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ORIGINAL PAPER

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Application of *BARE*-1 retrotransposon markers to the mapping of a major resistance gene for net blotch in barley

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Abstract Net blotch, which is caused by the fungus *Pyrenophora teres* Drechs. f. *teres* Smedeg., presents a serious problem for barley production worldwide, and the identification and deployment of sources of resistance to it are key objectives for many breeders. Here, we report the identification of a major resistance gene, accounting for 65% of the response variation, in a cross between the resistant line CI9819 and the susceptible cv. Rolfi. The resistance gene was mapped to chromosome 6H with the aid of two recently developed systems of retrotransposon-based molecular markers, REMAP and IRAP. A total of 239 *BARE*-1 and *Sukkula* retrotransposon markers were mapped in the cross, and the 30-cM segment containing the locus with significant resistance effect contained 26 of the markers. The type and local density of the markers should facilitate future map-based cloning of the resistance gene as well as manipulation of the resistance through backcross breeding.

Key words *BARE*-1 retrotransposon · Barley · Net blotch resistance · Linkage mapping · Quantitative trait locus (QTL)

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Introduction

Net blotch of barley (*Hordeum vulgare* L.), which is caused by the fungal phytopathogen *Pyrenophora teres* Drechs. f. *teres* Smedeg., constitutes one of the most serious constraints on barley production world-wide (Shipton et al. 1973), reaching as far north as the Arctic Circle in Finland (Mäkelä 1975). It may lead to grain yield losses of up to 40% in severe infections (Steffenson 1997). Deployment of improved host-plant resistance is a principal goal of breeders in many countries (Robinson and Jalli 1996, 1997; Jalli and Robinson 2000).

Several barley lines with major-gene resistance to net blotch have been identified. The results of classical genetic analyses indicate that resistance is controlled by 1–3 loci, depending on the barley accession and the net blotch isolate used for testing (Mode and Schaller 1958; Wilcoxson et al. 1992; Douiyssi et al. 1996; Ho et al. 1996). The genetic background of the susceptible parent used in crosses and the infection environment both influence the host resistance reaction (Khan 1969). At least three independently segregating resistance genes, *Rpt1*, *Rpt2* and *Rpt3*, have been localized by trisomic analysis on chromosomes 3H, 1H, and 2H, respectively (Bockelman et al. 1977). Quantitative resistance for net blotch has also been reported (Arabi et al. 1990; Steffenson and Webster 1992; Steffenson et al. 1996; Robinson and Jalli 1997).

The availability of molecular marker systems that are independent of phenotype and can be used to produce dense maps offers tools for the mapping and selection of net blotch resistance genes. A single dominant gene for resistance against one of the six tested pathogen isolates has been localized on chromosome 3H in cv. Igri, using RFLP (restriction fragment length polymorphism) markers (Graner et al. 1996). In a cv. Steptoe × Morex cross, one locus for seedling resistance was identified on chromosome 4H and a further two on 6H, whereas adult plant resistance was controlled by seven QTLs (quantitative trait locus/loci) on chromosomes 2H, 3H (two loci), 4H, 5H, 6H, and 7H (Steffenson et al. 1996). In

another study, twelve QTLs were identified, with the response depending on the leaf used for testing and the elapsed time after infection; these were mapped with RFLP and AFLP (amplified fragment length polymorphism) markers (Richter et al. 1998). Recently, major resistance genes that confer resistance to the spot form of net blotch were located on 7H with RFLP markers (Williams et al. 1999) and on 2H with RAPD markers (Molnar et al. 2000).

All molecular marker systems except RFLP are based on prevalent, widespread elements in the genome. Retrotransposons are highly abundant and dispersed components of the barley (Suoniemi et al. 1996; Vicient et al. 1999) and other plant genomes and are ubiquitous (Flavell et al. 1992; Voytas et al. 1992; Suoniemi et al. 1998; Noma et al. 1999) throughout the plant kingdom. Resembling retroviruses in their structure and life cycle, retrotransposons remain as integral parts of the chromosome but produce daughter copies which are then inserted into new loci (Boeke et al. 1985; Löwer et al. 1996; Labrador and Corces 1997; Kumar and Bennetzen 1999). This integration process creates joints between genomic DNA and the conserved retrotransposon termini, the long terminal repeats (LTRs). Several techniques that produce marker bands from retrotransposon insertion loci have recently been developed and applied in barley (Waugh et al. 1997; Gribbon et al. 1999; Kalendar et al. 1999).

Here, we have used two retrotransposon-based marker systems, REMAP (Retrotransposon-Microsatellite Amplified Polymorphism) and IRAP (Inter-Retrotransposon Amplified Polymorphism), to map net blotch resistance. At the same time the general usefulness of IRAP and REMAP markers for mapping was demonstrated and their distribution on barley chromosomes determined. Linked retrotransposon markers may be useful for marker-assisted selection and could facilitate development of near-isogenic lines for future studies.

Materials and methods

Net blotch isolates

Finnish *P. teres* isolates (P7, P8, P40, and P58) were used as previously described (Robinson and Jalli 1997). Conidia of these isolates were placed on 2.3% lima-bean agar plates (LBA, 2.3% Bacto-Lima Bean Agar, Difco, BD Microbiology Systems, Franklin Lakes, N.J.) and were then kept under near-UV light (on a 12-h photoperiod) at 18 °C for 2 weeks. Petri dishes were then flooded with 100 ml of distilled water to produce a spore suspension for inoculation.

