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ORIGINAL PAPER

Mapping of five resistance genes to sugar-beet powdery mildew using AFLP and anchored SNP markers

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Abstract Sugar-beet powdery mildew, caused by the fungus *Erysiphe betae*, now occurs in all sugar-beet growing areas and can reduce sugar yield by up to 30%. Powdery mildew resistant plants from three novel sources were crossed with sugar beet to generate segregating populations. Evaluation of resistance was carried out in artificially inoculated field and controlled environment tests. The resistance level in two of the sources was found to be significantly higher than that in currently available sugar-beet cultivars. AFLP analysis was used in combination with bulked segregant analysis to develop markers linked to the resistant phenotype in each population. Five dominant major resistance genes were identified and assigned the proposed symbols *Pm2* to *Pm6*. *Pm3* conferred complete resistance to powdery mildew; the other genes conferred high levels of partial resistance. From the use of anchoring SNP markers, two genes were located to chromosome II and three to chromosome IV. Two of the genes on chromosome IV mapped to the same location and one of the genes on chromosome II mapped to the same region as the previously identified *Pm1* gene. With the availability of these genes there is now excellent potential for achieving durable resistance to sugar-beet powdery mildew, thus reducing or obviating the need for chemical control.

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Introduction

Sugar beet (*Beta vulgaris* subsp. *vulgaris*) is a member of the family *Amaranthaceae* which also contains the crop species spinach (*Spinacea oleracea* L.) and quinoa (*Chenopodium quinoa*). It is diploid with $2n = 18$ chromosomes and a haploid genome size of 758 Mb (Arumuganathan and Earle 1991). Cultivated forms of the species, including fodder beets, leaf beets and garden beets, are sexually compatible with wild sea beet (*B. vulgaris* subsp. *maritima*). The species is outbreeding and highly heterozygous with a multi-allelic gametophytic self-incompatibility system. Sugar beet is susceptible to diseases which impact on the sugar yield. Sugar-beet powdery mildew (reviewed by Francis 2002), caused by the fungus *Erysiphe betae*, now occurs in all sugar-beet growing areas and in warm, arid climates can reduce sugar yield by up to 30% (Weltzien and Ahrens 1977). The disease is characterised by white dust-like colonies that develop over leaf surfaces following germination of wind-borne conidia. Sulphur or triazole fungicides are currently applied to the crop for disease control.

Whitney et al. (1983) identified partial resistance to powdery mildew in sugar-beet germplasm that conferred a slow-mildewing phenotype. Partial resistance has been introduced into breeding lines such as C39 (Lewellen 1995) and reportedly developed within commercial hybrids by the sugar-beet industry (Lewellen and Schrandt 2001). Wolf et al. (2006) reported that the epidemic onset of powdery mildew in cultivars with a low susceptibility was delayed by two weeks and the infection severity reduced by 20–60%. Whitney (1989) confirmed in controlled greenhouse evaluations that two *B. vulgaris* subsp. *maritima* accessions, WB242 and WB97, had individual plants that showed high resistance. WB242 was originally collected in the Loire estuary in France but the origin of WB97 is

unknown. The resistance from these accessions was backcrossed into sugar-beet breeding lines and in field evaluations was shown to be inherited as a single, dominant, major gene with the proposed symbol *Pm* (Lewellen and Schrandt 2001). The allelism of the resistance from these two sources was not determined. Although *Pm* conditioned a high level of resistance, disease developed on mature leaves late in the season. Janssen et al. (2003) mapped the resistance gene derived from WB242 to an interval of 6.4 cM between anonymous flanking AFLP markers on chromosome II. To date this is the only monogenic form of powdery mildew resistance that has been mapped with molecular markers in sugar beet. Marker analysis of *B. vulgaris* subsp. *maritima* accession PI 504236 revealed the presence of two QTLs that were significantly associated with resistance (Francis 2002). Janssen et al. (2003) carried out QTL mapping in two anonymous populations with resistance scores in both approximating a normal distribution. Five QTLs were identified in population A that together explained 27% of the phenotypic variation. In population B 19% of the variation was explained by two loci.

