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High-resolution mapping of the leaf rust disease resistance gene *Lr1* in wheat and characterization of BAC clones from the *Lr1* locus

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Abstract Leaf rust is the most common disease in wheat production. There are more than 45 specific resistance genes described and used in wheat breeding to control epidemics of leaf rust, but none of them has been cloned. The leaf rust disease resistance gene 1 (*Lr1*) is a good model gene for isolation by map-based cloning because it is a single, dominant gene which is located in the distal region of chromosome 5DL of wheat. As the first step towards the isolation of this gene we constructed a high-resolution genetic map in the region of the *Lr1* locus by saturation mapping of two large segregating F_2 populations (Thatcher*Lr1* × Thatcher, Thatcher*Lr1* × Frisal). The resistance gene *Lr1* was delimited in a 0.16-cM region between the RFLP markers ABC718 and PSR567 (0.12 cM from ABC718 and 0.04 cM from PSR567). A genomic BAC library of *Aegilops tauschii* (D genome) was screened using the RFLP markers ABC718 and PSR567. Five positive BAC clones were identified by ABC718 and four clones by PSR567. Two NBS-LRR type of resistance gene analogs, which encode proteins highly homologous to the bacterial blight disease resistance protein Xa1 of rice, were identified on BAC clones isolated with PSR567. Polymorphic BAC end probes were isolated from both ends of a 105-kb large BAC clone identified by ABC718. The end probes were mapped at the same locus as ABC718, and no recombination event was found within 105 kb around ABC718 in our analysis of more than 4,000 gametes.

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Introduction

Bread wheat (*Triticum aestivum* L. em. Thell.) is one of the most important food crops in the world. More than 570 million metric tons are produced (http://hordeum.msu.montana.edu/genome) worldwide each year, which contribute about 20% of the calories ingested by the world population (FAOSTAT home page; http//apps.fao.org/). However, the production of wheat is significantly affected by different diseases. Leaf or brown rust disease caused by the fungal pathogen *Puccinia recondita* f. sp. *tritici* is the most common disease in wheat production. Epidemics of this disease can lead to severe losses of grain yield and decreased nutritional quality. The use of resistance genes is an effective, economical and ecological method to control epidemics of leaf rust disease. More than 45 different resistance genes derived from wheat and species related to wheat have been described (Knott 1989; McIntosh et al. 1995) and used in wheat breeding (Johnson and Lupton 1987). For some of the leaf rust resistance (*Lr*) genes, molecular markers have been developed for marker-assisted selection in breeding (Feuillet and Keller 1998). However, to date no *Lr* gene has ever been isolated, and the molecular basis of resistance is not yet understood.

Common wheat is an allohexaploid ($2n = 6x = 42$) containing the three genomes A, B and D. It has an extremely large genome (16×10^9 bp/1C, Bennett and Smith 1976) with more than 80% repetitive DNA sequences. These properties of the wheat genome make cloning of agronomically important genes by genetic methods very difficult. Fortunately, the highly repetitive DNA sequences in the wheat genome are not randomly distributed. There are gene-rich and gene-poor regions in the wheat genome (Gill et al. 1996a, b). Gene-rich regions contain less repetitive DNA sequences and undergo recombination much more frequently than gene-poor

regions. The highest gene density (one gene per 4–5 kb) has been found at the *Lrk* locus in wheat (Feuillet and Keller 1999). Comparison of the gene composition at orthologous *Lrk* loci in wheat, barley and rice showed that the high density of genes is conserved at syntenic loci of large and small grass genomes (Feuillet and Keller 1999). Therefore, gene-rich regions in the wheat genome may be amenable to molecular manipulations as are the small genomes of plants such as rice (Faris et al. 2000).

The leaf rust disease resistance gene 1 (*Lr1*) described by Ausemus et al. (1946) was identified in the hexaploid wheat cultivar Malakoff (Dyck and Samborski 1968) and is present in a number of wheat cultivars (Knott 1989; McIntosh 1988). Plants containing the *Lr1* resistance gene show a typical hypersensitive reaction after infection with an avirulent pathogen. *Lr1* is a single, dominant gene and was mapped to the long arm of chromosome 5D by monosomic analysis (McIntosh et al. 1965). Genetic and physical localization of restriction fragment length polymorphism (RFLP) markers linked to *Lr1* indicated that this gene is located at the very distal end of chromosome 5DL of wheat (Feuillet et al. 1995). Comparative mapping analysis using the RFLP markers tightly linked to *Lr1* showed that there was no conserved colinearity in the region of the *Lr1* locus to a rice chromosome (Gallego et al. 1998). Physical mapping of chromosome 5DL with deletion lines showed that 1.6 µm of the distal part of the chromosome (24% of the physical length of 5DL) covered more than 70 cM of genetic distance (52% of the genetic map of 5DL). This result indicates that the distal part of chromosome 5DL undergoes recombination much more frequently than the rest of 5DL (Feuillet et al. 1995).