Plant material

A cross was made between the susceptible six-row spring barley cv. Rolfi and the resistant barley line CI9819. Altogether 119 doubled haploid lines were produced from the F_1 by anther culture as described previously (Manninen 1997). Five seeds of each doubled haploid line were sown in pots (6 cm diam.) of nutrient-supplemented peat. The pots were arranged in a randomized block design of four replicate blocks in a greenhouse maintained at 18–21 °C with a 12-h photoperiod. The pots were watered by hand. In addition, seeds were produced from the same cross which were not used for production of doubled haploids. Seedlings from ten F_1, 149 F_2, 49 BC_1 plants, and the parents, were tested for their infection response (IR) in a similar way. Barley-wheat (*Triticum aestivum* L.) addition lines were obtained from B. S. Gill (Kansas State University, Manhattan, Ks.). Seeds of these lines were germinated and grown for 2 weeks in a growth chamber before harvesting leaves for DNA isolation.

Inoculation and scoring

The isolate P8 was used for inoculation of F_1, F_2 and BC_1 plants, but a mixture of four isolates was used to inoculate the doubled haploid lines. Conidial density was assessed in a hemocytometer and aqueous suspensions were prepared at 2×10^4 conidia/ml. Two weeks after sowing, when seedlings were at growth stage GS12/13 (Tottman and Makepeace 1979), relative humidity in the greenhouse was raised to 100% and seedlings were inoculated with the conidial suspension at 0.5 ml per pot using a compressed air sprayer. The high humidity was maintained for 2 days until the first net blotch symptoms appeared. The infection response (IR) was recorded on the second leaf of single seedlings from each pot according to a ten-point scale (Tekauz 1985). The response was recorded when the entire IR range was evident in the material. Plants with IR scores ≤ 5 were considered resistant and values > 5 were taken to indicate susceptibility when the resistant:susceptible ratios were analyzed using the chi-squared (χ^2) test.

DNA preparation

The DNA of the doubled haploid lines was extracted from foliage of 10- to 14-day-old seedlings with a modified CTAB method (Poulsen et al. 1993); CsCl density gradient centrifugation was omitted and an RNase treatment was added to the protocol. From the barley-wheat addition lines, DNA was prepared as described previously (Kalendar et al. 1999).

RAPD, RFLP and microsatellite molecular markers

For RAPDs (randomly amplified polymorphic DNA), amplification reactions were performed as before (Manninen and Nissilä 1997), and the products electrophoretically separated on 1.4% agarose (50 V, 16 h). Clones for RFLP analysis were kind gifts from A. Graner (Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany), M. Sorrells (Cornell University, Ithaca, N.Y.), and A. Kleinhofs (Washington State University, Pullman, Wa.). Southern blotting and hybridization for RFLPs was done on Hybond N+ membranes (Amersham Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's instructions (Version 2).

The microsatellite primers described previously (Becker and Heun 1995; Liu et al. 1996; Russell et al. 1997) were used. The microsatellites were amplified in 20-µl reaction mixtures containing 50 ng of template DNA, 0.1 µM each primer; 200 µM each dNTP, 10 mM TRIS-HCl pH 8.8, 50 mM KCl, 0.1% Triton X-100, 2.0 mM $MgCl_2$, and 1.0 U Dynazyme polymerase (Finnzymes, Espoo, Finland). Most of the microsatellites were amplified according to the touchdown PCR program A of Liu et al. (1996), but, for BMS90 and HVCSG and for BMS18 and BMS64, the PCR program of Russell et al. (1997) was used with annealing temperatures of 58 °C and 60 °C, respectively. Microsatellite products were resolved on an ALF DNA sequencer and analyzed with the Fragment Manager computer program (Amersham Pharmacia Biotech).

REMAP amplifications

The REMAP products are derived from PCR amplifications between a primer matching a retrotransposon LTR and a primer that

anneals to simple sequence repeat (SSR) regions, anchored at the 3′ end, as described previously (Kalendar et al. 1999). The LTR primers were derived from the 5′ LTR of the *BARE*-1a sequence (Accession No. Z17327, nt 309-2137). The *BARE*-1 LTR primers were: LTR-A, a reverse primer (5′-ggaattcatAGCATGGATAA-TAAACGATTATC-3′; lower case letters indicate a sequence included to facilitate cloning) complementary to nt 369–391; LTR-B, a reverse primer (5′-CATTGCCTCTAGGGCATAATTCCA-ACA-3′) complementary to nt 309–335; LTR-Z, a forward primer (5′-ctcgctcgcccaCTACATCAACCGCGTTTATT-3′) matching nt 1993–2012. Primer LTR-S is a reverse primer and is complementary to nt 10662–10685 of *BARE*-1a (5′-GATAGGGT-CGCATCTTGGGCGTGAC-3′). This is part of an insertion element within the 3′ LTR referred to here as *Sukkula*. The SSR primers included: $(GA)_9C$, $(CA)_{10}G$, $(AC)_9C$, $(AC)_9G$, $(AC)_9T$, $(AG)_9C$, $(CT)_9C$, $(TG)_9A$, $(TG)_9C$, $(CAC)_7T$, $(CAC)_7A$,$(CTC)_6G$, and $(GCT)_6A$.

