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RFLP mapping of three new loci for resistance genes to powdery mildew (*Erysiphe graminis* f. sp. *hordei*) in barley

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Abstract Three new major, race-specific, resistance genes to powdery mildew (*Erysiphe graminis* f. sp. *hordei*) were identified in three barley lines, 'RS42-6*O', 'RS137-28*E', and 'HSY-78*A', derived from crosses with wild barley (*Hordeum vulgare* ssp. *spontaneum*). The resistance gene origining from wild barley in line 'RS42-6*O', showed a recessive mode of inheritance, whereas the other wild barley genes were (semi)-dominant. RFLP mapping of these three genes was performed in segregating F_2 populations. The recessive gene in line 'RS42-6*O', was localized on barley chromosome 1S (7HS), while the (semi)-dominant genes in lines 'RS137-28*E', and 'HSY-78*A', were localized on chromosomes 1L (7HL) and 7L (5HL), respectively. Closely linked RFLP clones mapped at distances between 2.6 cM and 5.3 cM. Hitherto, specific loci for powdery mildew resistance in barley had not been located on these chromosomes. Furthermore, tests for linkage to the unlocalized resistance gene *Mlp* revealed free segregation. Therefore, these genes represent new loci and new designations are suggested: *mlt* ('RS42-6*O'), *Mlf* ('RS137-28*E'), and *Mlj* ('HSY-78*A'). Comparisons with mapped QTLs for mildew resistance were made and are discussed in the context of homoeology among the genomes of barley (*H-vulgare*), wheat (*Triticum aestivum*), and rye (*Secale cereale*). Duplications of RFLP bands detected in the neighbourhood of *Mlf* and *mlt* might indicate an evolutionary interrelationship to the *Mla* locus for mildew resistance.

Key words *Hordeum vulgare* ssp. *spontaneum* *Erysiphe graminis* f. sp. *hordei* · Mildew resistance · RFLP mapping · Homoeology

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Introduction

Powdery mildew caused by *Erysiphe graminis* D. C. f. sp. *hordei* is an obligate parasite and one of the most important diseases of barley in temperate climates. Based on the gene-for-gene hypothesis of Flor (1955), which was confirmed for powdery mildew of barley by Moseman (1959), many race-specific powdery mildew resistance genes from different origins have been recognized in barley (Moseman 1955; Wiberg 1974). Mapping studies have localized these genes on chromosomes 4 (4H), 5 (1H), and 6 (6H) (Jørgensen 1993). Recently, the *MlLA*-mildew resistance gene was mapped on chromosome 2 (2H) by means of RFLP markers (Hilbers et al. 1992; Giese et al. 1993).

RFLP (Restriction Fragment Length Polymorphism) mapping is a powerful means to localize genes in plant genomes without knowledge of their function or their sequence (Beckmann and Soller 1983; Tanksley 1983). Many resistance genes of graminacious species have been marked with RFLP clones, e. g. the complex resistance locus *Rp1* for resistance to *Puccinia sorghi* in maize (Hulbert and Bennetzen 1991), the gene *Xa21* for resistance to bacterial blight in rice (Ronald et al. 1992), the *ym4* gene for resistance to barley yellow mosaic virus or barley mild mosaic virus (Graner and Bauer 1993), the genes *Pm1, Pm2, Pm3* (Hartl et al. 1993, 1995; Ma et al. 1994), *Pm4* (Ma et al. 1994) and *Pm12* (Jia et al. 1994) for resistance to *E. graminis* in wheat.

Loci for resistance to powdery mildew of barley, such as *Mla* (Schüller et al. 1992), *MlLA* (Hilbers et al. 1992), *mlo* (Hinze et al. 1991) and *Mlg* (Görg et al. 1993), which are widely used in barley breeding, have also been marked with RFLP clones. One of the long-term aims is to isolate these genes by map-based cloning (Paterson and Wing 1993).

Accessions of *H. vulgare* ssp. *spontaneum* lines from Israel have repeatedly been described as a very rich gene pool for powdery mildew resistance (Moseman 1955; Fischbeck et al. 1976). Many resistances were identified,

but allelism or close linkage with already known loci for mildew resistance has been determined for only some of them (Jahoor 1987; Jahoor and Fischbeck 1987 a, b).

The objective of the present study was to identify new major genes for powdery mildew in barley lines derived from *H. vulgare* ssp. *spontaneum*, and localize them by the application of molecular markers.