In view of the difficulties encountered when isolating genes from hexaploid wheat based only on map position, it has been proposed that agronomic genes which are genome-specific (i.e. present only in one of the three genomes in wheat) are amenable to cloning using the diploid progenitors of wheat (Kam-Morgan et al. 1989; Gill and Gill 1994). Recently, Stein et al. (2000) reported on successful physical chromosome walking at the *Lr10* resistance locus in hexaploid wheat using a genomic BAC library of *T. monococcum* (A^m genome). This showed that isolation of genes in gene-rich regions of wheat might be feasible using a subgenome map-based cloning approach. The diploid goat grass (*Aegilops tauschii* Schmal.; *Ae. squarrosa* L. $2n = 2x = 14$) is the donor of the D genome of bread wheat (Kihara 1944; McFadden and Sears 1946). The D genome of *Ae. tauschii* shows almost complete homology to the D genome of bread wheat (Boyko et al. 1999). A genomic BAC library of this genome has been constructed (Moullet et al. 1999), thus, allowing the physical characterization of loci in *Ae. tauschii* which are orthologous to regions in the D genome of hexaploid wheat. Here, we report on the genetic fine mapping of the *Lr1* resistance gene in two large segregating populations and on preliminary characterization of bacterial artificial chromosome (BAC) clones isolated with markers flanking the *Lr1* gene.

Materials and methods

Plant material

For genetic mapping of the leaf rust disease resistance gene 1 (*Lr1*), the near-isogenic lines (NILs) Thatcher and Thatcher*Lr1* (Dyck and Samborski 1968) and the Swiss cultivar Frisal were used. The spring wheat varieties Thatcher and Frisal are susceptible against leaf rust disease, while Thatcher*Lr1* is resistant. Two segregating F_2 populations of Thatcher*Lr1* × Thatcher and Thatcher*Lr1* × Frisal were developed and used for genetic mapping. F_2 seeds were individually sown in Jiffy pots (4×5-cm Hole, Jiffy A/S, Denmark) in plastic plates (each plate contained 25 Jiffy pots). The 10-day-old seedlings were artificially infected as described by Schachermayr et al. (1995) with the leaf rust isolate 90035, which is avirulent for the resistance gene *Lr1*. The phenotypes (resistant or susceptible) of the F_2 plants were evaluated 10 days after infection. Subsequently, the F_2 plants were sprayed with the fungicide Opus Top (Dr. R. Maag SA, Dielsdorf, Switzerland) and further cultured in a greenhouse. After DNA analysis, only recombinant plants between markers and *Lr1* were selected. In the F_3 generation, 20 seeds of each recombinant F_2 plant were sown, and the resistance of the seedlings was evaluated by artificial infection as described above.

RFLP and BAC analysis

For screening of RFLP markers for polymorphisms, about 5 g of leaf tissue was collected from 4- to 6-week-old plants of Thatcher, Thatcher*Lr1* and Frisal and frozen in liquid nitrogen. Extraction of genomic DNA from the leaf tissue was performed as described by Graner et al. (1990). Seven restriction endonucleases (*Eco*RI, *Hin*dIII, *Xba*I, *Dra*I, *Eco*RV, *Bam*HI and *Bgl*II) were used for the digestion of genomic DNA. A 20-µg aliquot of total DNA was incubated with 50 u of an endonuclease in a final volume of 35 µl at 37 °C for 5 h. The DNA fragments were separated on a 0.75% agarose gel and transferred from gels to Hybond-N^+ membranes (Amersham, Germany) according to the manufacturer's instructions. The DNA was fixed on the membranes by baking at 80 °C for one hour.

For RFLP analysis of segregating F_2 populations, about 200 mg of leaf tissue from each F_2 plant was harvested 3–4 days after evaluation of the resistance phenotype. Isolation of genomic DNA was conducted following the protocol described by Stein et al. (2001). DNA digestion, fragment separation by gel electrophoresis and DNA transfer from gel to nylon membranes were carried out as described above.

For Southern hybridization, DNA probes were labelled by the random hexamer method with [^{32}P]dCTP (Feinberg and Vogelstein 1983) at 37 °C for 1–2 h and denatured by addition of a 0.4 *N* NaOH solution to a final concentration of 0.2 *N*. After prehybridization of the membranes at 65 °C for 4–6 h, the labelled probes were added to the hybridization solution and allowed to hybridize for 16–20 h. The membranes were washed twice for 20 min in a washing solution containing 0.1% SDS and 0.5 × SSC (1 × SSC: 0.15 *M* NaCl plus 0.015 *M* sodium citrate). Membranes were placed in plastic sheets and exposed to Kodak BioMax MS X-ray films for 16–20 h or to Fuji X-ray films for 2–4 days. BAC isolation and analysis was done as described by Stein et al. (2000).