Luterbacher et al. (2004) screened up to 600 *Beta* accessions for resistance to powdery mildew in field and glasshouse evaluations. Significant differences in resistance were observed within the section *Beta* ($P < 0.05$) and resistant accessions were crossed with susceptible sugar-beet breeding lines to generate segregating populations for mapping. We report here the mapping of five resistance genes to sugar-beet powdery mildew from three of these novel sources. The availability to breeders of new resistance sources increases the potential for breeding durable disease resistance and reduces or obviates the requirement for chemical control.

Materials and methods

Plant material

Population PM-MAR, consisting of 83 individuals, was produced by crossing a single resistant plant of *B. vulgaris* subsp. *maritima* (accession collected at Fayyum Harfosh Farm, Matruh, Egypt) with a plant of the susceptible male-sterile sugar-beet breeding line SfHS. Genetic male-sterility, conferred by the single recessive gene a_1 (Owen 1952), was used to ensure the production of hybrid seed. Population PM-GBT, consisting of 150 individuals, was produced by crossing a single resistant hybrid plant with a plant of line SfHS. The resistant hybrid parent was derived by crossing a single resistant plant of the garden beet variety 'Simender' (accession collected in Denmark) with a plant of the susceptible male-sterile sugar-beet breeding line CALE and then crossing a single resistant progeny plant with a plant of line SfHS. Population PM-LBT, consisting of 96 individuals, was produced by crossing a single powdery mildew resistant plant of the leaf beet variety 'Merolakhano' (accession collected at Zakros, Crete, Greece) with a plant of line SfHS. Seeds of line SfHS and CALE were obtained from Lion Seeds Ltd., Maldon, Essex, UK The International Database for *Beta* (IDBB; http://www.genres.de/idb/beta) codes for the original resistant accessions of populations PM-MAR, PM-GBT and PM-LBT were 9749, 6453 and 3123, respectively.

The sugar-beet cultivars Dominika, Latoya and Harry were on the Recommended List for 2006 produced by the UK's National Institute for Agricultural Botany (NIAB). They scored 8, 7 and 1 out of 9 for resistance to powdery mildew respectively. 3R-64 was developed as a breeding line with resistance to virus yellows (Stevens and Thomas, 2005) with apparent high susceptibility to powdery mildew.

Resistance evaluation

Original accessions were evaluated for powdery mildew resistance in small-plot field trials conducted at Broom's Barn in growing seasons from 1997 to 2000 as part of the screening programme described by Luterbacher et al. (2004). The three original accessions appeared to be segregating for resistance to powdery mildew. In developing the PM-GBT population, grandparent and parent populations harbouring resistance were evaluated in the same way in 2001 and 2003. Every fourth plot and the space between parallel rows of plots was sown with the susceptible sugar-beet cultivar 'Sandra' to encourage uniform natural infection. Population PM-LBT was evaluated for resistance in a field trial at Broom's Barn in 2004 along with resistant and susceptible parent lines and the susceptible sugar-beet cultivar 'Roberta' as a control. Glasshouse-grown seedlings were transferred to a netted tunnel to harden-off two weeks after sowing and transplanted to the field site seven weeks later. Powdery mildew infected plants were placed as inoculators in the centre row of the trial plots on 12 July (17 weeks after sowing). Plants were scored for powdery mildew infection at 16, 31 and 59 days after inoculation (DAI). Populations PM-MAR and PM-GBT were evaluated for resistance in a controlled environment (CE) room maintained with 16 h light (22°C) and 8 h dark (17°C). Resistant and susceptible parent lines and the susceptible sugar-beet cultivar 'Roberta' were also included. At four weeks after sowing, plants were inoculated with powdery mildew by brushing the leaves with infected plants. Plants were scored for powdery mildew infection at 23 DAI (PM-GBT evaluation) or 25 DAI (PM-MAR evaluation). All accessions and populations were scored subjectively using a 0–6 infection scale (Table 1). For QTL analysis, Microsoft Excel was used to convert the infection scores to percentage of leaf

Table 1 Powdery mildew disease assessment scale

Score	Description
0	No colonies obvious
1	One or a few colonies (<20)
2	Colonies coalescing on some leaves. Most leaves infected. *Ca*. 5% leaf area infected
3	Large areas of infected leaf either with high density of discrete colonies or coalesced areas. 25–50% leaf area infected
4	50–75% of leaf area infected. Younger leaves reasonably clear of infection
5	75% or more of leaf area infected. Profuse sporulation. Younger leaves infected
6	Almost total leaf cover

area infected using the following Gompertz equation: = 110 × EXP(−EXP(0.68 × (*x*. − 3.61))) where EXP denotes exponential and *x* is the Excel spreadsheet cell number containing the infection score. This equation was derived by comparing infection scores based on field observations with the percentage of leaf area infected as detected using a leaf area meter.