Materials and methods

Plant material

'RS137-28*E', 'RS42-6*O', 'HSY-78*A', and 'D * 1B-87B' are random sampled (RS) barley lines from the F_7 bulks between accessions of *H. vulgare* ssp. *spontaneum* ('137-28', '42-6', 'HSY-78', '1B-87') collected in Israel, and barley cultivars ['Elgina; (E), 'Oriol' (O), 'Aramir' (A), 'Diamant' (D)], and are therefore called wild barley derived lines (Jahoor 1987). In each generation from F_2 to F_7 single-plant selections were made for mildew resistance derived from the original wild barley lines, and the agronomic type of cultivated barley. In the F_7, homozygous lines have been extracted and test crossed with different barley cultivars and barley lines. The testcrosses with the cultivars 'Roland' and 'Koral', possessing the genes *Mla9* or *Mla13*, respectively, served to study the mode of inheritance and genetic relationship of the wild barley genes to the highly polymorphic *Mla* locus. Testcrosses with the NIL (near-isogenic line) 'P19' (Kølster et al. 1986), carrying the gene *Mlp*, and with the line 'RS170-35*A', carrying the gene *Mlp3* (Jahoor et al. 1989), were performed to study the genetic relationship to genes of the *Mlp* locus. F_2/F_3 populations from crosses between 'RS137-28*E', 'RS42-6*O', 'HSY-78*A', and the cultivars 'Pallas' or 'Gitte' served as mapping populations for the localization of the resistance genes derived from wild barley with RFLP markers.

Tests with isolates of powdery mildew

The mildew tests were performed at the seedling stage in detached leaves placed upon agar (Aslam and Schwarzbach 1980). In order to prevent contamination, the seedlings were raised in a growth chamber at 18°C with permanent light for 8 days. The detached leaves were placed in plastic plates upon agar (5%) containing 30 mg/l of benzimidazol in order to delay leaf chlorosis and 30 µg/ml of Ampicillin for protection against bacteria. The leaves were inoculated with appropriate isolates derived from single conidia of powdery mildew maintained at the Department of Agronomy and Plant Breeding, TUM Weihenstephan. During the incubation period of 9 and 11 days employed for European and Israeli isolates respectively, the plates were kept under the same controlled conditions as used to raise the seedlings. Mildew infection readings were after 9 or 11 days, respectively, according to the scoring scale 0 (fully resistant) to IV (fully susceptible) described by Torp et al. (1978).

RFLP analysis

For RFLP analysis, 80 F_2 plants were randomly selected from each cross. The genomic DNA was isolated according to the CTAB procedure described by Saghai-Maroof et al. (1984). The DNA was digested with the restriction enzymes *Bam*HI, *Eco*RI, *Hin*dIII, *Eco*RV, and *Xba*I following the manufacturer's recommendations (Pharmacia Uppsala). The digested DNA (12 µg/lane) was subjected to electrophoresis in 0.75% agarose gels (Seakem FMC), and subsequently transferred to a Biodyne B Nylon membrance (Pall, Portsmouth) as described by the supplier (Pall Croporation, Dreieich).

All RFLP clones used in this investigation originated from the MWG collection (Graner et al. 1991; Jahoor et al. 1991). The inserts of the recombinant plasmides from the stock of MWG clones were labelled with α-^{32}P-dCTP by random priming (Feinberg and Vogelstein 1983), and subsequently used as probes. Hybridization and further treatments of the memberane were conducted with reference to Jahoor et al. (1991). The exposure time of the X-ray film was 1–2 weeks at −70 °C.

Linkage analysis

The two-point analyses were performed using the LINKAGE-1 program (Suiter et al. 1983). The multipoint analyses of data from the RFLP and powdery mildew loci were performed with MAPMAKER (Version 3.0/Exp) (Lander et al. 1987; Lincoln and Lander 1992). The recombination values in % were converted into centiMorgans (cMs) by applying the Kosambi function (Kosambi 1944). The standard errors of the recombination fractions were only calculated for two-point analyses and are therefore only given in % units.

