Nucleotide-sequence comparison of the products amplified from genomic DNA of Frisal (*Fr I* and *Fr II*) and Th*Lr35* (*ThLr35 I* and *ThLr35 II*). Sequences were amplified with the primers BCD260F1 and BCD260R2. An additional 29 bp was found in the Th*Lr35* II allele. The *arrowheads* indicate the primer sequences used to develop the dominant STS marker. The *boxed* nucleotides indicate the additional *Dde*I restriction site (CTNAG) in the allele on chromosome 2B of Th*Lr35*. Differences between the two classes of Frisal sequences are outside the DNA region shown in the Figure

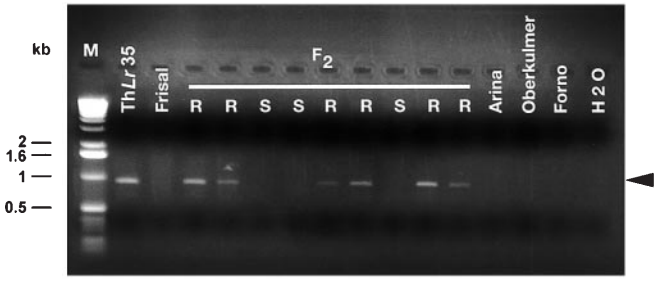

Fig. 6 STS marker for the *Lr35* resistance gene. The amplification products from genomic DNA with the primer combination BCD260F1/35R2 are shown. The DNA was isolated from the resistant parent Th*Lr35* (R.L.6082), the susceptible parent Frisal, nine F_2 individuals (*R* resistant individual, *S* susceptible individual), two winter wheat varieties Arina and Forno and the spelt variety Oberkulmer. The arrowhead indicates the dominantly inherited DNA fragment of 931 bp, which showed a complete linkage with the resistance gene *Lr35*. The size marker (M) is the 1kb ladder

This CAPS marker showed dominant inheritance in the mapping population and complete linkage to the *Lr*35 resistance gene.

The 1.5-kb fragments amplified with BCD260F1 and BCD260R2 from the parents Th*Lr35* and Frisal were subcloned and sequenced (Fig. 5). Five independent clones from the resistant line Th*Lr35*, as well as from Frisal, were analyzed. Two different types of sequences were obtained for Th*Lr35* which most likely correspond to two homoeologous chromosomal locations of BCD260/0.9. One type of sequence derived from Th*Lr35* had an additional insertion of 29 bp at position 906 compared to the second type (Fig. 5). This insertion includes the *Dde*I restriction site which allowed the development of the CAPS marker. Thus, we conclude that the sequence with the insertion corresponds to the resistance locus on chromosome 2B. Two different classes of sequences were also generated from the genomic DNA of Frisal, but none showed the 29-bp insertion (Fig. 5).

A specific primer (35R2) was designed from the 29-bp insertion in Th*Lr35* (Fig. 5). In combination with primer BCD260F1 it led to the amplification of a single fragment of 0.9 kb in the resistant line Th*Lr35*, whereas no product was amplified in Frisal. The 0.9-kb fragment was amplified from all the phenotypically resistant F_3 families whereas no amplification product could be obtained from the susceptible F_3 families (Fig. 6). Thus, this STS marker showed no recombination in the F_2 progeny and was completely linked with the resistance gene *Lr35*.

Validation of the CAPS and STS markers in wheat breeding material

A set of 48 European breeding lines derived from different breeding programs (Siedler et al. 1994) was used to test the specificity of the markers developed for the resistance gene *Lr35*. These breeding lines are known not to contain *Lr35*, as until now this resistance gene has not been exploited in wheat breeding programs. With the CAPS marker all the breeding lines showed the characteristic pattern of the susceptible variety Frisal (data not shown). The additional 450-bp fragment obtained with Th*Lr35* was not detected in any of these lines. Moreover, the use of the STS marker in the breeding lines did not result in any amplification product (Fig. 6, data not shown). We conclude that the two primers BCD260F1 and 35R2 are amplifying a specific sequence only in lines containing the *Lr35* resistance gene.