Amplifications were performed in 20-μl reactions containing 20 mM TRIS-HCl pH 8.8, 50 mM KCl, 2 mM $MgCl_2$, 0.01% Tween-20, and 20 ng of template DNA. For LTR-A, 200 μM dNTPs, 1 U *FIRE*Pol DNA polymerase (OY Soolis, Tartu, Estonia), and 200 nM (4 pmol) each of the SSR and LTR primers included. The reaction cycle consisted of: 94 °C for 2 min; 30 cycles of 94 °C for 30 s, 55–58 °C (depending on the particular SSR primer) for 30 s, and 72 °C for 2 min; and a final elongation step of 72 °C for 5 min. For LTR-B or LTR-Z, the reaction contained instead 150 nM (3 pmol) each of the SSR and LTR primers, 200 μM dNTPs, and 0.7 U *FIRE*Pol DNA polymerase. The reaction program was the same as for LTR-A except that 29 cycles were used. The annealing temperature was, respectively, 55 °C and 57–58 °C for dinucleotide- and trinucleotide-repeat SSR primers. Reactions were carried out in either a Mastercycler Gradient (Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) or a PCT-225 DNA Engine Tetrad (MJ Research, Waltham, Mass.) thermocycler in 0.2-ml tubes. Thermostable polymerases from other suppliers (Promega, Epicentre) gave identical results for REMAP as well as for IRAP and ISSR (Inter- Simple Sequence Repeat amplified polymorphism).

IRAP and ISSR amplifications

The IRAP amplifications were carried out as previously described (Kalendar et al. 1999), in the same buffer as above for REMAP. The reactions contained: 200 μM dNTPs, 200 nM (4 pmol) each primer (combinations of LTR-A and LTR-Z, or LTR-S), and 1 U *FIRE*Pol DNA polymerase. The reaction program consisted of: 94 °C for 2 min; 30 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 2 min; and a final elongation step of 72 °C for 5 min. The ISSR amplifications were carried out as before (Kalendar et al. 1999) and as above for IRAP, except that the reactions contained 0.7 U *FIRE*Pol DNA polymerase and the annealing temperature varied from 55–58 °C depending on the SSR primer.

REMAP, IRAP and ISSR markers

For detection of REMAP, IRAP, and ISSR products, one-fifth of the reaction mixture was subjected to electrophoresis (80 V, 7–8 h) in 2% agarose (Result LE, BIOzym, Landgraaf, The Netherlands) and visualized by ethidium bromide staining. Gels were photographed, the negatives scanned and digitized, and difference tables for the presence or absence of marker bands prepared.

Linkage map and QTL mapping

The segregation of the molecular markers in the doubled haploid progeny was tested against an expected 1:1 ratio using χ^2 analysis. Because the χ^2 analysis was repeated 233 times, the 5% significance threshold was replaced by a Bonferroni corrected significance threshold of 2.5×10^{-4}. The program JoinMap 2.0 (Stam and Van Ooijen 1995) was used for map construction. A LOD threshold of 9.5 was used for associating the markers into linkage groups. Recombination fractions were converted to centiMorgans with Haldane's mapping function. A map was constructed for each linkage group using the first- or second-round maps calculated with jump thresholds from 3.0 to 5.0. Based on the previously mapped anchor markers (RFLPs and microsatellites), and results from amplification of markers in the barley-wheat addition lines, the linkage groups were joined to form chromosomes. The orientation and distances between the linkage groups within each chromosome were estimated by keeping the maps of the groups fixed and allowing JoinMap to construct maps from joined data with a jump value of 5.0.

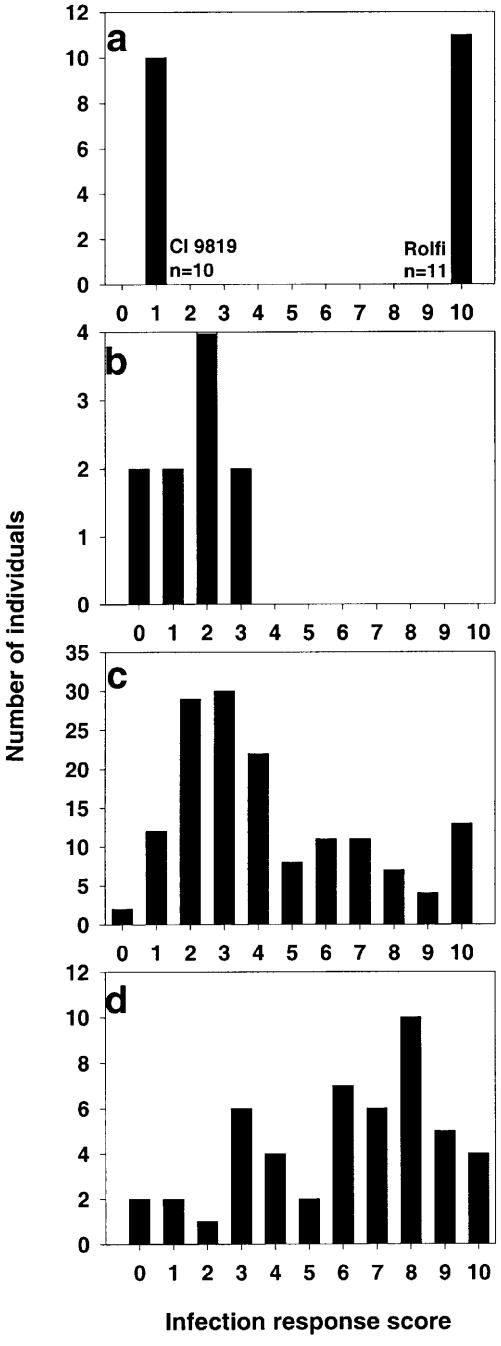

Fig. 1a–d Frequency distributions for the net blotch infection responses of seedlings. **a** Parents of the cross. **b** F_1 generation, $n = 10$. **c** F_2 generation, $n = 149$. **d** BC_1 generations, $n = 49$

The MQTL version 0.98 computer program (Tinker and Mather 1995) was used for QTL analyses. The average of the IR scores from four replicate blocks was used as the phenotypic value for each doubled haploid line. Both simple interval mapping (SIM) and simplified composite interval mapping (sCIM) were used. Epistatic interactions were analyzed with SIM. A genome-wide 5% error rate threshold for the test statistics was computed using 1000 permutations.