Microsatellite analysis

Wheat microsatellite markers from chromosome 5DL of wheat developed by Röder et al. (1998) were used in this study. One primer of each primer pair was labelled using 5′-IRD 700 or 5′-IRD 800 (MWG-Biotech, Germany). Each 10-µl polymerase chain reaction (PCR) reaction consisted of the following reagents: 2 µl 15 ng/µl total genomic DNA, 1 µl 10× PCR buffer containing 15 m*M* $MgCl_2$, 0.5 µl 10 m*M* dNTPs, 0.1 µl 5 pmol/µl labelled primer and 0.2 µl 10 pmol/µl unlabelled primer, 0.1 µl of 5 U/µl *Taq* DNA polymerase (Sigma, St. Louis, Mo.) and 6.1 µl H_2O. The PCR re-

actions were carried out in a thermal cycler as follows: 94 °C for 4 min, then 35 cycles at 94 °C for 1 min, 55 °C or 60 °C (dependent on primer pairs) for 1 min, 72 °C for 1 min, finally, an elongation step at 72 °C for 5 min. The amplified DNA fragments were separated by electrophoresis (48 samples each time) on a 7% polyacrylamide gel in an automatic DNA Sequencer Li-Cor 4200 according to the instructions of the manufacturer.

Linkage analysis

Linkage estimation was based on the recombination frequency (defined as the added number of recombination events among the total number of gametes analysed). The recombination frequency was transformed to map units [centiMorgans (cM)] without using the Kosambi function as most of the markers analysed were very closely linked.

Results

Polymorphic DNA markers for genetic mapping of the *Lr1* gene

The generation of a saturated genetic map around the target gene is the first step of map-based cloning. Two segregating F_2 populations, Thatcher*Lr1* × Thatcher and Thatcher*Lr1* × Frisal, were constructed and used for fine mapping of the *Lr1* gene. To find polymorphic DNA markers for genetic mapping of the *Lr1* gene, we selected eight RFLP markers on chromosome 5DL of wheat, one on chromosome 5DL of *Ae. tauschii* and ten from syntenic regions of barley (chromosome 5H) and oat (data not shown). The genomic DNA of the three parents (Thatcher*Lr1*, Thatcher and Frisal) was digested with seven restriction enzymes and analysed by Southern hybridization. Most of the RFLP markers investigated (14 of 19) were monomorphic and could not be used for genetic mapping. The RFLP marker PSR567 showed polymorphisms in both populations. The RFLP marker BCD1421 was polymorphic only between Thatcher*Lr1* and Frisal, while TAG621 and ABC718 revealed a polymorphism only between Thatcher*Lr1* and Thatcher.

Microsatellite markers are frequently more polymorphic than RFLP markers. Five microsatellite markers on chromosome 5DL of wheat were analysed on the three parents. Two of them (GWM269, GWM654) showed polymorphisms between Thatcher*Lr1* and Frisal. The polymorphic fragments amplified were 128 bp (Thatcher*Lr1*) and 144 bp (Frisal) with GWM269, and 138 bp (Thatcher*Lr1*) and 128 bp (Frisal) with GWM654. The microsatellite marker GWM272 amplified polymorphic fragments only between Thatcher*Lr1* (128 bp) and Thatcher (136 bp).

Genetic fine mapping of the *Lr1* gene

For fine mapping of the *Lr1* gene, two segregating populations Thatcher*Lr1* × Thatcher and Thatcher*Lr1* × Frisal were analysed. In the population Thatcher*Lr1* × Thatcher, 2,826 F_2 plants were evaluated for phenotypical segregation of resistance and susceptibility by artificial infection with leaf rust. A total of 723 F_2 plants showed susceptibility to the leaf rust disease, and 2,103 plants revealed resistance ($\chi^2 = 0.36$). In the population Thatcher*Lr1* × Frisal, 832 F_2 plants were scored for the phenotype: 602 plants were resistant and 230 plants were susceptible ($\chi^2 = 3.11$). The segregation ratio of resistance to susceptibility corresponds to a ratio of 3:1 in both segregating populations. Thus, the resistance is controlled by a single dominant gene.