The original accessions of the PM-MAR and PM-GBT populations were compared with three sugar-beet cultivars and the breeding line 3R-64 in a CE room resistance evaluation trial carried out as described above. For each population twenty plants were scored for powdery mildew infection at 23 DAI and the scores converted to percentage of leaf area infected. The percentage of leaf area infected per plant was compared between populations by a one-way analysis of variance and a test for the least significant difference (LSD). Statistical analyses were carried out using GenStat® Release 8.2 (VSN International, Hemel Hempstead, UK).

DNA extraction

Genomic DNA was extracted from snap-frozen leaf tissue using the Nucleon Phytopure Plant DNA Extraction Kit (Amersham Biosciences, Little Chalfont, UK) with the addition of 10 mM 2-mercaptoethanol to Reagent 1. After cooling samples on ice, DNA was isolated by phenol:chloroform:isoamylalcohol extraction and isopropanol precipitation (Sambrook et al. 1989) then dissolved in 50 µl sterile distilled water (SDW). RNA was degraded by addition of 1 µg RNase (Roche, Welwyn Garden City, UK) and incubation for 15 min at 37°C. The quantity and quality of DNA were assessed by agarose-gel electrophoresis using 0.8% agarose with known concentrations of uncut lambda DNA (Roche). Gel images were captured using the GeneGenius gel documentation system with GeneSnap software (Syngene, Cambridge, UK). DNA concentrations were calculated using GeneTools software (Syngene).

Fluorescent AFLP analysis

AFLP analysis was performed as described by Trybush et al. (2006). Reactions were carried out essentially as described by Vos et al. (1995) but using fluorescent, multiplex technology. Polymorphic bands were named according to the selective primers used to amplify them and the size (in base pairs) of the fragments scored. Each population was analysed using 18 primer combinations, including some previously identified as highly polymorphic (data not shown) and some identified by bulked segregant analysis (see below).

Bulked segregant analysis

Bulked segregant analysis (BSA) was performed, as described by Michelmore et al. (1991), in order to identify markers co-segregating with powdery mildew resistance in each population. The diluted DNA samples (5 ng/µl) of eight resistant (*R*) and eight susceptible (*S*) individuals were pooled into *R* and *S* bulks that were screened with 144 AFLP primer combinations. The primer combinations that gave the most number of polymorphic bands between the bulks were tested on the bulk individuals to eliminate false positive markers prior to screening against the whole population.

SNP genotyping

Anchoring SNP genotyping was performed essentially as described by Möhring et al. (2004) but with minor adaptations. The SNP markers presented by these authors were assigned to chromosomes according to Butterfass (1964) and Schondelmaier and Jung (1997). PCR primer and extension primer core sequences were as described and markers 1–3 and 4–6 were separately multiplexed for each linkage group. Multiplex PCR amplification was performed in a 5 µl volume containing 10 µg genomic DNA, 200 µM dNTPs (Promega, Southampton, UK) and 0.1 µM each primer with 1X PCR Buffer, 1.5 mM $MgCl_2$ and 0.025 U HotStarTaq® DNA Polymerase (Qiagen, Crawley, UK). All thermocycling was performed using a PCR Express Thermal Cycler (ThermoHybaid, Ashford, UK). Two µl PCR product were purified with 0.8 µl ExoSAP-IT (Amersham Biosciences) according to the protocol provided. Primer extension was performed in a 5 µl volume containing 1.5 µl purified PCR product, 0.25 µM each extension primer and 0.25 µl SNaPshot Multiplex Ready Reaction Mix (Applied Biosystems, Warrington, UK). Extension primers 1–3 and 4–6 from each LG (Möhring et al. 2004) had sizes of 24, 30 and 36 nucleotides respectively and were not HPLC-purified. Three µl extension reaction product were purified with 0.5 U CIP (New England Biolabs,

Hitchin, UK) according to the protocol provided. Prior to separating fragments, 0.75 µl purified sample were mixed with 0.25 µl GeneScan™–120 LIZ™ size standard and 9 µl Hi-Di Formamide (Applied Biosystems), denatured at 95°C for 5 min and placed on ice. Fragments were separated, sized and scored as described in the fluorescent AFLP protocol.