Results

Net blotch resistance tests

Frequency distributions of net blotch infection response for parents, F_1, F_2, and BC_1 generations inoculated with the isolate P8 are shown in Fig. 1. The resistant line CI9819 exhibited low IRs (1) and the susceptible parent cv. Rolfi high IRs (10). The IR values from 0 to 5 were considered to indicate resistance and IRs from 6 to 10 susceptibility. The F_1 individuals were all resistant, indicating dominance of the resistance gene(s). The resistant:susceptible ratio in the F_2 generation fitted the 3:1 ratio expected for segregation of one dominant gene ($\chi^2 = 2.74$, $P = 0.098$). In the BC_1 generation, a deviation from the expected 1:1 ratio for one dominant gene was observed ($\chi^2 = 4.59$, $P = 0.032$) with more susceptible lines emerging than expected.

The doubled haploid lines were scored for net blotch resistance in a separate experiment using a mixture of four Finnish isolates of *P. teres*. A mixture was used to ensure infection since the aggressiveness of isolates may change during cultivation in vitro. A mixture of isolates also mimics the natural situation in the field; the goal of this study was to map net blotch resistance genes that are effective under field conditions. The mean IRs for each line were based on four individuals and varied from 2.25 to 8.0. In this experiment, the IR for CI9819 was 1 and, for cv. Rolfi, 8–9. A continuous distribution with one peak that was skewed towards the resistant values was observed for the IRs of the doubled haploid lines.

Markers

Six different types of molecular markers, RFLP, RAPD, REMAP, IRAP, ISSR, and microsatellites, were applied to the mapping population. In the RFLP analysis, 74 clone and enzyme combinations were tested and 32 (43%) established a difference between the parents. Twenty-nine microsatellite loci were screened and 16 polymorphic ones (55%) were found. From the 283 RAPD primers tested, 171 (60%) found at least one difference between the parents; together these 171 primers revealed 303 band differences between the parents.

In the REMAP analyses, which detect insertional retrotransposon polymorphism with respect to nearby microsatellites, 24 combinations of SSR primers in the classes $(AC)_n$, $(CA)_n$, $(AG)_n$, $(GA)_n$, $(TG)_n$, $(CT)_n$, $(CAC)_n$,$(CTC)_n$, and $(GCT)_n$, with a single additional nucleotide at their 3′ ends to anchor them, were used together with LTR primers. Most experiments employed LTR-A and LTR-Z, primers complementary to the