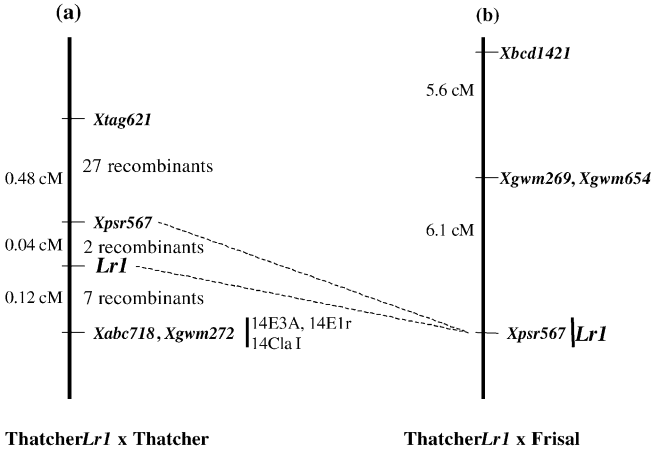

Fig. 1a, b High-resolution genetic maps of the leaf rust disease resistance gene *Lr1* in the distal region of chromosome 5DL of wheat. Map resulting from the analysis of; **a** 2826 F_2 plants of the cross Thatcher*Lr1* × Thatcher, **b** 832 F_2 plants of the population Thatcher*Lr1* × Frisal. 14E3A, 14E1r and 14ClaI are three BAC-end probes which could only be mapped in the population Thatcher*Lr1* × Thatcher

Three RFLP markers, ABC718, TAG621 and PRS567, and one microsatellite marker GWM272, were polymorphic between Thatcher*Lr1* and Thatcher and were used for mapping. Figure 1a shows the genetic fine map of the cross Thatcher*Lr1* × Thatcher. The *Lr1* gene was mapped between RFLP markers ABC718 and PSR567, and it is very tightly linked with both markers. 7 seven recombination events between ABC718 and the *Lr1* gene and two between PSR567 and *Lr1* were found in the analysis of 5,652 gametes (2,826 F_2 plants). The RFLP marker TAG621 was mapped on the same side as PSR567 and is 0.48 cM proximal from PSR567 and 0.52 cM from the *Lr1* gene. The microsatellite marker GWM272 was mapped at the same locus as ABC718. No recombination events were observed between the two markers in the analysis of 5,652 gametes.

The two RFLP markers BCD1421 and PSR567 as well as the two microsatellite markers GWM269 and GWM654 were polymophic between Thatcher*Lr1* and Frisal and used for analysis of the F_2 population of Thatcher*Lr1*×Frisal. A total of 832 F_2 plants were analysed, and the mapping results are shown in Fig. 1b. PSR567 was found to cosegregate with the resistance gene *Lr1* in the analysis of 832 F_2 plants. BCD1421 was located at more than 11 cM from *Lr1* proximal to the centromere. Both microsatellite markers GWM269 and GWM654 were mapped at 6.1 cM from the resistance gene.

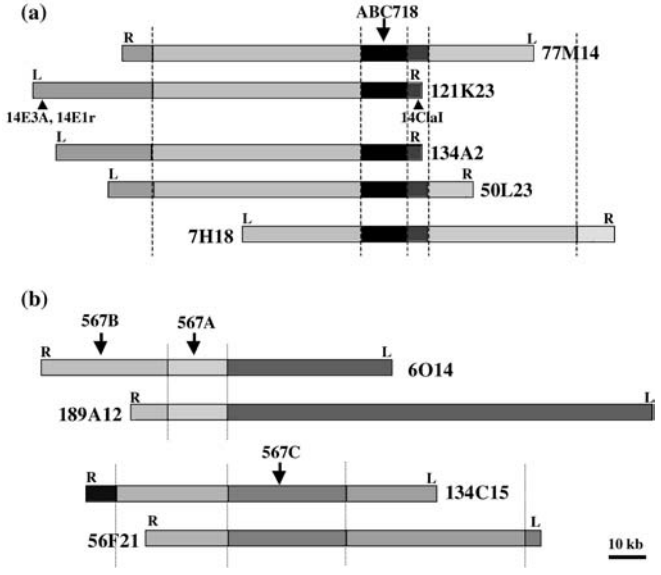

Fig. 2 Schematic representation of BACs isolated with RFLP probes ABC718 (**a**) and PSR567 (**b**) from the genomic BAC library of *Aegilops tauschii*. BAC DNAs were digested with restriction enzyme *Not*I, and fragments were separated by pulse field gel eletrophoresis. Different patterns indicate different fragments. *Dotted lines Not*I sites, *L* and *R left* and *right end* of BACs respectively, *arrows* fragments which hybridized with RFLP probes ABC718 or PSR567, *arrowheads* reveal locations of the polymorphic BAC-end probes

