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Chromosome landing at the *Mla* locus in barley (*Hordeum vulgare* L.) by means of high-resolution mapping with AFLP markers

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Abstract The complex *Mla* locus of barley determines resistance to the powdery mildew pathogen *Erysiphe graminis* f. sp. *hordei*. With a view towards gene isolation, a population consisting of 950 F_2 individuals derived from a cross between the near-isogenic lines 'P01' (*Mla1*) and 'P10' (*Mla12*) was used to construct a high-resolution map of the *Mla* region. A fluorescence-based AFLP technique and bulked segregant analysis were applied to screen for polymorphic, tightly linked AFLP markers. Three AFLP markers were selected as suitable for a chromosome-landing strategy. One of these AFLP markers and a closely linked RFLP marker were converted into sequence-specific PCR markers. PCR-based screening of approximately 70 000 yeast artificial chromosome (YAC) clones revealed three identical YACs harbouring the *Mla* locus. Terminal insert sequences were obtained using inverse PCR. The derived STS marker from the right YAC end-clone was mapped distal to the *Mla* locus.

Key words Barley · *Mla* locus · AFLP · High-resolution mapping · Chromosome landing

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Introduction

Resistance in plants is frequently controlled by the interaction between dominant resistance (R) genes in the host and dominant avirulence (Avr) genes of the pathogen. This system is in agreement with Flor's gene-for-gene hypothesis (1955) which was first confirmed for barley powdery mildew by Moseman (1959). It is assumed that the gene product of an R gene recognizes specific signal molecules encoded by the Avr genes of the pathogen (Keen 1992). Moreover, race-specific resistance genes are often clustered in the plant genome. These complex loci are believed to be either clusters of closley linked genes or many alleles of a single resistance gene. Such a grouping has been shown in numerous well-documented plant-pathogen systems in agriculturally important crops including flax-*Melampsora lini* (Islam and Shepherd 1991), lettuce-*Bremia lactucae* (Farrara et al. 1987), maize-*Puccinia sorghi* (Richter et al. 1995), wheat-*Puccinia recondita* f. sp. *tritici* (McIntosh et al. 1995) and wheat-*Erysiphe graminis* f. sp. *tritici* (Zeller et al. 1993). In the case of powdery mildew of barley (*Erysiphe graminis* f. sp. *hordei*) multiple allelism has been confirmed for different resistance loci (Jørgensen 1994). At the *Mla* locus on the short arm of chromosome 1H of barley 34 different alleles or several closely linked loci (Kintzios et al. 1995) have been identified to-date and therefore detailed genetical studies have been focused on this genomic region. Rare recombination events between different *Mla* alleles were described supporting the idea of a close linkage of different resistance genes (Jahoor et al. 1993; Mahadevappa et al. 1994). To clarify the arrangement and function of the different *Mla* alleles it will be necessary to reveal the molecular organisation and the sequence of this genomic region and to identify the resistance gene. Since the first cloning of a plant race-specific R gene in tomato, *Pto*, (Martin et al. 1993) the molecular structure of several R genes from

different plant species has been described (Wenzel 1998). Generally, only the effect of the R genes on the phenotype can be recognized, and no information exists about the gene product. Therefore two strategies for the molecular cloning of R genes are followed: transposon tagging and map-based cloning. Whereas the first approach requires a functional transposon system in the species of interest, the map-based cloning strategy can be used for all plant species with sexual reproduction. This technique includes high-resolution mapping of the R gene of interest, physical mapping, chromosome walking by the aid of high-molecular-weight (HMW) DNA clones like YACs and BACs to construct a contig containing the target gene, identification of a potential coding region via the sequencing of subclones or the screening of a cDNA library, and finally verification of the candidate clone by a complementation experiment (Wing et al. 1994). Following this strategy several race-specific R genes have been isolated (Baker et al. 1997). The development of new PCR-based marker techniques like AFLPs (Vos et al. 1995) and the availability of near-isogenic lines (NILs) or the use of bulked segregant analysis (Michelmore et al. 1991) facilitates the finding of closely linked molecular markers followed by high-resolution mapping using large progenies. Together with YAC libraries (Kleine et al. 1993, 1997) all these tools are available for a map-based cloning approach in barley. Since chromosome walking can be complicated by the occurence of chimeric or rearranged HMW clones, and moreover in plants with large and complex genomes by the amount of DNA, the frequency of repetitive DNA and the non-finding of useful endclones, chromosome landing (Tanksley et al. 1995) is the most promising strategy to establish the physical coverage of the region of interest. Thus, one or more molecular markers are required within a physical distance from the target gene that is less than the average insert size of the YAC or BAC library being used for screening with the relevant markers. The detection of flanking molecular markers on a single genomic clone implies landing on the target gene without walking. The feasibility of this approach within large genomes was demonstrated through the successful cloning of the *mlo* resistance gene in barley (Büschges et al. 1997). In the present paper we describe the construction of a high-resolution genetic map around the *Mla* locus by the combined application of the AFLP technology and NILs. Subsequently a YAC clone was isolated covering the complete *Mla* region, providing the first step towards the map-based cloning of *Mla*.