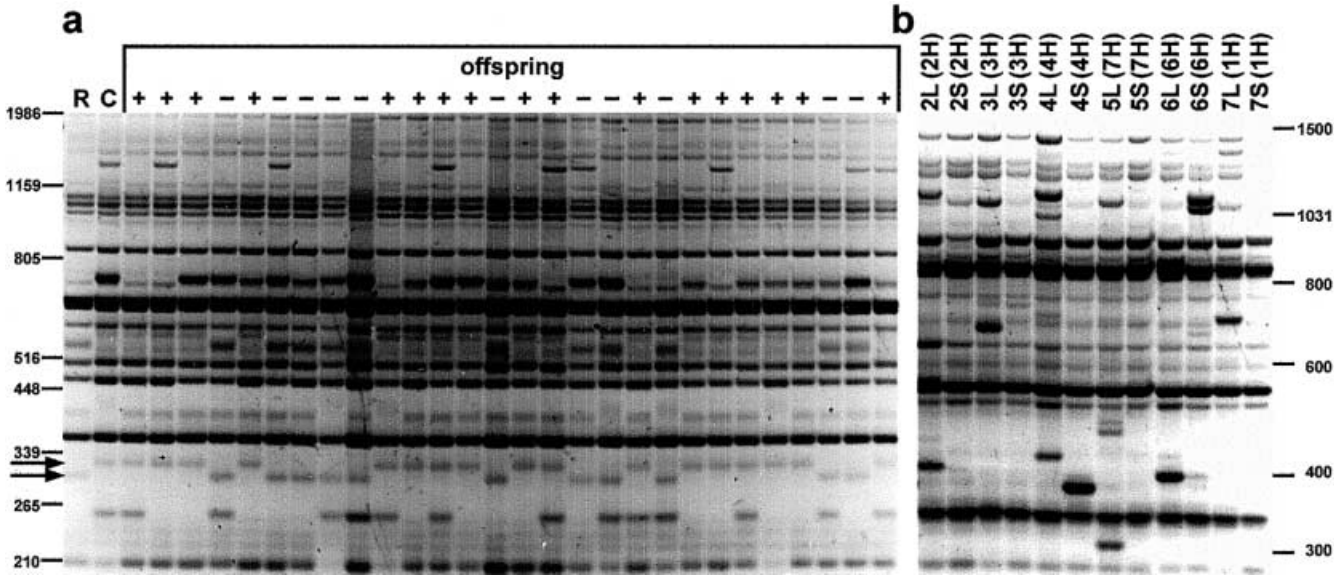

Fig. 2a, b Banding patterns generated by *BARE*-1 and *Sukkula* retrotransposon markers. **a** REMAP amplification with $(CTC)_6G$ and LTR-A primers. The parental lines cv. Rolfi and CI9819 are labeled "R" and "C", respectively, and are shown adjacent to 28 of the doubled-haploid offspring. The offspring phenotypes are marked (+) for resistant and (−) for susceptible. The *twin arrows* indicate the bands corresponding to the two alleles for REMAP marker REMAP-B5 (6H) linked to the major QTL for net blotch resistance. **b** REMAP amplification in barley-wheat addition lines, carried out with the same primers as in **a**. Lanes are labeled with the donor barley chromosome arm, the receiving wheat chromosome being denoted in *parentheses*. For all amplifications, the products have been stained with ethidium bromide following agarose gel electrophoresis; the gel is shown as a negative image. Size markers for **a** are indicated (in bp) on the *left* and those for **b** are shown on the *right*

BARE-1 retrotransposon LTR and facing outward from the upstream and downstream ends, respectively.

One set of analyses was made with the SSR primer $(GCT)_6A$ and the LTR-S, which is an insertion sequence within the *BARE*-1a 3′ LTR (Manninen and Schulman 1993) that behaves as an independent retrotransposon (our unpublished data) which we have named *Sukkula* (“shuttle” in Finnish). The reactions generated 20–40 bands, the product lengths varying from 100 to 3000 bp. For mapping, 215 polymorphic REMAP bands (~9 bands per primer combination) were chosen. A typical gel resolving REMAP amplification products in resistant and susceptible doubled-haploid offspring is displayed in Fig. 2a; results for the same REMAP primer combination applied to the barley-wheat addition lines are shown in Fig. 2b.

For the IRAP analyses, two combinations of primers were used, LTR-Z with LTR-A, and LTR-S with itself. The first combination generates products from *BARE*-1 LTRs integrated in all possible insertional orientations (head-to-head, head-to-tail, tail-to-tail), whereas the LTR-S reactions visualized only those *Sukkula* elements present in head-to-head orientation. Twenty-four polymorphic bands were used for mapping. The *Sukkula*-based IRAP displayed a high degree of polymorphism, 15 bands out of a total 35 scored.

Linkage map construction

Altogether 322 markers were analyzed in the progeny and 306 (215 REMAPs, 39 ISSRs, 24 IRAPs, 15 microsatellites, 8 RFLPs, and 5 RAPDs) were placed on the linkage map (Fig. 3). Previously mapped RFLP and microsatellite markers were used as anchors. A LOD score as high as 9.5 was used for constructing the linkage groups; with lower LOD thresholds, anchors from different chromosomes were grouped together. Twenty linkage groups were formed and 13 of these were assigned to chromosomes. All of the unassigned linkage groups except one contained three or fewer markers; the exception contained six markers and covered 42.9 cM. Nine markers were not linked to any other marker at a LOD score of 9.5. In total, 29 markers could not be assigned to any of the chromosomes.