Discussion

Development of molecular markers for the detection of *Lr35*

In this work, the goal was to develop a molecular marker for the adult plant leaf rust resistance gene *Lr35* which is derived from a wild relative of wheat, *T. speltoides*. Linkage analysis showed that three co-dominant RFLP probes (BCD260, PSR540 and WG996) were completely linked with the resistance gene *Lr35*. A tight linkage of probes was expected due to the low level, or complete lack, of pairing and the reduced rate, or absence, of recombination between wheat and alien chromatin (Zeller and Hsam 1983; Dyck and Kerber 1985). Complete linkage between molecular markers and introgressed leaf rust resistance genes from alien species into wheat has already been found for *Lr9* (Schachermayr et al. 1994; Autrique et al. 1995), *Lr19* (Autrique et al. 1995), *Lr24*

(Autrique et al. 1995; Schachermayr et al. 1995; Dedryver et al. 1996), *Lr25* and *Lr29* (Procunier et al. 1995) and *Lr28* (Naik et al. 1998). In the case of the *Lr32* gene which was transferred from *T. tauschii*, the D-genome donor of wheat, recombination between the resistance gene and two RFLP markers was observed (Autrique et al. 1995). For *Lr35*, 11 RFLP markers showed a close linkage to the resistance gene. Between the most distant probes KSUF11 and FBB47 a genetic distance of 3.3 cM was found. This indicated that there was recombination between these loci as well as between them and the resistance gene. Interestingly, the probes which span 3.3 cM in the Th*Lr35*×Frisal cross cover approximately 39 cM in the cross Synthetic×Opata. This shows that recombination is about 10-fold lower than in a cross involving hexaploid wheat. These results suggest that pairing and crossovers between chromosome 2B and a fragment of chromosome 2S introgressed into Th*Lr35* are possible but at a reduced rate. This is in agreement with the hypothesis that the S genome of *T. speltoides* is the B-genome donor of hexaploid wheat as previously suggested by Daud and Gustafson (1996) and Maestra and Naranjo (1998). With a *T. speltoides*-specific probe, Daud and Gustafson (1996) detected a signal in the genome of tetraploid and hexaploid wheat. In contrast, signals in the genome of the *sitopsis* species, which have been proposed previously as the B-genome donor of wheat, were barely detected, Maestra and Naranjo (1998) showed that homoeologous pairing occurred between the chromosomes of *Triticum aestivum* and *T. speltoides*. A pattern of preferential pairing of two types, A–D and B–S, confirmed that the S genome is very closely related to the B genome of wheat. When characterizing wheat-alien translocations by C-banding analysis, Friebe et al. (1996) could not determine the translocation breakpoint of chromosome 2B in the *Lr35* donor line R.L. 5711. These authors suggested that the translocation chromosome 2B was composed of several fragments derived from chromosome 2S of *T. speltoides* and that recombination could occur between the translocated parts. We found that the relative position of the 13 probes used in our analysis was not identical between the cross Synthetic×Opata and the cross Th*Lr35*×Frisal. A decreased recombination frequency and different arrangements of the fragments transferred from *T. speltoides* could explain the different order of the markers in the two maps.

Marker-assisted selection for *Lr35*

RFLP markers are very reliable markers for plant breeding but are also labour intensive and expensive (Mohan et al. 1997). The high number of plants that has to be analysed in plant breeding programs requires a rapid diagnostic assay. PCR provides a simple and fast screening method adapted to MAS (marker-assisted selection). PCR-based STS markers, which identify a short and unique sequence at a known genetic locus are reliable and efficient diagnostic tools. STS markers have been successfully used to detect polymorphisms in cereals (D'Ovidio et al. 1990; Weining and Langridge 1991; Williams et al. 1991; Tragoonrung et al. 1992; Talbert et al. 1994; Schachermayr et al. 1994, 1995).

One of the probes (BCD260/0.9) which showed complete linkage with the *Lr35* resistance gene was converted into two different PCR-based markers: CAPS and STS markers. Although the CAPS marker is a PCR-based marker, an additional step for restriction-enzyme digestion makes it less practical than the STS marker which only needs a PCR reaction. The insertion of 29 bp in Th*Lr35* with the additional *Dde*I restriction site allowed us to convert the RFLP marker into a STS marker. Thus, large populations in breeding programs can now be screened for the presence or absence of *Lr35* in a fast and easy way. Since no STS could be amplified from all the European breeding lines we have tested here, we conclude that this STS marker is highly specific for the *Lr35* resistance gene.

The pyramiding of different resistance genes can be supported by the use of molecular markers in classical breeding programs. In the case of the resistance gene *Lr35*, a combination with other genes should be achieved. No virulence has been reported in Canada, South Africa, Australia and Switzerland (Kerber and Dyck 1990; Kloppers and Pretorius 1995; Kolmer 1997; R.F. Park, personal communication; own data) until now. The use of this gene as a single resistance source would certainly lead to the emergence of virulent races which would overcome the resistance in a short time. With the help of the STS marker, the combination of *Lr35*with other leaf rust resistance genes which are active at the seedling and/or the adult stage should facilitate more efficient breeding for durable resistance against this disease.

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