Materials and methods

Plant material

Two near-isogenic lines (NILs) 'P01' and 'P10', developed by Kølster et al. (1986), and the recurrent parent 'Pallas' were screened to isolate polymorphic molecular markers which are tightly linked to the *Mla* locus. NIL 'P01' contains a large donor fragment from 'Algerian' carrying *Mla1,* whereas NIL 'P10' contains a small donor fragment from 'Arabische' carrying *Mla12.* High-resolution mapping was based upon the segregating data of an F_2 population with 943 individuals derived from a cross between 'P01' and 'P10'.

Powdery mildew tests

Powdery mildew tests were carried out at the seedling stage using detached leaves placed on agar containing 30 mg/l of benzimidazol and 50 mg/l of ampicillin. The F_2 seedlings were raised under controlled conditions in a growth chamber to avoid contamination. Inoculation was performed with the powdery mildew isolates 'Ar4' and '201–60' differentiating the alleles *Mla1* and *Mla12.* Further analysis was conducted according to Jahoor et al. (1993). To confirm the results and to obtain a complete segregation of all posssible F_2 classes, approximately 20 000 seedlings from the F_3 generation were re-tested with the same isolates as described above.

RFLP and AFLP analysis

Genomic DNA from young leaves was extracted and purified using the CTAB method (Saghai-Maroof et al. 1984). Yeast high-molecular-weight DNA was isolated as described by Michalek et al. (1997).

Cleavage with different restriction enzymes, Southern blotting and hybridisation were performed according to Jahoor et al. (1991). All RFLP clones used in this investigation originated from the MWG collection (Graner et al. 1991; Jahoor et al. 1991).

AFLP analysis was carried out as described in the AFLP plant-mapping kit (PE Applied Biosystems). For selective amplification *Eco*RI and *Mse*I primers with three additional nucleotides were employed. For multifluorophore fragment analysis on an ABI Prism™ 377 DNA Sequencer the *Eco*RI primers were either labelled with 5-carboxy-fluorescein (5-FAM), 2′,7′-dimethoxy-4′,5′-dichloro-6-carboxy-fluorescein (JOE), or N,N,N′,N′-tetramethyl-6-carboxy-rhodamin (TAMRA). For calculating fragment sizes the internal Genescan-500 ROX length standard (PE Applied Biosystems) labelled with 6-carboxy-X-rhodamin (ROX) was used. Semi-automated AFLP fragment analysis was performed with GeneScan™ analysis software version 2.0.2 and Genotyper™ analysis software version 2.0 (PE Applied Biosystems). For isolation of specific AFLP fragments, the *Eco*RI primer was labelled with [γ-^{33}P] ATP (Amersham) using 0.1 U of T4 polynucleotide kinase (Pharmacia) for 30 min at 37°C. AFLP reactions, electrophoresis and visualisation were carried out with minor modifications according to Vos et al. (1995). The AFLP fragments were excised from the dried polyacrylamide gel after exposure and eluted with 50 µl of H_2O. Ten microliters of the suspended DNA were re-amplified with the specific AFLP primers. The amplification products were cloned into the pGEM-T Easy vector (Promega) and sequenced by Thermo Sequenase chemistry (Amersham).

YAC analysis

The YAC libraries used for screening were described by Kleine et al. (1993, 1997). YAC library screening was carried out as described by Michalek et al. (1997). Inverse PCR (IPCR) was performed with several modifications according to Silverman (1993) to isolate YAC insert terminal sequences. The PCR products were cloned and sequenced as described above.

STS and CAPS analysis

The RFLP marker MWG2197, the flanking AFLP marker ACT/CAA-271, and the YAC ends YAC2197 Al and YAC2197 Ar,

Table 1 Primer sequences, PCR conditions and restriction endonucleases for converted RFLP, AFLP and YAC end clones

Marker	Clone type	Primers	PCR conditions	Restriction endonucleases
aMWG 2197	RFLP	5′-TACTGAAGATTCGTCTGTGAAGG-3′ 5′-AAAGAAAAGAAACAAGCCAT-3′	94°C, 30 s 55°C, 30 s 72°C, 1 min } × 30 cycles	*Scr*f I
*aACT**CAA	AFLP	5′-TCCCACCGTTCCCATCCA-3′ 5′-CACTGAGTTCCGATTCAAGCAC-3′	94°C, 30 s 60°C, 30 s 72°C, 1 min } × 30 cycles	–
aYAC2197 Al	YAC end clone	5′-TCAACTAGAGGCTTACTAGGGAC-3′ 5′-GACCAAAATATGGTAGATTCATT-3′	94°C, 30 s 55°C, 30 s 72°C, 1 min } × 30 cycles	–
aYAC2197 Ar	YAC end clone	5′-ATTCAACATCAACCGGCTTATA-3′ 5′-GCATCGGCCCATTACTTTC-3′	94°C, 30 s 60°C, 30 s 72°C, 1 min } × 30 cycles	*Mae*I

were converted into STS markers. For the detection of polymorphisms, amplicons of the RFLP marker MWG2197 and the right YAC end were digested with appropriate restriction endonucleases. All primer sequences, PCR conditions and restriction endonucleases used for STS or CAPS analysis are listed in Table 1.