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ISYDCLPFDLKKCFSYCGLFPEDHWFTSSEINHFWVA
VGIIDSDHQADRNYLEELVDNGFLMKQFDWRDRWWYV
MHDLMHELSKSVSAQECHNISGFDFRADAISQSVRHL
SINIEDRYDANFEKEMCKLRERIDIANLRTLMICRRY
EEERIAKILKDS

FKEINSLRVLFIAVSTPES-FPYR
FSKLIHLQYLKISSSYKDGEISLPST
LSRFYHLKFLDLDDWNGRSDLPED
FSHLENLHDFRAGSELHSNIRNVGK
MKHLQELKEFHVRKESMGFELSELGAL
SELEEELTVLGLEHVATKEEATAAK
LMLKRNLKQLELLWSGRDGPTTDAGI
LDALQPHSNLRVLTIANHGGTVGPSWLCLDMW
   LTRLETLILEGISWST-LPPFGT
   LPNLKGLKLKKISGMHQFGKC
   CMRLKTVEFYEIPELAEWPVEPKCHS
FPSIEEIKCIDCPNLRVMPFSE
V-SCTNLRRLEVSGCPKMS-LPS
MPYTSTLTSCDVKRCDSERLLYDGKELVVSGYGGALT
FHNLDKVEDMTVGKCDGLFPQELDD
SFVFHSVESLKLDVSH-LTSSKSSPSKV
LNCFPALSVLHIDGCEECVMQ
FPSSSSLQELTFFQCKGVVLVPVENGGGI
QEDKSLLQSLRIFICGKLLSRWPMGMGESETI
CPFPASLRILDVEEEPSMKSMAL
LSNLTSLTGLTLNACSNLTVDGFNP
LIAVNLIR-LQVRGCNTLAADMLSEVASQRAK
LLPAGSF-RLEVLRVDDISGLLVAPICN
LLAPA-LRILEFRSDGRTESFTEEQDKA
LQLLTSLEKLHFFICEGLQSLPQG
LHRLSSLKELHVRYCPNIRSMPKEG
  LPVSLRKLYMSDCSAEIDEQIEKIKRTNPDFL

L--L--L--L-L--(N/C/T)-(-)L--IP--
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Fig. 3 Amino acid sequence of the open reading frame encoded by 567B-3.2 at the locus 567B. Twenty-seven imperfect repeats of the LRR motif were identified. *Bold letters* Amino acids fitting the consensus sequence L--L--L--L-L--(N/C/T)-(-)L--IP-- proposed by Jones and Jones (1997) for cytoplasmic LRRs (where a *hyphen* stands for any amino acid)

Characterization of the BAC clones isolated by the RFLP marker ABC718

The RFLP marker ABC718 developed from barley cDNA is very tightly linked and distal to the *Lr1* gene (0.12 cM). Southern hybridization showed that ABC718 has three copies in the genome of hexaploid wheat. They were located on chromosomes 5D, 5B and 7B by analysis of the nullitetrasomic lines of Chinese spring (data not shown). There is only one copy in the genome of *Ae. tauschii* (data not shown). ABC718 was used to screen the genomic BAC library of *Ae. tauschii*. Five positive BAC clones (7H18, 77M14, 121K23, 134A2 and 50L23) were identified with this marker (Fig. 2a). The insert length of the BAC clones is between 90 kb and 105 kb. Analysis with the restriction enzyme *Not*I showed that the five BAC clones span more than 150 kb around the ABC718 locus and that BAC clones 121K23 and 134A2 shared the same right ends (Fig. 2a). ABC718 was located in the 12-kb large fragment (sole common fragment of the five BAC clones), which was 3.5 kb distant from the right end of BAC clones 121K23 and 134A2. Large end fragments of four BAC clones (77M14, 121K23, 134A2 and 50L23) were isolated by plasmid rescue and cleaved into small fragments with *Hin*dIII combined with *Eco*RI for left ends and with *Cla*I for right ends. Twenty-one fragments were isolated from the end regions of the four BAC clones: 12 were repetitive or at least contained a repetitive sequence; eight were low-copy sequences, but did not show polymorphisms between the three parents; one end fragment (14ClaI) isolated from the right end of BAC clones 121K23 and 134A2 (Fig. 2a) is a single-copy fragment in the wheat genome and is polymorphic between Thatcher*Lr1* and Thatcher. In order to develop polymorphic markers from the left end of the BAC clone 121K23, which is the largest BAC clone (105 kb) among the five clones, about 7.5 kb of DNA was isolated in three *EcoRI*-fragments (3.5 kb, 2.6 kb and 1.3 kb). The 2.6-kb fragment contained a repetitive sequence and could not be used. Five RFLP probes were developed from the 1.3-kb and 3.5-kb fragments by PCR amplification with specific primers. Three fragments were monomorphic in both mapping populations and two (14E1r and 14E3A) (Fig. 2a) were low copy (more than five copies) and revealed polymorphisms between Thatcher*Lr1* and Thatcher.