Results

Mode of inheritance and tests for allelism with the *Mla* locus

Two test crosses were performed for an assessment of the inheritance patterns of the wild barley resistance genes involving the *H. vulgare* ssp. *spontaneum*-derived lines 'RS137-28*E' and 'HSY-78*A'. The F_2 seedlings were inoculated with selected powdery mildew cultures that were avirulent to these lines and virulent for the genes *Mla7*, *Mla12*, *Mla9*, and *Mla13* present in cultivars 'Elgina', 'Aramir', 'Roland', and 'Koral', respectively. The latter two cultivars were used as test-cross parents. In both test-crosses, a 3:1 segregation was obtained for the wild barley derived mildew resistance (Table 1). Since infection types of the resistant F_2 progenies varied constantly between immunity and intermediate reaction types, a clear classification for homozygous and hetereozygous progenies was not possible. In both crosses, therefore, all resistant progenies were pooled for segregation analysis. Apparently, the degree of dominance is not complete. The testcross with line 'RS42-6*O', and 'Gitte' was examined in the same way but with isolates virulent to 'Oriol' (*Mla7*) and 'Gitte' (*Mla1*). For this test-cross, a recessive inheritance was established (Table 1). The F_2 generation of the fourth test-cross '(D* 1B-87B) * Roland' was tested with 'We-3', avirulent to wild barley resistance and virulent to *Mla9* carried by 'Roland'. An inheritance of two genes segregating in a 13:3 manner was obtained. This segregation is interpreted as an independent segregation of a (semi)-dominant and a recessive gene. For allelism tests with the *Mla* locus, isolates avirulent against both test-cross parents but virulent against the resistance genes derived from the original cultivars of the wild barley derived lines were employed, and free segregation was confirmed in all four cases (Table 1). It was therefore concluded that the resistance genes originating from the *H. vulgare* ssp. *spontaneum* lines, as far as they have been detected in the tests for allelism with the isolates used, are neither allelic nor linked to alleles of the *Mla* locus.

Table 1 Mildew reaction of parents and segregationg generations

Wild barley derived lines	Cultivar	Isolate	Infection types of the parents		Generation	Total number	Genotypes			Ratio expected	χ^2	P
							RR	RS	SS			
RS137-28*Elg.	Roland	Mo-4	0	$IV_{1,1}$[a]	F_2	599		436	163	3:1	1.56	0.20–0.30
RS137-28*Elg.	Roland	201/107	$I_{0,4}$	$I_{0,3}$	F_2	253		235	18	15:1	0.32	0.50–0.70
HSY-78*Ar.	Koral	Ru-3 + Ar-4	0	$IV_{0,7}$	F_2	217		164	53	3:1	0.04	0.90–0.95
HSY-78*AR.	Koral	201/107	0	0	F_2	217		204	13	15:1	0.02	0.90–0.95
D*1B-87B	Roland	We-3	$0 + I_{0,1}$	$IV_{1,2}$	F_2	647		543	104	13:3	3.04	0.05–0.10
D*1B-87B	Roland	Ar-4	$I_{0,1}$	$I_{0,1}$	F_2	416		390	26	61:3	2.27	0.10–0.20
RS42-6*Oriol	Gitte	201/107	0	$IV_{0,7}$	F_2	354		92	262	1:3	0.18	0.10–0.30
RS42-6*Oriol	Gitte	Mo-4	$I_{0,2}$	0	F_2	453		371	82	13:3	0.12	0.70–0.90
HSY-78*Ar.	Pallas	184/21	0	$IV_{0,8}$	F_2	145		116	29	3:1	1.93	0.10–0.30
HSY-78*Ar.	Pallas	184/21	0	$IV_{0,8}$	F_3	41	9	23	9	1:2:1	0.61	0.70–0.90
RS137-28*Elg.	Pallas	Ru-3	$I_{0,1}$	$IV_{1,0}$	F_2	198		160	38	3:1	3.56	0.10–0.05
RS137-28*Elg.	Pallas	184/21	$I_{0,1}$	$IV_{0,8}$	F_3	70	28	31	11	1:2:1	9.17	–
RS42-6*Oriol	Gitte	201/60	$II_{0,1}$	$IV_{0,7}$	F_2	124		34	90	1:3	0.39	0.50–0.70
RS42-6*Oriol	Gitte	201/60	$II_{0,1}$	$IV_{0,7}$	F_3	74	18	39	17	1:2:1	0.24	0.70–0.90

[a] Compared to the susceptible standard line SM4142 = $IV_{1,0}$

Test for linkage of the new loci

A half-diallel of the three lines 'RS137-28 * E', 'RS42-6 * O', 'HSY-78 * A', was conducted to determine linkage relationships between the *H. spontaneum* genes. The crosses were subjected to a mildew test with isolate '184/21', which is avirulent to the three *H. spontaneum*-derived resistance genes and virulent against all resistance genes in the cvs 'Aramir', 'Elgina' and 'Oriol'. The chi-square values confirmed free segregation for all three crosses (Table 2 a). In addition, two testcrosses were performed with the line 'D * 1B-87B' (Table 2 a). Susceptible plants were not observed with either 'RS137-28 * E', or with 'RS42-6 * O', suggesting that the (semi) dominant genes of 'D * 1B-87B' and 'RS137-28 * E', are either very closely linked or else are alleles of the same locus. The same conslusion applies to the recessive genes of 'D * 1B-87B' and 'RS42-6 * O'. In this way, three loci carrying resistance genes against powdery mildew are represented by 'RS137-28 * E', 'RS42-6 * O', and 'HSY-78 * A', which are independently inherited.