Linkage analysis

The genetic distances between the alleles *Mla1, Mla12* and the flanking RFLP markers MWG60, MWG68 were calculated with Mapmaker 3.0 software using the Kosambi mapping function with a LOD score of 3.0 (Kosambi 1944). The distance and localisation of the tightly linked RFLP and AFLP markers were specified in terms of the number of recombination events in the phenotype.

Results

Screening for closely linked AFLP markers

The near-isogenic character of the parental lines 'P01' and 'P10' allows direct screening for tightly linked molecular markers. For detecting polymorphic AFLP markers between the lines 'Pallas', 'P01' and 'P10', 256 combinations using 16 *Eco*RI + 3 and 16 *Mse*I + 3 primers were analysed. Approximately 18 000 fragments were amplified with an average number of 80 fragments per primer combination. Six fragments showing polymorphism between 'P01' and 'P10' were amplified from the DNA of 'P01' and four fragments from the DNA of 'P10'. Pools of 20 homozygous resistant plants and 20 homozygous susceptible plants from the segregating F_2 population were used in a bulked segregant analysis to confirm these results. Out of the ten previously detected polymorphic markers only three also revealed polymorphisms between the phenotypic pools (Fig. 1) and these were designated ACT/CAA-271, ACT/CCC-101 and AGG/CTG-151 according to the additional nucleotides of the selective primer pair used for AFLP amplification and the fragment length (bp).

High-resolution mapping

Leaf segments from 943 F_2 plants of a cross between the NILs 'P01' and 'P10' were inoculated with two single-spore powdery mildew isolates ('201-60' avirulent on 'P01' containing *Mla1* and virulent on 'P10' containing *Mla12,* 'Ar4' virulent on 'P01' and avirulent on 'P10'). The observed ratio for the two *Mla* alleles was consistent with a 3 : 1 segregation as expected for dominant resistance genes. In order to obtain a detailed F_2 classification, 20 leaf segments from all F_3 families were re-tested using the powdery mildew isolates. The segregation ratio of 1 : 2 : 1 was verified with a chi-square test ($\chi^2 = 4.577$; *df* 2; $P < 0.05$). Between *Mla1* and *Mla12* no recombination event was detected, supporting the assumption of multiple allelism.

Previous studies identified several RFLP markers with close linkage to the *Mla* locus (Schüller et al. 1992; Graner et al. 1994). The co-dominant RFLP markers MWG60 and MWG68 were selected as flanking anchors to screen for all recombination events around the *Mla* locus. Seventy five plants were detected exhibiting recombination events in this interval. Thirty five recombinant individuals have a breakpoint between the distal marker MWG60 and *Mla1*/*Mla12*, and 40 between the proximal marker MWG68 and *Mla1*/*Mla12.* Two recombinant plants possessed a new allelic arrangement that could be caused by a double-crossover event or by gene conversion. A detailed description of all detected recombination classes is presented in Fig. 2. High-resolution mapping was conducted with segregation data obtained from these 75 recombinant plants. In a first step the co-dominant RFLP markers MWG2083 and MWG2197 were integrated in the mapping interval. The probe MWG 2083 showed four