Marker-trait association and linkage mapping

All segregating AFLP markers were tested for significant association with powdery mildew resistance using the non-parametric rank sum test of Kruskal–Wallis (Lehmann 1975) found in MapQTL® software 4.0 (Van Ooijen et al. 2002). For each individual test a significance level (*P*-value) of 0.001 was used. Linkage analysis was performed using JoinMap version 3.0 software (Van Ooijen and Voorrips 2001) that uses the estimation procedures for cross-pollinators as described by Maliepaard et al. (1997). The software was used to test markers for segregation distortion using a Chi-square test. Linkage groups were determined using a minimum LOD threshold of 4.0 and map construction performed using the Kosambi mapping function with the following JoinMap parameter settings: Rec = 0.4, LOD = 1.0, Jump = 5. A third round of ordering whereby problematic markers are forced onto a map was not employed; such markers were discarded. Resulting linkage maps were drawn using MapChart software (Voorrips 2001).

Results

Resistance evaluation

The results of powdery mildew resistance evaluation in three different populations are shown in Fig. 1. In each case there appeared to be segregation for resistance, with some individuals having a low infection score and others having an infection score as high as the susceptible control cultivar Roberta. In the PM-GBT and PM-LBT populations there appeared to be strong segregation of the phenotype, suggesting the presence of resistance genes of large effect. In the PM-MAR population there appeared to be a large number of resistant plants compared with susceptible plants, suggesting that more than one resistance gene was segregating. The susceptible parent lines were generally as susceptible as Roberta and the resistant parent lines contained individual plants with low infection scores. The PM-LBT plants were also evaluated for resistance at 31 and 59 DAI (data not shown). The number of resistant plants (with an infection score of 0 or 1) diminished at each subsequent time point; at 59 DAI most plants were as susceptible as Roberta.