Tests for allelism with the *Mlp* locus

Three testcrosses were performed to test allelism or linkage of the three new genes with the *Mlp* locus (Table 2 b), which has not so far been localized. For 'RS42-6 * O' a test-cross was performed with 'D * 1B-87B', which carries a gene allelic or closely linked to the recessive gene of 'RS42-6 * O' (Table 2 a). The test-crosses with the NIL 'P19' were previously carried out by Jahoor et al. (1989). The chi-square test resulted in the rejection of the null-hypothesis for free segregation in a 15:1 manner only for the cross '(RS137-28* E) * P19'. The corresponding linkage distance was calculated to be $36.5 \pm 3.8\%$ recombination units. This, however, contradicts the results of other crosses involving the *Mlf* locus (data not shown) inclusive of the cross with 'D * 1B-87B' (Table 2 b), which showed independent segregations from the *Mlp* locus.

Powdery mildew tests for the F_2 mapping populations

The F_2 plants of the mapping populations were infected with isolates 'Ru-3', '184/21', or '201/60' characterized by avirulence against the resistance genes derived from wild barley. The segregations confirmed a monogenic mode of inheritance (Table 1).

The plants re-grew, and 80 young plants of each cross were randomly selected for RFLP analysis. Their progenies (ten seedlings from the F_3 family of each single F_2 plant) were tested with isolates '184/21' or '201/60', respectively. The expected 1:2:1 segregations were verified except for the cross '(RS137-28 * Elg) * Pallas' ($\chi^2 = 9.17$) in which there were more homozygous resistant plants than theoretically expected (Table 1).

Table 2a Segregation of mildew reaction of crosses among *H. spontaneum*-derived lines

Parent 1	Parent 2	Isolate	Infection types of the parents		Generation	Total number	Resistant	Susceptible	Ratio expected	χ^2	P
HSY-78*Ar.	RS137-28*Elg.	184/21	0	$I_{0,1}$	F2	258	241	17	15:1	0.05	0.90–0.95
HSY-78*Ar.	RS42-6*Oriol	184/21	0	I-$II_{0,3}$	F2	197	152	45	13:3	2.16	0.10–0.30
RS137-28*Elg.	RS42-6*Oriol	184/21	$I_{0,1}$	I-$II_{0,3}$	F2	244	210	34	13:3	3.71	0.05–0.10
D*1B-87B	RS42-6*Oriol	184/21	0	I-$II_{0,3}$	F2	117	117	0	55:9	19.15	–
D*1B-87B	RS137-28*Elg.	184/21	0	$I_{9,1}$	F2	424	424	0	61:3	404.13	–

Table 2b Segregation of mildew reaction of test-crosses for tests of linkage or allelism with the *Mlp* genes

Parent 1	Parent 2	Isolate	Infection types of the parents		Generation	Total number	Resistant	Susceptible	Ratio expected	χ^2	P
HSY-78*Ar.	P19 (*Mlp*)	184/21	0	I-$II_{0,3}$	F2	347	330	17	15:1	1.88	0.15–0.20[a]
RS137-28*Elg.	P19 (*Mlp*)	Or-4	0	$II_{0,3}$	F2	692	669	23	15:1	9.75[a]	–
RS145-1*(Ar. (*Mlp3*)	D*1B-87B	184/21	$II_{0,6}$	0	F2	336	317	19	61:3	0.70	0.40–0.50

[a] Unpublished results from the study of Jahoor et al. (1989)

Tests of probes for polymorphism

Over 200 different probes were hybridized to select polymorphic markers between the lines of the mapping populations. A total of 178 RFLP markers was successfully evaluated by screening blots containing the DNA of the parents. The degree of polymorphism within the corresponding crosses ranged from 35.4% to 50.6% (Table 3). The range was much larger when the degree of polymorphism was calculated for the indivudual chromosomes. The minimum was 7.7% for chromosome 4 (4H) between 'RS42-6 * O' and 'Gitte', and the maximum 78.9% for chromosome 5 (1H) between 'HSY-87 * A' and 'Pallas'.