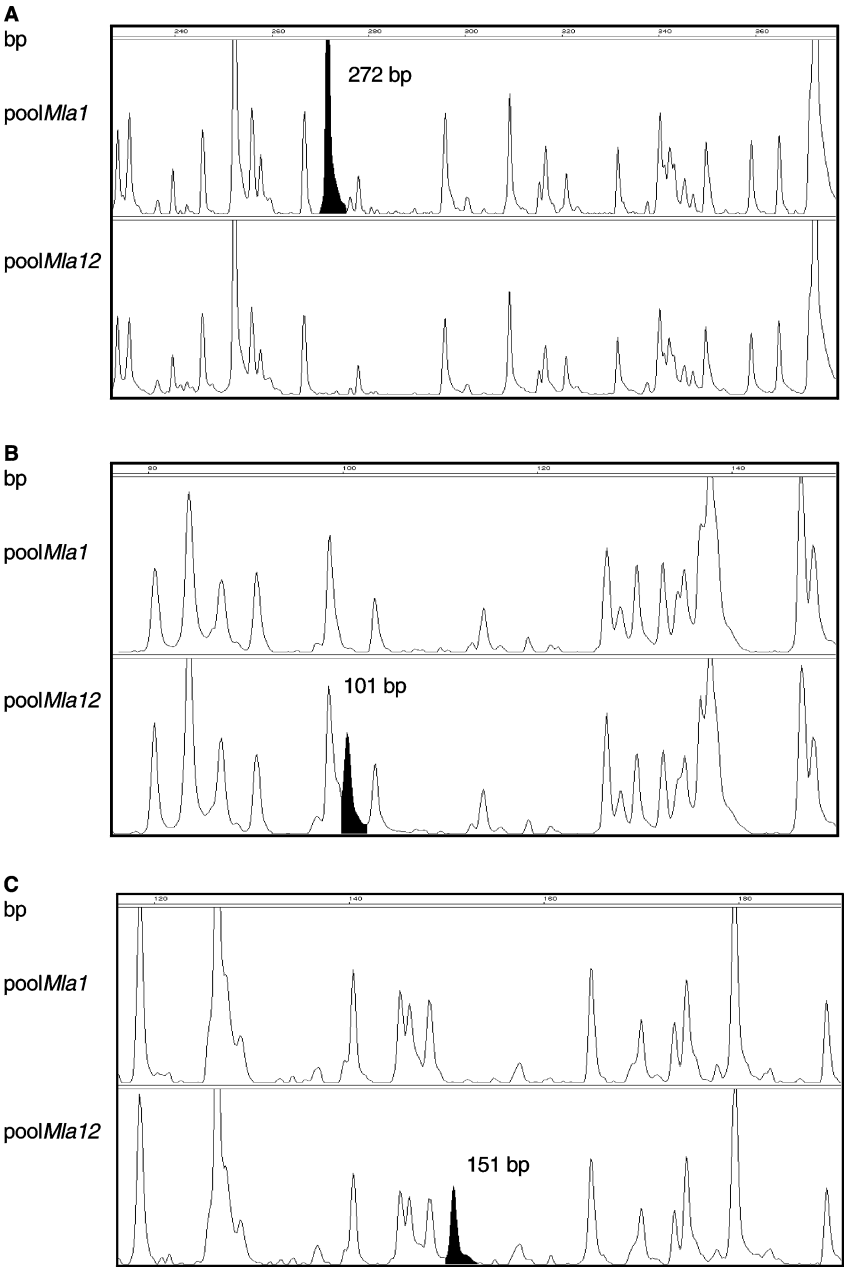
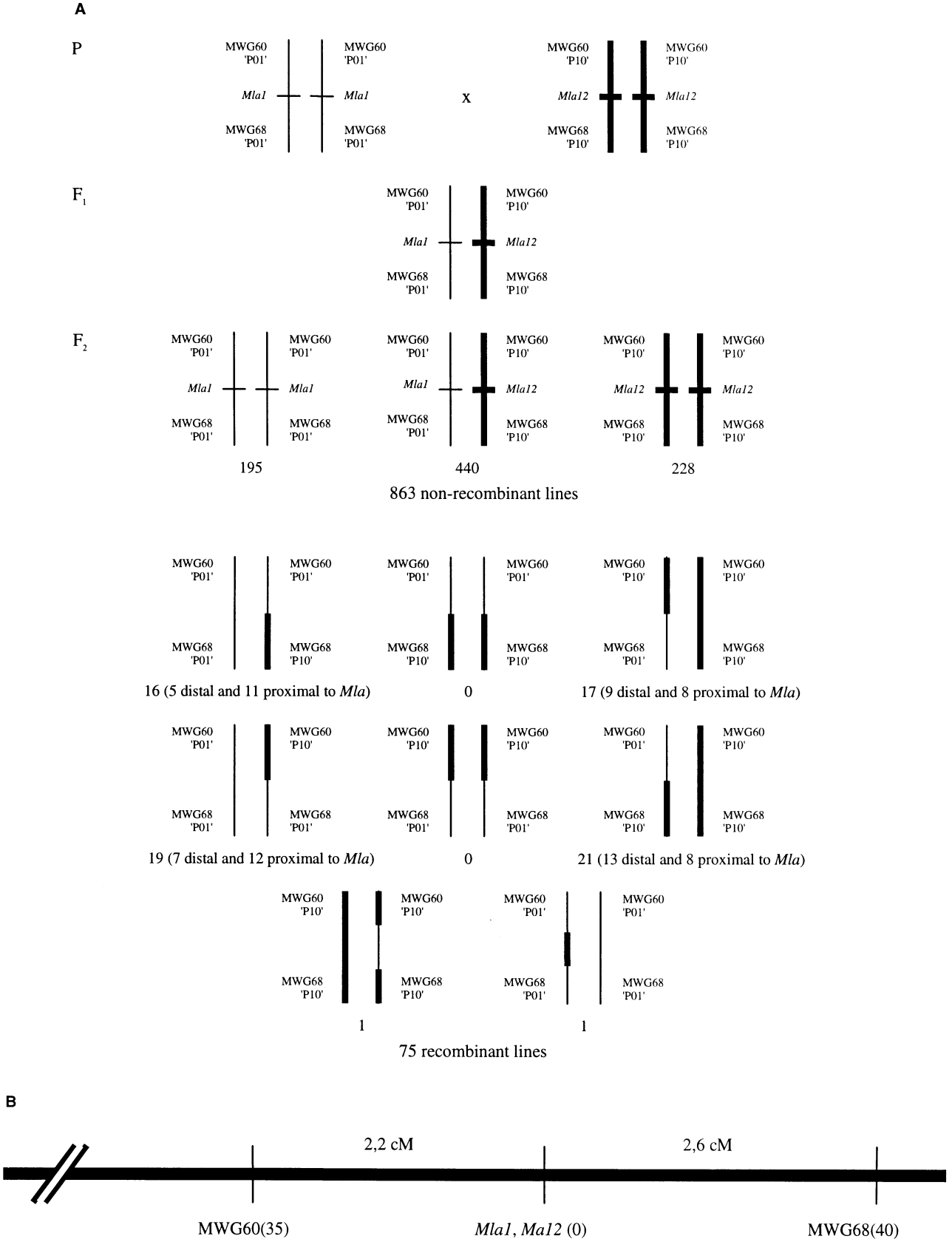