The physical distance of 14ClaI is about 4–16 kb away from ABC718, whereas 14E1r and 14E3A are separated by more than 80 kb (Fig. 2a). The three probes were mapped in the population Thatcher*Lr1* × Thatcher, and the three polymorphic fragments were located on chromosome 5D of wheat. More than 4,000 gametes (more than 2,000 F_2 plants) of Thatcher*Lr1* × Thatcher were analysed by Southern hybridization. No recombina-

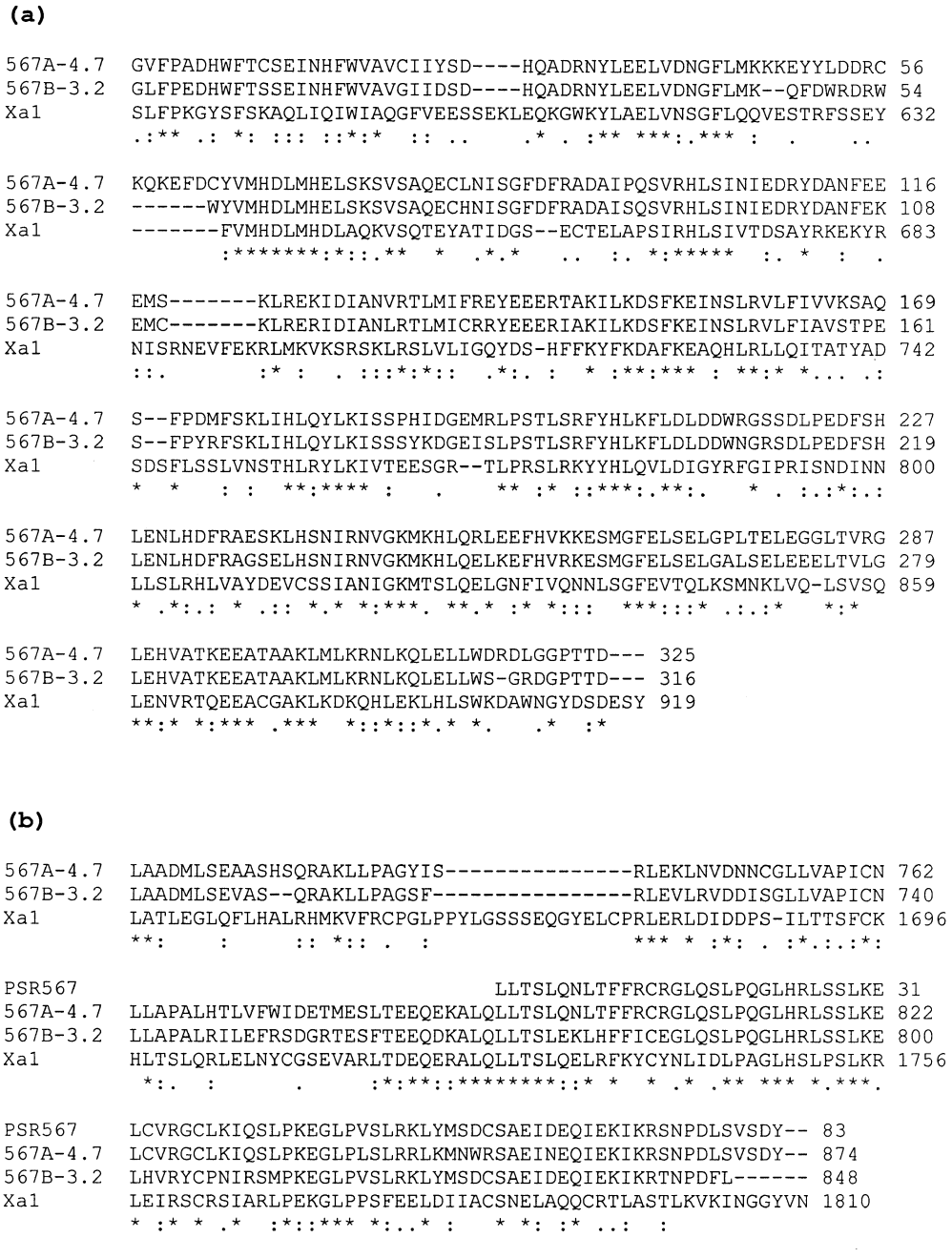

Fig. 4a, b Amino acid sequence comparison of two putative proteins encoded by 567A-4.7 and 567B-3.2 with the Xa1 protein (accession no. AB002266) as well as with the sequence encoded by the probe PSR567. The most homologous regions of the amino acid sequences are given. **a** The region from the beginning of the sequence to the seventh LRR motifs of 567B-3.2, **b** The C-terminal region. *Asterisk* Identical amino acids, *two vertical dots* and *one dot* mean similarity of amino acids

tion events were observed between ABC718 and the three BAC end probes. They all mapped at the same locus as the RFLP marker ABC718 (Fig. 1a).