RFLP analyses in segregating progenies

For detection of linkage between RFLP probes and the mildew resistance genes in each cross, polymorphic RFLP probes were selected which were not farther than 40 cM apart in existing molecular maps (Graner et al. 1991, 1993). The raw data received from both the mildew tests in the F_3 progeny and the evaluations of the autoradiograms of the F_2 plants were computed in two separate procedures: the data in %, including the standard errors, were derived from the two-point analyses (Table 4), the data in cM resulted from multi-point analyses (Fig. 1 a, d, e). Chromosome regions for which linkage were detected were enriched with further RFLP probes. The linkage groups containing the resistance genes are shown in Fig. 1 a and the levels of significance to reject either the null-hypothesis or free segregation of the loci, respectively, is indicated by the corresponding contingency coefficients from two-point analyses (Table 4). The minimal log likelihood of odds (LOD) in the multi-point analyses was pre-set at 3.0 within the linkage groups (Fig. 1 a, d, e). The order of the probes corresponded in all cases with the published RFLP maps (Graner et al. 1991, 1993).

Linkage group 1, which contained seven polymorphic clones and the recessive resistance gene of the line

Table 3 Survey of tested and cross-specific polymorphic clones

Chrom.	(RS42-6*Oriol) *Gitte	(RS137-28*Elg) *Pallas	(HSY-78*Ar) *Pallas	Sum[a]
1H	9[b]	11	15	19
2H	8	10	10	21
3H	4	10	5	21
4H	1	7	6	13
5H	9	14	9	30
6H	8	9	9	21
7H	24	29	16	53
Sum	63	90	70	178
%[c]	35.4	50.6	39.3	

[a] Sum of probes of the corresponding chromosome tested on the parental blots
[b] Sum of polymorphic probes of the corresponding chromosome
[c] Degree od polymorphism for each cross (columns 2–4)

Table 4 Two-point recombination values given in % recombination fractions (upper diagonal) and chi-square contingency values (lower diagonal) of RFLP loci linked to the mildew resistance loci *Mlt* (a), *Mlj* (b) and *Mlf* (c)

a	MWG35	MWG851a	*mlt*	MWG555a	MWG47	MWG530	MWG564	MWG89
MWG35		0.0	4.6 ± 1.9	6.5 ± 3.0	9.6 ± 2.6	18.4 ± 3.7	22.1 ± 4.1	28.6 ± 6.0
MWG851a	67.5		4.4 ± 2.5	5.2 ± 2.8	11.6 ± 3.8	24.0 ± 5.4	28.0 ± 5.8	34.0 ± 8.2
mlt	75.2	66.2		2.8 ± 2.0	9.0 ± 2.5	20.0 ± 3.8	23.9 ± 4.2	27.3 ± 5.9
MWG555a	67.1	26.9	68.1			18.8 ± 4.8	14.9 ± 4.3	22.6 ± 6.7
MWG47	69.2	57.3	72.9	63.1		8.1 ± 2.3	13.8 ± 3.0	18.9 ± 4.6
MWG530	58.8	43.4	61.2	51.5	76.6		17.0 ± 3.3	15.9 ± 4.1
MWG564	51.9	32.1	52.4	53.6	66.3	63.4		11.0 ± 3.4
MWG89	40.0	26.4	52.3	44.5	61.0	65.4	72.5	

b	MWG37	MWG7	MWG13	MWG54	MWG592	*Mlj*	MWG999	MWG956
MWG37		5.7 ± 3.7	5.3 ± 2.6	6.5 ± 3.2	17.1 ± 4.8	16.5 ± 5.0	27.6 ± 6.5	32.2 ± 8.8
MWG7	64.6		0.0	2.4 ± 2.4	8.4 ± 4.1	6 .9 ± 4.1	13.6 ± 5.4	22.0 ± 6.5
MWG13	77.2	71.6		1.2 ± 1.2	8.6 ± 3.0	11.5 ± 3.7	16.8 ± 4.5	21.5 ± 4.9
MWG54	75.9	70.7	80.7		8.7 ± 3.2	11.0 ± 4.0	15.7 ± 4.6	21.6 ± 5.2
MWG592	62.7	74.3	73.6	59.7		6.2 ± 2.7	11.0 ± 3.6	13.3 ± 3.7
Mlj	60.0	55.3	71.6	70.7	74.3		13.7 ± 4.4	16.3 ± 4.5
MWG999	42.8	54.4	61.1	63.5	68.6	57.8		3.4 ± 1.9
MWG956	40.2	55.3	58.5	70.7	69.8	56.1	78.3	

c	MWG86	MWG70	MWG539	*Mlf*	MWG53
MWG86		19.9 ± 3.4	17.8 ± 3.5	23.5 ± 4.3	33.9 ± 6.8
MWG70	63.8		1.3 ± 0.0	6.8 ± 2.2	30.9 ± 6.5
MWG539	62.4	80.5		6.0 ± 2.1	29.7 ± 6.4
Mlf	55.1	75.2	76.2		17.6 ± 5.2
MWG53	28.1	32.4	35.0	51.1	