Fig. 1 A–C Electropherograms showing polymorphic AFLP patterns detected with bulked segregant analysis for alleles *Mla1* and *Mla12*. **A** Primer combination E-ACT M-CAA, fluorescence dye 5-FAM. **B** Primer combination E-ACT M-CCC, fluorescence dye 5-FAM. **C** Primer combination E-AGG M-CTG, fluorescence dye JOE

recombination events relative to the *Mla* locus, two of which cannot be explained by a single double-strand recombination event. With MWG2197 only two plants with recombination events were detected, again suggesting the occurrence of a double crossover or a gene conversion. For PCR-based screening of the YAC libraries the clone MWG2197 was converted into a STS marker. The PCR products of the lines 'P01' and 'P10' were monomorphic. However, after sequencing the parental alleles, a point mutation creating a *Scr*fI restriction site was detected in line 'P10'. Based on this result the RFLP marker was converted into a CAPS marker, designated aMWG2197 (Fig. 3). Mapping of aMWG2197 confirmed the segregation results of MWG2197. Subsequently, the dominant AFLP markers were integrated in the map. ACT/CCC-101 co-segregated with the *Mla* locus, whereas ACT/CAA-271 showed two proximal, and AGG/CTG-151 one

Fig. 2 A, B Linkage analysis with flanking RFLP markers. **A** Derivation of mapping population from a cross between NILs 'P01' and 'P10'. All detected recombination events are shown within a interval, flanked by the co-dominant RFLP markers MWG60 and MWG68. **B** RFLP linkage map of the *Mla* region on chromosome 1HS. Map distances are given in centimorgans (*cM*). *Numbers in brackets* represent the recombination events relative to the *Mla* locus


A
P
MWG60 'P01'
MWG60 'P01'
Mla1
Mla1
MWG68 'P01'
MWG68 'P01'
x
MWG60 'P10'
MWG60 'P10'
Mla12
Mla12
MWG68 'P10'
MWG68 'P10'
F1
MWG60 'P01'
MWG60 'P10'
Mla1
Mla12
MWG68 'P01'
MWG68 'P10'
F2
MWG60 'P01'
MWG60 'P01'
Mla1
Mla1
MWG68 'P01'
MWG68 'P01'
195
MWG60 'P01'
MWG60 'P10'
Mla1
Mla12
MWG68 'P01'
MWG68 'P10'
440
MWG60 'P10'
MWG60 'P10'
Mla12
Mla12
MWG68 'P10'
MWG68 'P10'
228
863 non-recombinant lines
MWG60 'P01'
MWG60 'P01'
MWG68 'P01'
MWG68 'P10'
16 (5 distal and 11 proximal to Mla)
MWG60 'P01'
MWG60 'P01'
MWG68 'P10'
MWG68 'P10'
0
MWG60 'P10'
MWG60 'P10'
MWG68 'P01'
MWG68 'P10'
17 (9 distal and 8 proximal to Mla)
MWG60 'P01'
MWG60 'P10'
MWG68 'P01'
MWG68 'P01'
19 (7 distal and 12 proximal to Mla)
MWG60 'P10'
MWG60 'P10'
MWG68 'P01'
MWG68 'P01'
0
MWG60 'P01'
MWG60 'P10'
MWG68 'P10'
MWG68 'P10'
21 (13 distal and 8 proximal to Mla)
MWG60 'P10'
MWG60 'P10'
MWG68 'P10'
MWG68 'P10'
1
MWG60 'P01'
MWG60 'P01'
MWG68 'P01'
MWG68 'P01'
1
75 recombinant lines
B
2,2 cM
2,6 cM
MWG60(35)
Mla1, Mla12 (0)
MWG68(40)

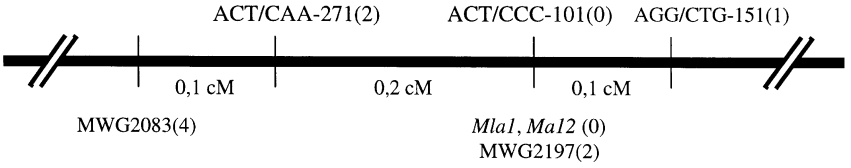


Fig. 3 High-resolution map of the *Mla* region on chromosome 1HS. Map distances are given in centimorgans (cM). Numbers in brackets represent the recombination events relative to the *Mla* locus

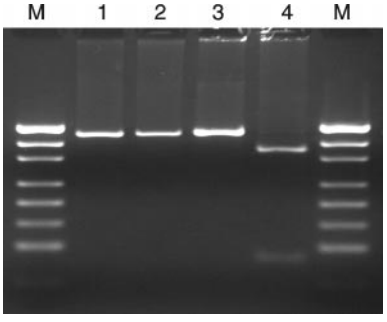


Fig. 4 Bulked segregant analysis of CAPS marker aMWG2197. *1* Pool *Mla*1, *2* pool *Mla12*, *3* pool *Mla1* digested with *Scr*fI, *4* pool *Mla12* digested with *Scr*fI, *M* pUC19 DNA/*Msp*I

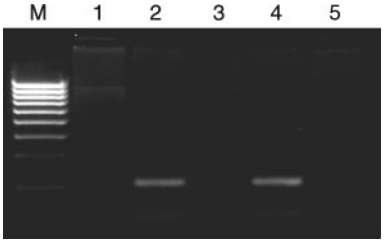


Fig. 5 Bulked segregant analysis of STS marker aACT/CAA. *1* 'Pallas', *2* 'P01', *3* 'P10', *4* pool Mla1, *5* pool Mla12, *M* 100 bp ladder

distal, recombination breakpoints relative the *Mla* locus. The high-resolution map is shown in Fig. 4. Since the fragments of ACT/CCC-101 and AGG/CTG-151 were too short to design adaequate primers, only ACT/CAA-271 could be converted into a STS marker, designated aACT/CAA. Its dominant character in the mapping population results from amplifying a PCR product only from the DNA of parent 'P01' (Fig. 5). Segregation analysis exhibited no difference to previous AFLP mapping.