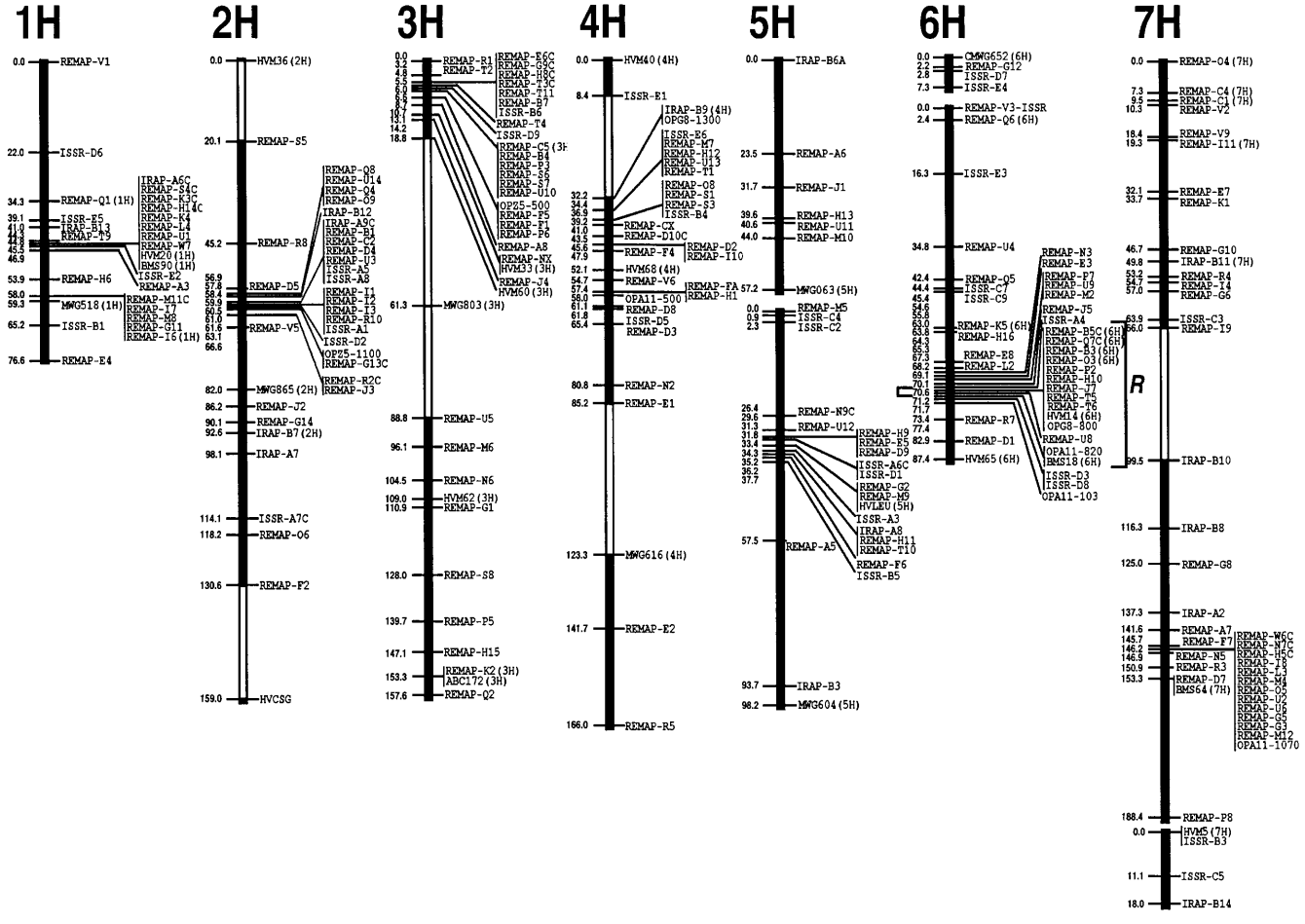

Fig. 3 Linkage map of Rolfi H CI9819 doubled haploid progeny. Markers joined by a *black bar* were grouped with a LOD of 9.5. Cumulative distances between markers are given in centiMorgans. Previously determined chromosome assignments are shown in *parentheses after* the name of the marker. The response peak of the major QTL for seedling resistance to net blotch is indicated by a *pair of brackets* (*R*) next to map positions and the marker labels on chromosome 6H

The linkage groups were joined to form chromosomes based on the anchor markers and the markers generated with the REMAP, IRAP, and ISSR systems from the barley-wheat addition lines. The linkage map of the barley genome covers 1016 cM (an average of 6.5 cM between each locus) with all seven chromosomes represented (Fig. 3). Dense clusters of markers were observed on chromosomes 2H, 6H, and 7H and gaps longer than 30 cM were present on chromosomes 3H, 4H, 5H, and 7H. Chromosomes 5H, 6H, and 7H are displayed as two parts because the orientation of the parts was not resolved unambiguously. For 29% of the mapped markers, segregation was distorted ($P < 2.15 \times 10^{-4}$). The majority of these skewed markers were located on chromosomes 2H (REMAP-R8-REMAP-O6), 3H (REMAP-R1-HVM60), and 5H (REMAP-A6-REMAP-M10). An excess of Rolfi alleles was observed for all of the skewed markers, except for ISSR-C7 on chromosome 6H, where an excess of CI9819 alleles was detected.

QTL mapping

Two loci affecting seedling resistance to net blotch were found. One major resistance gene was observed on chromosome 6H (Fig. 4). This locus explained 65% of the variation observed between the IR scores of the doubled haploid progeny lines. The peak for the resistance effect was located by both SIM (TS = 120.4) and sCIM (TS = 165.1) between the microsatellite marker BMS18 and the ISSR marker ISSR-A4. This region contains a cluster of 10 retrotransposon markers, and three other markers. The 5% error rate threshold computed from 1000 permutations was 13.0 for SIM and 39.1 for sCIM. These correspond to LOD thresholds of 2.86 and 8.60. The difference between the IRs for the two homozygotes at this locus was estimated by MQTL to be 2.29. The observed IR frequency distributions for the two homozygotes at the BMS18 locus are shown in Fig. 5. While scanning the genome for epistatic interactions, the major resistance locus at BMS18 was held constant. An epistatic locus affecting net blotch resistance was found on chromosome 5H, lying between REMAP-N9 and REMAP-F6 (Fig. 6a). This locus had an effect only when the major resistance gene on 6H was represented by alleles from Rolfi, the susceptible parent (Fig. 6b). In both of the loci affecting resistance, CI9819 alleles increased seedling resistance to net blotch.

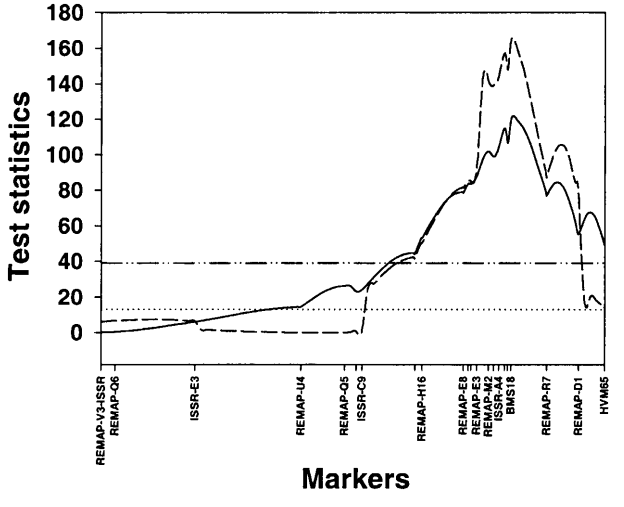

Fig. 4 QTL scan of the chromosome 6H map of Rolfi H CI9819 for seedling resistance to net blotch. Reference lines correspond to the 5% significance thresholds for SIM (*dotted line*) and sCIM (-..-..). Scans for both simple interval mapping (SIM, -) and simplified composite interval mapping (sCIM, - -) are represented. For simplicity, only 15 out of the 35 markers in this region of 6H are shown; the region between ISSR-A4 and BMS18 contains 13 markers