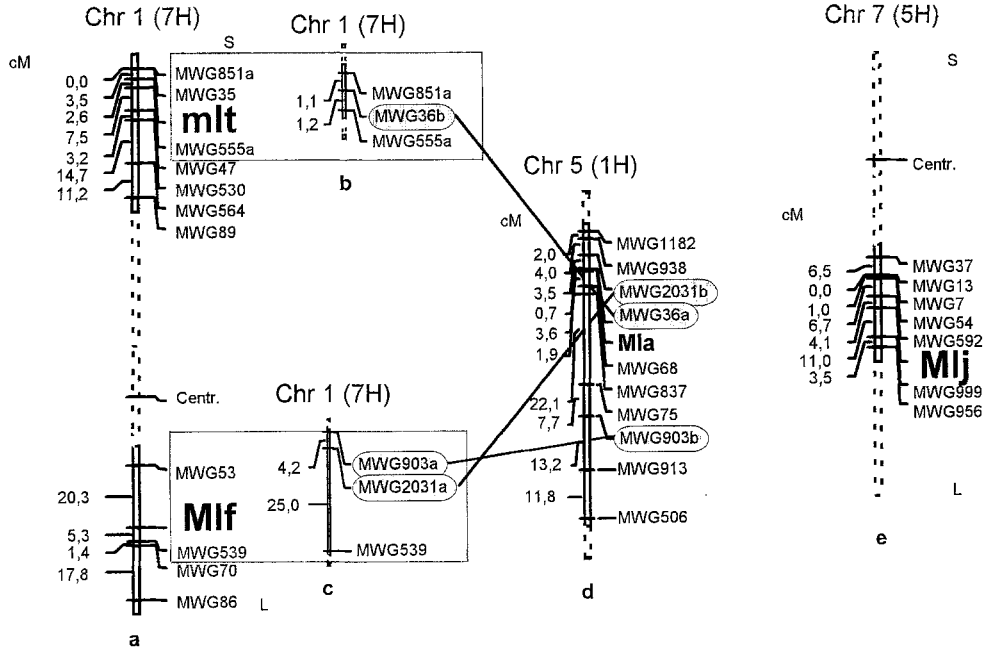

Fig. 1 *Centr.* = centromere, *S* = short chromosome arm, *L* = long chromosome arm. (*a, e*) RFLP mapping of the loci *mlt*, *Mlf* and *Mlj*, (*b, c*) mapping positions of MWG36b obtained by Kilian et al. (1995) and of MWG2031a and MWG903a by Graner et al. (1993) respectively in different mapping populations, (*d*) RFLP probes linked to chromosome 5 (1H) of the F_2 mapping population '(HSY-78*Ar)*Pallas'. □ cuts of regions of chromosome 1 (7H) from different mapping populations that carry at least one marker from the *Mla* region of chromosome 5 (1H)

'RS42-6*O', comprised a distance of 42 cM (Fig. 1 a). The clone MWG555a showed the closest linkage to this resistance gene (Table 4 a). The distance in recombination units was 2.8 ± 2.0% or 2.6 cM. The corresponding contingency coefficient was 68.1. Both the distances between the seven probes and the order were in correspondence with the existing RFLP map. Therefore, this linkage group was mapped to chromosome 1S (7HS).

Linkage group 2, which contained four RFLP probes and the (semi)dominant resistance gene of 'RS137-28' (Fig. 1 a), comprised a map distance of 45 cM. The

shortest map distance between marker and resistance was 5.3 cM calculated for MWG539. The recombination fraction was 6.0 ± 2.1%; the corresponding contingency coefficient amounted to 76.2 (Table 4 c). The distances and order between the probes corresponded to the existing RFLP map. Therefore, this resistance gene was mapped to chromosome 1L (7HL).

Linkage group 3 comprised a map distance of 32.8 cM and contained seven RFLP probes together with the '*spontaneum*' resistance gene of line 'HSY-78*A' (Fig. 1 e). The RFLP marker MWG592 showed the closest linkage with a distance of 6.2 ± 2.7% recombination units (Table 4 b) or 4.1 cM, respectively. The contingency coefficient was 74.3. The position of the low-copy probe MWG54 contained in this linkage group did not agree with the corresponding 'Vada/1B-87' RFLP map, where the position of this probe was originally on chromosome 3 (3H). Since all the other six RFLP probes mapped consistently on chromosome 7L (5HL), it seems justified to localize the *H. spontaneum*-derived resistance gene from this line on chromosome 7L (5HL).