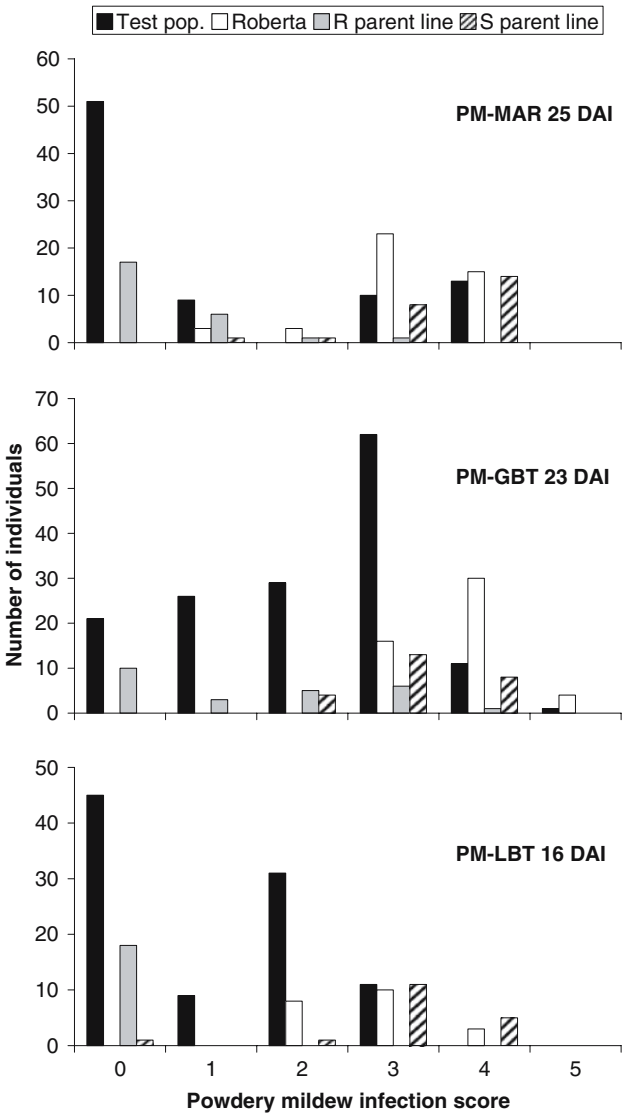

Fig. 1 Distribution of powdery mildew infection scores of three segregating populations (test pops.). The timing of resistance evaluation for each population is indicated in days after inoculation (*DAI*). The resistant (*R*) and susceptible (*S*) parent lines and the susceptible sugar-beet cultivar 'Roberta' were included as control populations

Figure 2 shows the results of evaluating the resistance levels in the PM-MAR and PM-GBT original accessions compared with three sugar-beet cultivars and the breeding line 3R-64. Both original accessions had a significantly lower ($P = 0.05$) infected leaf area per plant than the three sugar-beet cultivars. The cultivars were not significantly different from each other. 3R-64 had a significantly higher infected leaf area per plant than the other populations. Analysis of the infection score data (not shown) revealed that the two original accessions were segregating for resistance, with profiles similar to those of the respective resistant parent lines in Fig. 1.

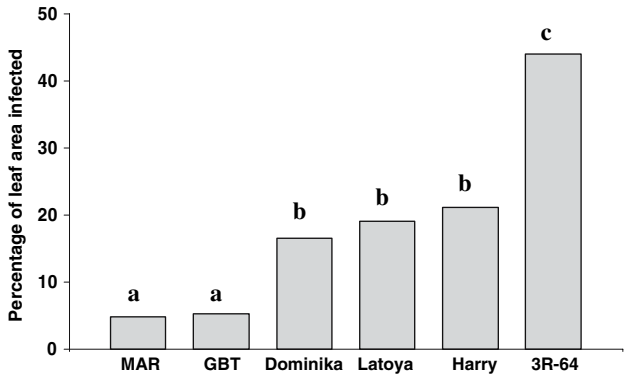

Fig. 2 Mean percentage of leaf area infected by powdery mildew in the PM-MAR and PM-GBT original accessions (*MAR* and *GBT* respectively) compared with three sugar-beet cultivars and the breeding line 3R-64. Columns marked with different letters indicate significant difference in infected leaf area per plant ($n = 20$) according to the LSD test ($P = 0.05$)

Marker-trait association and linkage mapping

To detect markers significantly associated with powdery mildew resistance a *P*-value of 0.005 was initially used in Kruskal–Wallis tests. The software handbook suggested using at least this significance level for the individual tests in order to obtain an overall level of about 0.05. At the $P = 0.005$ level two additional markers were identified as significant in the PM-GBT population. Linkage mapping revealed no gradient in the test statistic with adjacent markers, so the two markers were considered to represent false positives and a more stringent significance level of 0.001 was adopted in all tests. In total 203, 150 and 166 segregating AFLP markers were identified and tested in populations PM-MAR, PM-GBT and PM-LBT respectively.

The results of marker-trait association testing and subsequent linkage analysis are shown in Table 2 and Fig. 3 respectively. Markers identified as significant for powdery mildew resistance were mapped to chromosomes II and IV in the PM-MAR population and chromosome IV in the PM-GBT and PM-LBT populations by successful integration of anchored SNP markers. SNP markers that were not mapped here were either monomorphic or not detected. In each of the three populations the chromosome IV SNP markers were segregating in the susceptible female parent. In order to integrate these with the male-segregating AFLP markers into genetic linkage maps it was necessary to incorporate AFLP markers segregating in both parents (all three populations) and AFLP markers segregating in the susceptible female parent (populations PM-GBT and PM-LBT). After incorporation of additional markers the order of male-segregating markers was conserved in each case.