Discussion

Resistance to net blotch

Two loci affecting seedling resistance to net blotch in barley were mapped by using molecular markers – a major resistance gene on chromosome 6H accounting for 65% of variation and an epistatic locus on chromosome 5H giving a smaller effect. The previously mapped microsatellite markers HVM14 and HVM65 (Liu et al. 1996), which flank the resistance gene on 6H, confirm the localization of this gene to the centromeric region of the short arm of chromosome 6H. When one-LOD support intervals (Lander and Botstein 1989) were constructed for this major gene, the effective region was limited to less than 2.0 cM. The Ethiopian barley line CI9819 served as the source of resistance to net blotch in the cross with cv. Rolfi because this line had been shown to be resistant to *P. teres* isolates from several countries (Khan 1969; Bockelman et al. 1977; Robinson and Jalli 1996). Based on the small IR values scored for the F_1

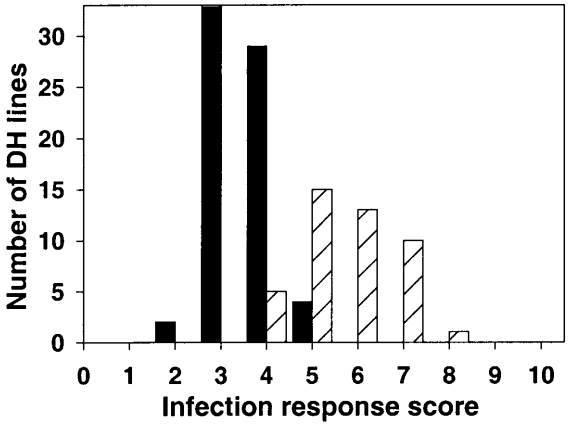

Fig. 5 Frequency distributions for the net blotch infection response scores in the doubled haploid lines carrying alleles from Rolfi (*hatched bars*) or CI9819 (*solid bars*) at the microsatellite locus BMS18

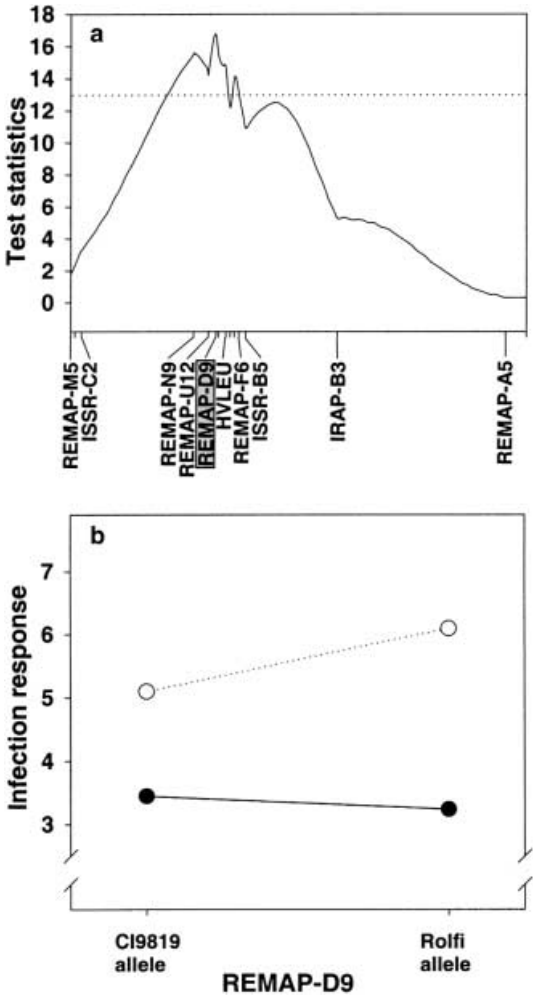

Fig. 6a Scan of the chromosome 5H map of Rolfi H CI9819 for epistatic interactions affecting seedling resistance to net blotch. Locus BMS18 was held constant during the scan. The reference line corresponds to the 5% significance threshold. **b** Phenotypic values for the different epistatic genotypes. The *closed circles* represent DH lines with CI9819 alleles and *open circles* DH lines with Rolfi alleles at the BMS18 marker locus

individuals, the major resistance gene on 6H was dominant.

Although the hypothesis of one dominant gene was supported by the results from the F_2 generation, the data for the BC_1 plants did not fit this hypothesis well, because the IRs were skewed towards the susceptible values. This could indicate that the effect of the genetic background hinders the expression of resistance genes in the BC_1 generation. Variability of the host reaction in the progenies of resistant varieties has been shown to be influenced by the susceptible parent used (Khan 1969). However, we were not able to map epistatic loci that inhibited the expression of resistance genes in the susceptible genetic background. The effect of the background genome may be due to the activity of several loci, with small individual contributions, which would be difficult to detect individually in a progeny population of the size used in this study. The epistatic locus located on 5H was a minor resistance gene, the effect of which was masked by the expression of the major resistance gene. As far as we know, epistatic interactions for net blotch resistance have not been mapped previously.

In accordance with our mapping of the major resistance gene to chromosome 6H, work by others has placed a significant QTL effect for seedling resistance in the same area of 6H in a cv. Steptoe × cv. Morex cross (Steffenson et al. 1996). In a different cross (Richter et al. 1998), one of 12 QTLs for seedling resistance, in the same region of 6H, was found both 7 and 9 days after inoculation. A QTL affecting field resistance to net blotch has also been mapped in the same chromosomal region of 6H (Spaner et al. 1998). As suggested by Robertson (1985) alleles with quantitative effects as well as alleles with qualitative effects may exist at the same locus. In contrast, earlier trisomic analysis placed three major resistance genes on chromosomes 1H, 2H, and 3H in CI9819, CI7584, and cv. Tifang (Bockelman et al. 1977), where we found no significant effects.