Discussion

Three *H. vulgare* ssp. *spontaneum*-derived barley lines ('RS42-6*O', 'RS137-28*E', 'HSY-78*A') were subjected to genetical analysis of their resistance to powdery mildew. Three genes were identified, which were inherited independently from each other (Table 2 a), from the *Mla* locus (Table 1), and also from *Mlp* (Table 2 b). On the basis of the data obtained for each of the three F_2 populations, linkage groups consisting of the corresponding resistance gene and flanking RFLP markers were constructed. Two linkage groups were assigned to barley chromosome 1 (7H), and one group to chromosome 7 (5H). None of the known loci for resistance genes to powdery mildew are located on these barley chromosomes and the resistance genes segregated independently from the *Mlp* gene for mildew resistance, which is not yet exactly localized. Therefore, these genes represent new loci for powdery mildew resistance in barley and new designations are proposed for them as follows: *mlt* for the recessive gene in barley line 'RS42-6*O' on chromosome 1S (7HS) closely linked to the RFLP marker MWG555a (d = 2.6 cM), *Mlf* for the (semi)dominant resisitance gene in 'RS137-28*E' on chromosome 1L (7HL) closely linked to MWG539 (d = 5.3 cM), and *Mlj* for the (semi)dominant resistance gene in 'HSY-78*A' closely linked to MWG592 (d = 4.1 cM) on chromosome 7 (5HL) (Fig. 1 a, e).

RFLP markers have also been used to determine chromosome regions for quantitative resistance to powdery mildew in the barley genome. Some of the QTLs mapped by Heun (1992), Saghai-Maroof et al. (1994), and Backes et al. (1995) seem to overlap with the chromosome regions of the newly identified loci in the present investigation. The RFLP markers MWG851 a and MWG555 a, which detected the QTL on chromosome 1S (7HS) (Backes et al. 1995), are involved in the same linkage group that has been established for *mlt* in the present investigation (Fig. 1 a). Saghai-Maroof et al. (1994) claimed to have detected QTLs in regions in which previously the major race-specific resistance genes *Mla, Mlg, mlo, MlLA,* and *Mlh* were located. Conversely, it may be hypothesized, that major genes for mildew resistance are present in QTL regions for quantitative resistance in European barley cultivars that coincide, and became re-established, with loci for major gene resistance by the newly effective genes (or alleles) at the *Mlf, Mlj,* and *mlt* loci derived from wild barley. Further evidence for coincidence of QTLs and major gene loci related to the interaction between barley and powdery mildew may be taken from the fact that neither a QTL nor a major resistance gene for powdery mildew have been attributed to chromosome 3 (3H). Such observations are in agreement with other host-pathogen interactions. For example, Leonards-Schippers et al. (1994) localized a QTL for resistance to *Phytophthera infestans* in potato to chromosomal segments to which the race-specific alleles of the *R1* locus for resistance to the same pathogen had been localized. Such results support the hypothesis of some authors, e.g. Ellingboe (1976), that the genetic base for quantitative resistance correlates with 'defeated' genes for race-specific defense reactions.

Results from cytogenetic research, as well as from the use of isozymes and DNA markers, have established homoeology between the chromosomes of the A, B, and D genomes of hexaploid wheat, including its wild relatives, as well as to chromosomes of the barley (H) and rye (R) genome (Miller and Reader 1987; Sharp et al. 1988, 1989; Chao et al. 1989; Gill et al. 1991; Anderson et al. 1992; Devos et al. 1992, 1993; Wang et al. 1992; Namuth et al. 1994). A large number of loci for resistance against *E. graminis* have been identified in hexaploid wheat (*Triticum aestivum*) and rye (*Secale cereale*). Some resistance genes were introgressed into bread wheat from rye and other species of the genus *Triticum* including the section *Aegilops* (Zeller et al. 1993). Loci for resistance to powdery mildew have been localized in each of the homoeologous groups of wheat chromosomes. If the loci *Mlf, Mlj,* and *mlt* are included, this now also applies to barley with the exception of chromosome 3. The resistance genes *Pm1* (A), *Pm9* (A), *Pm18* (A), *pm5* (B), *Pm15* (D), and *Pm19* (D) were localized on chromosome homoeologous group 7 in wheat (Tosa and Sakai 1990; Hart et al. 1993; Hartl et al. 1995; Lutz et al. 1995). Candidates for homoeologous loci are *mlt* and *pm5*. Both genes are located on the short arms of homoeologous group-7 chromosomes, and they are inherited recessively. The resistance genes *Pm1*, *Pm9*, and *Pm18* from a linkage group on chromosome arm 7AL (Hartl et al. 1995), to which the *Mlf* locus on chromosome 1L (7HL) may indicate homoeology. The wheat genes *Pm1* and *Pm18* are presumably allelic (Hartl et al.