Characterization of BAC clones isolated with the RFLP marker PSR 567

The RFLP marker PSR567, which was very tightly linked (0.04 cM) and proximal to *Lr1*, originates from genomic DNA of hexaploid wheat. The probe PSR567 results in six hybridizing fragments in *Ae. tauschii* and more than ten bands in *T. aestivum*. Only one of the fragments in Thatcher*Lr1* was polymorphic and could be assigned to 5D. Thus, there are possibly one or more sequences related to PSR567 on chromosome 5D of wheat which have not yet been mapped in relation to the *Lr1* gene. To better understand the complexity of the PSR567 sequence family and to get a sequence contig spanning the *Lr1* gene, we screened the *Ae. tauschii* BAC library with PSR567. Four positive BAC clones (6O14, 56F21, 134C15 and 189A12) were isolated that contain inserts of genomic DNA between 95 and 133 kb (Fig. 2b). Southern hybridization using probe PSR567 showed that BAC clone 6O14 contained two sequences corresponding to PSR567 while only one was identified in the other three BAC clones. Subsequent finger printing analysis indicated that the four BAC clones could be

divided into two groups (6O14 with 189A12 and 56F21 with 134C15). They contain three different sequences related to PSR567, named 567A, 567B and 567C (Fig. 2b). For further characterization of the BAC clones, we isolated 1.5–2-kb large DNA fragments around the three sequences corresponding to PSR567 from the four BACs by digestion with restriction enzyme *Hin*dIII and sequenced(AY 123939-41). In addition, the probe PSR567 was sequenced (AY 123938). The probe PSR567 is 1,285 bp in size, and blast results showed that the first 250 bp of the sequence encode an amino acid sequence which is homologous to the leucine-rich repeat (LRR) region of the bacterial blight-resistance protein Xa1 of rice (Genbank accession no. AB002266). Sequence comparison showed that fragments 567A, 567B and 567C have 95%, 89% and 82% of sequence identity with DNA sequence PSR567 in the coding region, respectively (data not shown). In the non-coding region, only the DNA sequence of 567A from BAC clones 6O14 and 189A12 shows a similarity to the sequence of PSR567 (data not shown), indicating that 567A is orthologous to the probe PSR567. The BAC clones isolated with ABC718 were hybridized with the end probes of the four BAC clones isolated with PSR567, and no overlap was observed.

For a more detailed characterization of the two putative genes 567A and 567B on BACs 6O14 and 189A12, 4.7 kb of DNA (567A-4.7) from 567A and 3.2 kb (567B-3.2) from 567B were isolated by restriction enzyme digestion or by plasmid rescue and sequenced (AY 123939–40). Sequence analysis revealed that the first 2,700 nucleotides of 567A-4.7 encode an open reading frame of 893 amino acids, whereas the first 2597 nucleotides of 567B-3.2 show an open reading frame of 863 amino acids (Fig. 3). The DNA sequences obtained are partial sequences of two putative resistance gene analogs (RGAs). The two sequences show more than 80% identity at the nucleotide level in the overlapping region and putatitively encode proteins which are homologous to the NBS-LRR type of resistance proteins. LRR motifs of 567A-4.7 and 567B-3.2 are present in the C-terminal domain of 650 amino acids (Fig. 4). The best homology was found with a putative bacterial blight resistance protein of rice (GENBANK accession no. AC079128) related to the Xa1 disease resistance protein. Comparison with Xa1 itself showed that the best homology is found in the regions from the beginning of the sequences to the seventh LRR motif and then again in the C-terminal region of the proteins (Fig. 4). The amino acid sequences encoded by 567A-4.7 and 567B-3.2 showed 78% identity and 88% similarity to each other.

Discussion

As a first step towards map-based cloning we have fine mapped the *Lr1* locus using two large F_2 populations. A high-resolution map in the region of the *Lr1* gene was constructed. The resistance gene *Lr1* was mapped between the RFLP markers ABC718 and PSR567 and is very tightly linked with the two markers (0.12 cM and 0.04 cM, respectively). PSR567 is the only probe which could be mapped in both populations, and very similar mapping positions were obtained.