YAC analysis

Approximately 70 000 clones from two YAC libraries were screened with aMWG2197 by a PCR-based approach. One positive clone with an insert of 300 kb was found in a pYAC4 library constructed from *Eco*RI partially digested DNA of the barley cultivar 'Franka' (Kleine et al. 1993). This clone was not analysed further due to its instability. Three independent positive clones, each 180 kb in size, were isolated from a pYAC-RC library constructed from *Mlu*I completely digested DNA of the barley cultivar 'Franka' (Kleine et al. 1997). They were designated YAC2197 A, B and C, respectively. To test if any of the identified AFLP markers are located on these YAC clones, AFLP analysis was carried out. The AFLP markers ACT/CAA-271, ACT/CCC-101 and AGG/CTG-151 were amplified with the DNA of all three YAC lysates but not with the DNA of the host strain as a target (Fig. 6 A–C). With respect to the localisation of ACT/CAA-271 and AGG/CTG-151, the observations indicate that all three YAC clones encompass the *Mla* region. This finding was confirmed by hybridisation experiments, resulting in specific positive signals for flanking AFLP markers with YAC DNA from YAC2197 A (Fig. 7 A–C). Furthermore a PCR product was amplified from YAC DNA with the STS primers from aACT/CAA, and the sequencing data of this amplicon were identical with the data of the AFLP fragment.

To elucidate the orientation of YAC insert terminal regions relative to the *Mla* locus, IPCR was used to isolate the end fragments of the YAC2197 A insert. The restriction enzymes *Hae*II and *Alu*I were employed for the right arm, and *Eco*RV and *Sau*3AI for the left arm. Only with *Sau*3AI and *Alu*I were suitable PCR products with a size of 242 bp and 635 bp, respectively, obtained via IPCR. The sequence of the *Sau*3AI fragment showed high similarity to the long terminal repeats of the BARE-1 copia-like retroelement of barley. The repetitive character of this element did not allow the conversion of the left end fragment into a specific STS marker. On the other hand, the *Alu*I fragment, designated YAC2197 Ar, could be converted into a STS marker. After the digestion of amplicons from parental DNA with the restriction enzyme *Mae*I a polymorphism was revealed, enabling genetic mapping. CAPS marker aYAC2197 Ar exhibited two recombination events relative to the phenotype and was localised between the markers MWG2083 and ACT/CAA-271, distal to *Mla*.

Discussion

The most time-consuming step of high-resolution mapping is the discovery and localisation of tightly linked