Analysis of the gradients in the Kruskal–Wallis test statistic (Table 2) suggested that two resistance genes were present on chromosome II in PM-MAR and one resistance gene was present on chromosome IV in each population. The proximal marker with the highest test statistic for each of the five resistance genes (highlighted with proposed gene symbols in Fig. 3) was in coupling phase with resistance in each case. The mean percentage of leaf area infected per plant varied between these proximal markers from 0.0 to 8.8 for resistant genotypes. The former infection level suggested complete resistance to powdery mildew. Although none of the proximal markers displayed significant segregation distortion, resistant genotypes were fewer than susceptible genotypes in four out of five cases. The percentage of phenotypic variation explained by each of the five proximal markers was 19, 27, 19, 16 and 26% respectively for *Pm2* to *Pm6*. The PM-LBT marker data were also compared with the powdery mildew infection data at 31 and 59 DAI. Similar results were obtained at 31 DAI as at 16 DAI but at 59 DAI none of the markers were significant for resistance.

Discussion

Through construction of marker linkage maps and use of bulked segregant analysis we have successfully mapped resistance to sugar-beet powdery mildew from three novel sources and identified five resistance genes. We have shown that these genes confer significantly higher levels of resistance than available in current sugar-beet cultivars. Marker analysis and linkage mapping were achieved using a combination of AFLP and SNP approaches.

Resistance in PM-MAR

The results of resistance mapping (Table 2) in population PM-MAR confirmed that the skewed segregation observed in this population (Fig. 1) was due to the presence of more than one segregating resistance gene. Through the use of anchored SNP genotyping, two resistance genes were identified on chromosome II and one on chromosome IV. These genes have the proposed symbols *Pm2*, *Pm3* and *Pm4* (Fig. 3). Based on the R^2 values of proximal markers, these genes explained an estimated 65% of the phenotypic variation including a probable small linkage effect between *Pm2* and *Pm3*. The three resistance genes exhibited a dominant phenotype; presence of just one of the three resulted in a large reduction in infected leaf area per plant when individuals carrying proximal markers for the other genes were removed from the analysis. *Pm2* and *Pm4* appeared to confer partial yet strong resistance to powdery mildew. Since the proximal markers for these two genes are located in dense marker clusters, it is expected that they are closely linked with the respective resistance genes and therefore strongly reflective of them. Clustering of AFLP markers on linkage groups is a common phenomenon in a wide range

Table 2 Associating male-parent segregating AFLP markers with powdery mildew resistance