Common wheat is hexaploid and has a huge genome size, with more than 80% of the genome consisting of repetitive sequences. It is hardly possible to make a long-distance chromosome walk for cloning of genes as described in model plants such as *Arabidopsis* and rice. Finding markers very tightly linked to the target gene by saturation mapping with large segregating populations can greatly reduce the problems of physical chromosome walking. The RFLP markers ABC718 and PSR567 flank *Lr1*, and the genetic distance between these markers is 0.16 cM. In wheat, the average physical length per one genetic unit (centiMorgan), based on a genome size of 16,000 Mb and a genetic map of 3,700 cM (Faris et al. 2000), is about 4.4 Mb. However, the physical distribution of recombination events along chromosomes is not random; for example the base pair to centiMorgan ratios ranges from 30 to more than 550 kb/cM in *Arabidopsis* (Schmidt et al. 1995), 120 kb to 1 Mb/cM in rice (Umehara et al. 1995) and 118 kb to 22 Mb/cM in hexaploid wheat (Gill et al. 1996a). Analysis of wheat homoeologous group 5 by cytogenetic ladder mapping using deletion lines and RFLP markers (Gill et al. 1996a) showed that wheat genes were distributed in clusters which were present mainly in the distal parts of the chromosomes and that most of the recombination events occurred in the gene cluster regions. Physical mapping of chromosome 5DL of wheat with deletion lines (Feuillet et al. 1995) showed that 24% of the physical length of the distal part of the chromosome corresponded to 52% of the genetic map of 5DL. Recombination frequency in this region was more than two fold higher than other parts of 5DL. According to a total 235 μm length of the haploid chromosomes of wheat (Gill et al. 1991) containing 16 million kb of DNA (Bennett and Smith 1976), the relationship between genetic and physical distance in the distal part of wheat 5DL is about 1,370 kb/cM. Thus, the physical distance between the RFLP markers ABC718 and PSR567 might be as small as 220 kb, a size of one or two chromosome walking steps with BAC clones. However, with recombination frequencies varying within short chromosomal regions (see below), the distance might also be larger.

Although the genome size of *Ae. tauschii* (4,024 Mbp/1C, Arumuganathan and Earle 1991) is smaller than the D genome of wheat (4.7×10^9 kb/1C, Lee et al. 1997), the development of polymorphic probes from BAC ends still proved to be very difficult and frequently was not successful. About 60% (12/21) of the end fragments isolated in this study were repetitive sequences and could not be used for physical mapping. In most cases, fragments which did not contain repetitive sequences did not show polymorphism. Nevertheless, polymorphic probes were developed from both end regions of the 105-kb large BAC clone 121K23. All of

them were mapped at the same locus as ABC718, indicating that no recombination events occurred in more than 4,000 gametes. It is well known that the physical distribution of recombination is uneven along the chromosome even at the microlevel. Stein et al. (2000) studied recombination frequency in the 230-kb region of the *Lr10* resistance locus and found that three recombination events occurred within 30 kb while no recombination could be identified in the rest of the region.

The RFLP marker PSR567 derived from genomic DNA of wheat was previously located on 5DL (one copy), 5BL (two copies) as well as on 7BS of wheat (Xie et al. 1993). Sequence analysis showed that 83 amino acids encoded by the first 250 nucleotides of probe PSR567 showed a LRR motif which is found in disease resistance proteins. Analysis of two larger DNA sequences isolated around the putative genes 567A and 567B, which both hybridized with the probe PSR567, revealed coding regions with high homologies to the nucleotide binding site-LRR type of resistance proteins encoded by the bacterial blight resistance gene *Xa1* of rice. We conclude that the two genes *567A* and *567B* putatively encode resistance gene analogs, although the amino acid sequences obtained from genomic sequences are not complete. The two RGAs are separated from each other by a physical distance of 10–50 kb and show high sequence homology (88%), suggesting that there is a small resistance gene family clustering around the *Lr1* locus. Most of the resistance genes molecularly characterized in plants are present in gene families and arranged in gene clusters (Hulbert et al. 2001). Such gene clusters can span over 100 kb. Therefore, the RGA gene family detected by PSR567 in the D genome might be a resistance gene family including *Lr1* because the RFLP marker PSR567 was found only two recombination events (0.04 cM) away from the *Lr1* gene among the more than 5,600 gametes investigated. Very similar to our finding at the *Lr1* locus on chromosome 5DL, Huang and Gill (2001) recently described a cluster of RGAs at the *Lr21* locus on chromosome 1DS. This cluster was detected by the probe KSUD14 derived from *Ae. tauschii*. As in the case of PSR 567, probe KSUD14 also encoded a partial RGA.

In future investigations we will focus on the use of new, high genome-coverage BAC libraries of *Ae. tauschii* with large inserts (J. Dvorak, personal communication) for establishing a physical contig with the flanking markers. Using the probes and the recombinant plant lines developed in the study described here, we should be able to span the *Lr1* locus with very few chromosome walking steps.

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