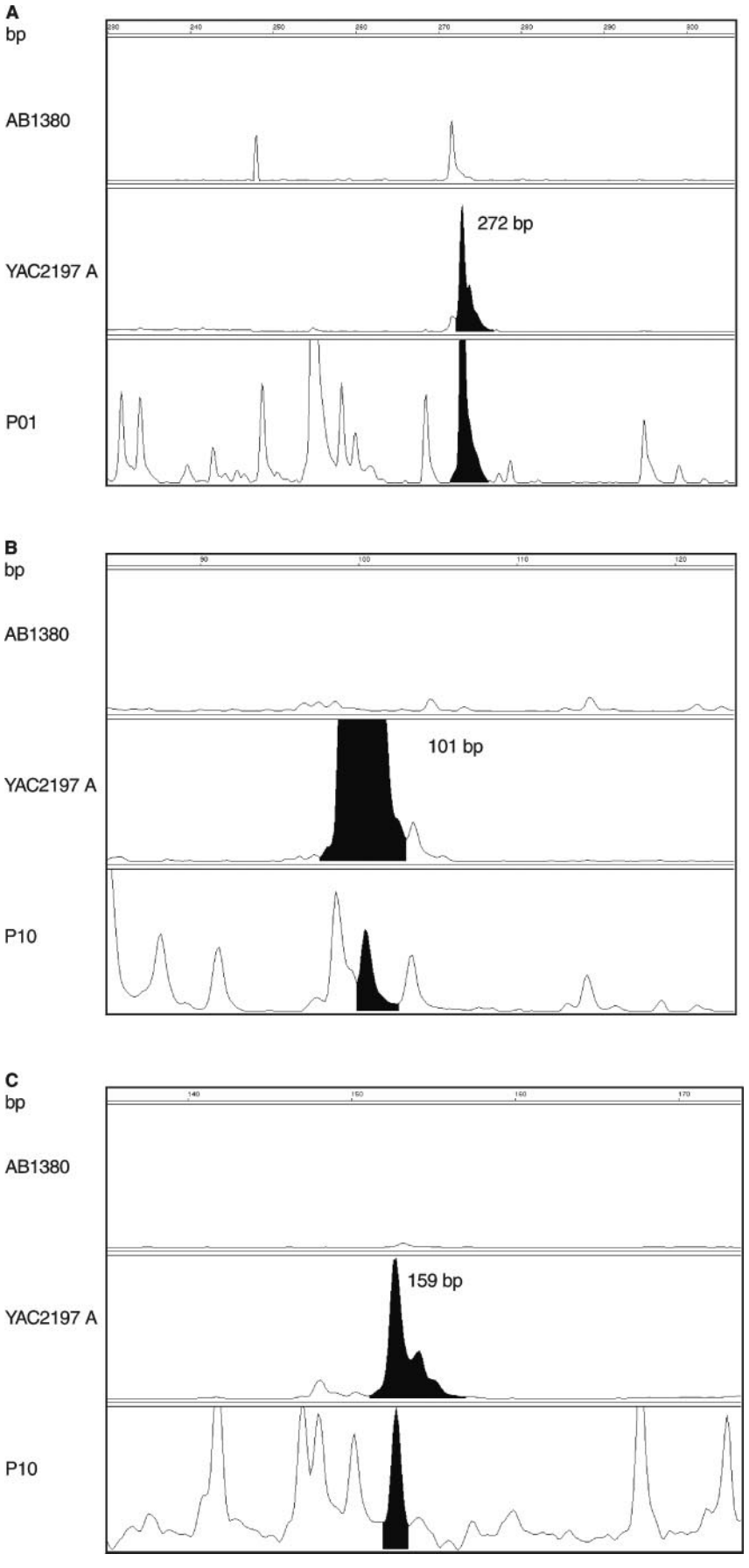


Fig. 6 A–C AFLP analysis with DNA from YAC2197 A and host strain AB1380. Electropherograms of the corresponding parental line are shown as a positive control. **A** Primer combination E-ACT M-CAA, fluorescence dye 5-FAM. **B** Primer combination E-ACT M-CCC, fluorescence dye 5-FAM. **C** Primer combination E-AGG M-CTG, fluorescence dye JOE

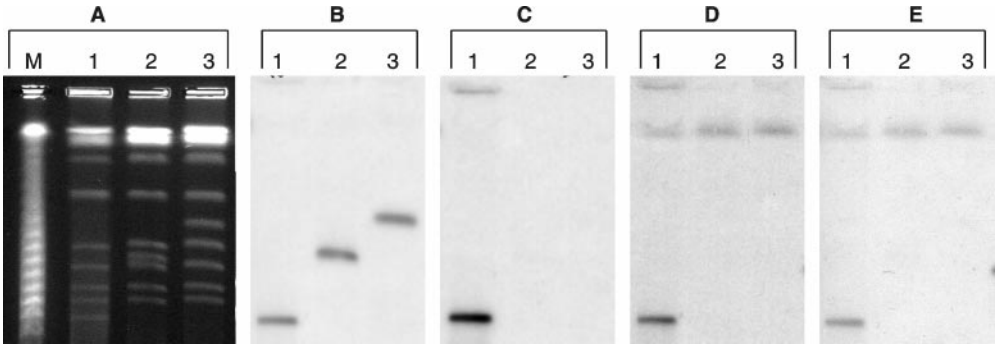


Fig. 7 A–E PFGE analysis of YAC clone YAC2197 A (*lane 1*) and two anonymous YAC clones (*lanes 2 and 3*). For a size standard a λ-ladder (*M*) was employed. **A** Ethidium bromide-stained pulsed-field gel. Autoradiographs with probes **B** MWG2197, **C** pBR322, **D** ACT/CAA and **E** AGG/CTG

molecular markers. For acceleration of this process, the application of a multifluorophore and semi-automated AFLP analysis on an ABI sequencer provides an expeditious and accurate method. Fluorescence-based semi-automated genotyping of microsatellites in humans was first reported by Ziegle et al. (1992) and, with modifications, this technique has been applied for AFLP fragment analysis, as demonstrated in the present study. In comparison with ^{33}P-labelled fragments the detection of fluorescently labelled fragments facilitates: (1) a higher throughput of samples, and (2) a higher sensitivity and resolution, resulting in more detectable fragments per primer combination in a size range from 100 to 600 bp (data not shown). Furthermore, errors occurring by manual sample- and data-handling are eliminated using semi-automated geno-typing. The segregation analysis of the STS marker aACT/CAA revealed no difference to the semi-automated segregation analysis of the AFLP marker ACT/CAA-271 with GeneScan™ and Genotyper™ analysis software, confirming its reliability.