Pop	Chr	Marker	Pos	Phase	*K**	*P*	Mean % infection by genotype		nr inf by genotype	
							+	−	+	−
PM-MAR	II	EAGA/MACA-183	0.0	C	5.4	–	7.4	18.4	51	26
		EAGC/MACA-317	12.3	C	2.9	–	9.3	14.3	54	23
		EAGA/MACA-242	30.5	R	0.5	–	14.4	7.2	42	35
		EACA/MACA-49	38.2	C	2.9	–	9.6	11.5	34	46
		EAGA/MCTT-114	48.7	C	9.4	–	3.2	16.5	31	49
		EAGA/MACT-85	53.2	R	13.3	**	17.4	3.5	45	36
		EACA/MACA-203	56.2	R	15.1	**	17.2	2.3	44	33
		EACA/MCAA-150	56.9	R	13.1	**	16.8	4.1	45	37
		EACA/MAGT-58	57.7	R	15.1	**	17.0	3.5	46	36
		EACA/MCAA-189	58.5	C	20.9	***	2.1	18.5	37	45
		EACA/MACA-126	58.8	R	19.1	***	17.6	2.2	43	34
		EAGA/MAGT-210	59.0	R	17.6	***	17.8	3.3	44	38
		EAGC/MCAA-100	59.3	C	16.3	***	3.4	17.4	37	45
		EAGA/MCTT-204	59.4	C	15.1	**	3.6	17.4	35	45
		EACA/MCTT-198	60.3	C	14.0	**	3.5	17.8	36	44
		EAGC/MATA-136	62.0	C	14.8	**	3.1	16.4	33	49
		EAGA/MATA-280	65.2	R	9.3	–	16.2	5.9	42	39
		EAGA/MACT-234	71.5	C	35.3	***	0.0	19.7	35	46
	IV	EACA/MATA-122	7.3	R	6.1	–	16.4	5.2	43	39
		EACA/MATA-185	13.6	C	8.0	–	4.5	15.8	34	48
		EACA/MACA-261	18.2	C	7.9	–	3.8	16.4	34	43
		EACA/MAGT-111	19.7	C	7.7	–	3.5	17.3	37	45
		EAGC/MCTT-237	21.0	R	9.7	–	18.6	3.4	42	38
		EAGA/MCTT-219	21.6	R	12.1	*	19.7	2.1	41	37
		EAGC/MCTT-153	23.0	C	14.0	**	2.2	18.9	35	44
		EAGC/MCTT-295	23.3	C	14.4	**	2.2	18.5	35	45
		EAGA/MCAA-76	24.1	C	12.1	*	2.2	17.3	34	48
		EAGA/MACT-276	25.5	C	9.5	–	2.2	17.3	33	48
		EAGC/MACT-224	29.1	R	9.2	–	19.0	3.2	41	40
		EAGA/MCTT-159	43.1	C	2.0	–	7.7	13.9	33	47
		EAGA/MCAA-99	43.7	C	2.0	–	7.9	13.1	32	50
		EACA/MCAA-70	47.4	R	0.1	–	11.5	10.6	46	36
		EAGA/MAGT-76	61.9	C	0.0	–	12.3	10.1	37	45
PM-GBT	IV	EAGA/MACA-322	0.0	R	7.4	–	19.5	14.0	45	76
		EACA/MACG-128	3.3	R	10.4	–	21.3	13.4	47	73
		EAGA/MACA-316	12.4	C	17.5	***	10.2	20.9	54	60
		EAGC/MATA-67	19.3	C	19.1	***	8.7	20.1	47	64
		EAGA/MAGT-105	21.1	C	24.9	***	8.8	21.9	53	71
		EACA/MACA-128	23.5	C	18.2	***	9.9	20.2	52	71
		EAGC/MACA-272	28.8	R	17.9	***	20.6	10.7	64	58
		EAGC/MACG-419	42.7	C	14.2	**	9.5	20.2	42	77
PM-LBT	IV	EACA/MACT-137	0.0	R	1.8	–	4.7	4.9	32	41
		EAGA/MATA-195	15.1	C	4.9	–	3.5	5.8	42	31
		EACA/MTAA-68	19.3	R	4.7	–	5.5	3.6	34	39
		EAGC/MACT-158	20.0	C	5.3	–	3.8	6.0	36	36

Table 2 continued

Pop	Chr	Marker	Pos	Phase	*K**	*P*	Mean % infection by genotype		nr inf by genotype	
							+	–	+	–
		EAGA/MACT-266	20.9	R	4.2	–	5.8	4.4	33	37
		EAGA/MAAC-152	25.6	R	7.7	–	6.2	3.5	34	38
		EAGA/MACA-181	26.5	R	6.5	–	5.6	4.0	35	39
		EAGA/MACA-264	43.8	C	21.7	***	1.7	8.8	42	32
		EACA/MACT-186	45.2	R	23.8	***	9.1	1.7	31	42
		EAGA/MACA-158	46.2	R	20.7	***	8.6	2.1	30	44
		EAGA/MCTA-210	56.6	C	25.1	***	1.1	9.1	40	31

Pop and *Chr* indicate the population and chromosome analysed respectively. Map positions (*pos*) are in centiMorgans; marker phase types are denoted as *C* (in coupling) or *R* (in repulsion). *K** denotes the Kruskal–Wallis test statistic and significant values are indicated by asterisks (* $P < 0.001$, ** $P < 0.0005$, *** $P < 0.0001$). Genotypic classes are indicated as +(AFLP marker present) or −(AFLP marker absent) for mean % leaf area infected and number of informative individuals (nr inf)