Differences in resistance gene number and loci may be attributed to the effects of the various environmental conditions and pathogen isolates used, as noted before (Bockelman et al. 1977; Ho et al. 1996). In the study reported here, efforts were made to control for this by mixing four Finnish net blotch isolates, even though only slight variations in virulence of the pathogen over the Nordic-Baltic area have been reported (Jalli and Robinson 2000). The IRs of the doubled-haploid progeny were evaluated separately with this mixture. The scoring stringency was reduced, Rolfi giving a score of 8–9 compared to 10 in the experiment with F_1, F_2, and BC_1 plants. Due to their continuous distribution, the IRs could not be meaningfully divided into susceptible and resistant classes. This may be caused by the use of a mixture of isolates in this experiment. The methods applied for testing seedling resistance yielded IRs of rank similar to those recorded for field-grown barley (Robinson and Jalli 1997).

Mapping with retrotransposon markers

Retrotransposons offer several advantages as molecular markers. The dispersed and abundant insertion loci of many retrotransposon families offer a rich source of marker bands. New copies are inserted but, unlike DNA transposons, not transpositionally removed, which facilitates phylogenetic analyses (Shimamura et al. 1997). Retrotransposon families vary in their activity in inserting daughter copies, allowing the matching of the family of elements used for marker generation to the phylogenetic depth required. The efficacy of retrotransposons as markers in diversity and linkage analyses has recently been established both for barley and for pea (*Pisum sativum* L.) (Waugh et al. 1997; Ellis et al. 1998; Flavell et al. 1998; Gribbon et al. 1999; Kalendar et al. 1999). Of the various techniques available, REMAP and

IRAP, applied here, have the advantage that no DNA digestions, ligations, or probe hybridizations are needed to generate the marker data, helping to increase the reliability and robustness of the results.

The major gene for net blotch resistance described here was localized with the aid of 239 *BARE*-1 and *Sukkula* retrotransposon markers, 78% of the total molecular markers used in the study. Although a set of *BARE*-1 insertion sites were mapped earlier by an anchored-AFLP method (Waugh et al. 1997), the present work represents the first use of a retrotransposon system to map a gene. It was shown earlier that retrotransposons are found near microsatellites (Kalendar et al. 1999; Ramsay et al. 1999). Of the SSR primers we used, only $(AGC)_6C$ failed to produce bands in combination with LTR primers, suggesting that microsatellites containing this repeat may have an unusual distribution.

In the cv. Rolfi H CI9819 doubled haploid lines, 29% of the markers showed distorted segregation, and the majority of these loci were located on chromosomes 2H, 3H and 5H. Segregation distortion is a common phenomenon in anther culture-derived doubled haploid progenies (Graner et al. 1991; Barua et al. 1993; Becker et al. 1995; Manninen 2000). Associations between anther culture response and molecular markers have been found on chromosomes 2H and 3H from a cv. Rolfi H cv. Botnia cross (Manninen 2000). Segregation distortion may at least partly be due to differences in genes affecting anther culture response.

A major feature of the *BARE*-1 and *Sukkula* markers is their clustering. Clustering has been observed also for AFLP markers in barley (Becker et al. 1995; Castiglioni et al. 1998) and elsewhere in high-density maps. On the low-resolution, cytological, level, *BARE*-1 is homogeneously dispersed throughout all chromosome arms but absent from centromeres, telomeres, and nucleolus-organizing regions (Suoniemi et al. 1996; Vicient et al. 1999). However, the organization of barley and other cereal genomes, on the fine scale, into gene islands and "repeat seas" (SanMiguel et al. 1996; Panstruga et al. 1998) may in part explain the clustering of either repetitive or single-copy markers at sufficiently high density. This organization is reflected in the tendency of *BARE*-1 to insert near microsatellites (Kalendar et al. 1999; Ramsay et al. 1999), and may increase the likelihood of clustering. Furthermore, not only *BARE*-1 (Suoniemi et al. 1997), but also other barley retrotransposons (Shirasu et al. 2000) tend to insert into each other. This "nesting" of integration evens has also been seen in maize (SanMiguel et al. 1996). The recent analysis of a 66-kb contiguous sequence (Shirasu et al. 2000) confirmed the clustering of retrotransposons and genes in barley and revealed a general pattern of nested insertions, in one case comprising four consecutively integrated elements.

In this mapping exercise, the clustering of markers was fortuitous. The particular segment covering 30 cM on chromosome 6H that had a significant effect on resistance when computed with sCIM contains 26 molecular markers. Several of these markers are co-dominant, indicating that manipulation of this locus in backcross breeding efforts should be relatively straightforward. Markers linked to the major resistance gene for the spot form of net blotch have been successfully used in a breeding program (Williams et al. 1999).

A dense map of molecular markers offers a starting point for map-based cloning of the resistance gene. This approach has successfully been used for cloning the *Mlo* gene for powdery mildew resistance in barley (Buschges et al. 1997; Simons et al. 1997). The conservation of the LTR termini (Suoniemi et al. 1997) contained within REMAP and IRAP marker bands facilitates the cloning and characterization of unique or low-copy-number sequences flanking the retrotransposon insertions. These sequences can then serve in the production of specific probes for isolation from large-insert genomic libraries of clones containing retrotransposon markers flanking the major net blotch resistance gene.

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