In previous studies NILs were successfully applied to isolate tightly linked markers for a gene of interest (Young et al. 1988; Hilbers et al. 1992; Schüller et al. 1992). Assuming a genome size of 1433 cM and an average chromosome length of 205 cM for barley (Graner et al. 1994), the ratio of donor chromatin to the entire chromatin of a BC_5F_6 generation should not be more than 3.7% (Muehlbauer et al. 1988). By surveying 18 000 AFLP fragments, 26 showed polymorphism between the recurrent parent 'Pallas' and 'P01' and 22 between 'Pallas' and P10, among them ten loci polymorphic for 'P01' and 'P10'. Therefore, an average portion of 0.1% of the donor chromatin can be calculated. This remarkably low level of polymorphism can be explained by the strategy employed by Kølster et al. (1986) to select against large donor elements at the *Mla* locus. Flanking hordein loci *Hor1* and *Hor2* were screened to select for the genotype of the recurrent parent 'Pallas'. Both NIL used in the present study exhibited the 'Pallas' genotype at these loci after backcrossing. Therefore, only small donor fragments remained at the *Mla* region of 'P01' and 'P10'. For 'P01' these findings were confirmed by RFLP analysis where even closely linked markers identified the RFLP pattern of 'Pallas' (Schüller et al. 1992). Furthermore, it can be estimated that only 36.6% of the detected polymorphic loci should be located on non marker chromosomes. Therefore, six out of the ten detected polymorphic AFLP markers ought to be located on the introgressed donor fragment on the marker chromosome. The fact that only three of ten polymorphic loci were tightly linked to the *Mla* locus can be explained by the existence of additional genes required for race-specific resistance. During the development of NILs such required genes remain unavoidably in the backcross population. Therefore these genomic regions show polymorphism by NIL screening but not by BSA analysis. Jørgensen (1988) described three mutagen-induced suppressor genes that modified the race-specific resistance reaction of *Mla12.* The three loci were not linked to the *Mla* locus. Freialdenhoven et al. (1994) found allelism for two of these suppressor genes located on chromosome 2H and later designated as *Rar1*. The other locus was designated *Rar2.* Jørgensen (1996) discovered that none of the three suppressors affect the phenotypic expression of the resistance gene *Mla1.* To clarify the coupling of the six unlinked AFLPs to such required genes for resistance it is necessary to localize them in an adaequate mapping population. For further analysis they will be converted into STS markers.

The co-dominant character of RFLP markers MWG60, MWG68, MWG2197, MWG2803, and of the *Mla* alleles allowed the detection of all possible recombination events. Therefore, even rare rearrangements were detected. For the genetic markers *Mla,* MWG2197 and MWG2083, located inside the mapping interval, two recombinant plants in each case were detected possessing an allelic composition different from that of bordering loci. Such recombination events without an exchange of closely linked flanking molecular markers could not be explained by a reciprocal crossover event, but rather by double a crossover or a gene conversion. Szostak et al. (1983) proposed a double-strand-break repair model, involving a repair mechanism for mismatches in heteroduplex DNA areas during meiosis to

be responsible for recombination. Non-crossover events were already assumed to occur at the resistance loci *Rp1* in maize (Hu and Hulbert 1994) and *mlo* in barley (Büschges et al. 1997). For the *Mla* locus Mahadevappa et al. (1994) described two recombination events without an exchange of flanking markers. Observations in yeast indicate that the frequency of gene conversions depends on their proximity to recombination initiation sites (Detloff et al. 1992), suggesting that the *Mla* region could be a hotspot for recombination. This speculation is supported by rearrangements or duplications occurring near the *Mla* locus (Shewry et al. 1990; Descenzo et al. 1994; 1996). The ratio between genetic and physical distance for the *Mla* region can be estimated as 0.57 cM/Mb, taking into consideration the fact that a YAC clone 170 kb in length covers a genetic map distance of 0.3 cM. Because for barley the average relationship of physical to genetic distance is about 3.7 Mb/cM (genome size of 5300 Mb, map length of 1433 cM), an approximately seven-fold higher recombination rate was observed.

The sequencing data of the flanking AFLP marker ACT/CAA-271 were employed for data-base searches using the BLAST programs (Altschul et al. 1990). The deduced amino-acid sequence showed 63% identity and 76% similarity to a putative resistance protein described by Leister et al. (1998). This R-gene candidate was derived via a PCR-based approach using degenerate primers designed for the conserved motifs of already known R genes: a nucleotide-binding-site (NBS) domain and a leucine-rich repeat (LRR) domain. Close linkage, as well as co-segregation, of resistance gene homologues with plant R genes was detected in several investigations (Kanazin et al. 1996; Leister et al. 1996, 1998; Yu et al. 1996; Feuillet et al. 1997; Lagudah et al. 1997). However, complex resistance loci can be composed of several R-gene homologues, as has been demonstrated for the *Cf9* locus in tomato (Parniske et al. 1997) and for the *M* locus in flax (Anderson et al. 1997). Clustering of R-gene homologues makes it difficult to ascertain that the tagged member of the multigene family encodes for the phenotypic expression of resistance. Since two recombination events were detected between the molecular marker ACT/CAA-271 and the *Mla* locus, the sequence is not a putative candidate for encoding *Mla1* or *Mla12*. Isolation of resistance-gene homologues is not necessarily a valuable tool for tagging resistance genes. However, they are probably suitable for finding closely linked genetical markers, the most critical step for map-based cloning.

We are currently screening cDNA libraries, generated from un-infected and infected leaves, with DNA from YAC2197 A, to isolate a candidate cDNA clone. We have detected recombinant plants generated via diallel crosses between several near-isogenic lines carrying different *Mla* alleles having a new specificity to different powdery mildew isolates. If such an altered phenotype is coincident with mutation sites of a cDNA clone strong arguments are afforded for a candidate encoding *Mla*. The definitive evidence requires a complementation experiment of a mutant phenotype by transformation.

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