1995). It is interesting to note that additional alleles have also been determined for *Mlf* (Schönfeld et al., in preparation). Homoeology may be assumed for *Mlj* on chromosome 7 (5H) in barley to the *Pm2* (D) locus in wheat and *Pm7* in rye. They all are positioned on chromosomes of homoeologous group 5 (Driscoll and Bielig 1968; McIntosh and Backer 1970). The indication for homoeology between *Mla* in barley and *Pm3* in wheat on group 1S chromosomes was discussed by Hartl et al. (1993). The loci for mildew resistance are not only found in homoeologous positions, but also show a high collinearity within the A, B, D, R and H genomes. It seems that most, if not all, rearrangements involving these loci have occurred before the evolutionary diversion of these genomes.

Differences in the map positions of low- and multi-copy probes obtained in different mapping populations have occasionally been reported (Sherman et al. 1995). During the present study seven low-copy clones were mapped to positions which differed from published RFLP maps. Two of the clones (MWG903a, MWG2031a) were linked within a distance of 4.2 cM and mapped on chromosome 1L (7HL) in the 'Igri/Franka' RFLP map (Graner et al. 1993). As shown in Fig. 1 c, d, duplications of these RFLP loci have been detected as MWG2031b and MWG903b which appear in loosely linked positions on chromosome 7 (7H) in the mapping populations '(RS137-28*E)*Pallas' and '(HSY-78*A)*Pallas' with the restriction enzyme *Eco*RI. The low-copy characteristics of both probes, together with the difference in linkage between them, indicate that these RFLP loci may have originated from independent duplication events.

Duplications of DNA sequences during the evolution of the barley genome would support our assumption that different loci for disease resistance may have descended from a 'prime' locus, that was subjected to inter- and intra-chromosomal duplication events. Ronald et al. (1992) have speculated on the mechanisms for the evolutionary development of 17 different race-specific *Xa* loci for specific resistance to *Xanthomonas oryzae* pv *oryzae*. They assumed duplications were an important mechanism for the genetic and physical evolution of this trait, based on the observation that, in many cases, molecular markers closely linked to the *Xa21* locus detected duplicated sequences in the rice genome. More recently, Ellis et al. (1995) have been able to demonstrate that inter- as well as intra-chromosomal duplications contributed to the evolutionary differentiation of the *L* and *M* loci for rust resistance in flax. For the *Mla* locus for mildew resistance on barley chromosome 5S (1HS) 31 alleles are at present known (Kintzios et al. 1995). Markers which are closely linked to this locus are multi-copy RFLP markers like MWG36a which mapped at a distance of 0.7 ± 0.7 cM to the *Mla* locus (Schüller et al. 1992). Supporting evidence for inter-chromosomal translocation or duplication of sequences is provided by the markers MWG203 1b and MWG36a which are closely linked (3.3 cM) (Fig. 1 d). The low-copy marker MWG2031a was first detected in a region of chromosome 1L (7HL) of the 'Igri/Franka' map, where *Mlf* was localized (Fig. 1 c). MWG36b was mapped between MWG555a and MWG851a by Killan et al. (1995) on chromosome 1S (7HS) (Fig. 1 b), a region where the *mlt* locus was localized in the present investigation on the basis of the common markers MWG555a and MWG851a. Both duplicated RFLP loci (MWG36a, b; MWG203a, b) relate together to the *Mla* locus but separately to the *Mlf* or *mlt* locus, respectively. Since many RFLP loci are represented by low- or multi-copy clones in the barley genome, more closely linked markers will have to be localized in the regions surrounding the loci for mildew resistance to substantiate the 'prime' locus hypothesis for *Mla*.

In any case, the new loci for mildew are valuable resources for future barley breeding, since they recombine with effective resistance genes or alleles from the *Mla*, *mlo*, and *MlLA* resistance loci that are presently used, and since they are effective against the whole range of virulence represented by European isolates (Jahoor and Fischbeck 1987a).

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