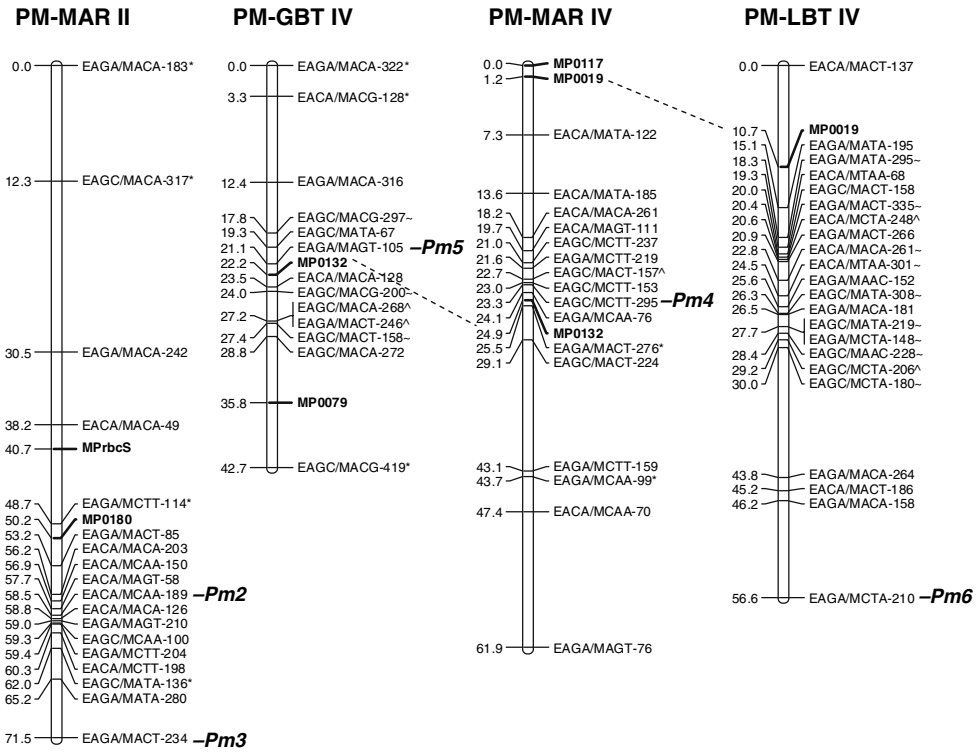

Fig. 3 Linkage mapping of markers for five powdery mildew resistance genes. Populations and chromosomes under analysis are indicated at the *top* of the linkage groups. Cumulative map distances are in centiMorgans and are indicated on the *left side* of the linkage groups. SNP markers are in *bold*. All other markers are AFLP markers; details of their nomenclature are described in Materials and methods. Markers showing significant segregation distortion ($P = 0.05$) are indicated by *asterisks*. ^ indicates AFLP markers segregating in both parents, ~ indicates AFLP markers segregating in the susceptible female parent only

of plant species including sugar beet (e.g. Schondelmaier et al. 1996) and may be explained by reduced recombination in chromosomal regions such as centromeres (Tanksley et al. 1992; AlonsoBlanco et al. 1998). *Pm3* appeared to confer complete resistance to powdery mildew. The previously mapped resistance gene derived from *B. vulgaris* subsp. *maritima* WB242 (Janssen et al. 2003) also conferred complete resistance to powdery mildew when tested in a greenhouse trial. We propose that the WB242 resistance gene is now referred to as *Pm1*. *Pm1* mapped to within 5 cM of the SNP marker MP0180 (T Kraft pers. comm.). In population PM-MAR the proximal marker for *Pm2* mapped to ~8 cM from marker MP0180. Therefore *Pm1* and *Pm2* could represent the same gene.

Resistance in PM-GBT and PM-LBT

The results of resistance mapping (Table 2) in populations PM-GBT and PM-LBT confirmed that the segregation patterns observed in these populations (Fig. 1) were due to the presence of dominant, major resistance genes. These genes have the proposed symbols *Pm5* and *Pm6* respectively (Fig. 3) and were both located to chromosome IV. The resistance level conferred by *Pm5* was not as strong as that

conferred by the other four genes in terms of percentage leaf area infected per plant and percentage of phenotypic variation explained. This could be due to the loss of minor or modifying genes during the two backcrosses to sugar beet undertaken in the development of the PM-GBT population. As with *Pm2* and *Pm4*, the proximal marker to *Pm5* is expected to be closely linked to the resistance gene due to marker clustering in this region of the linkage group. Since the PM-GBT population was created by backcrossing twice to a sugar-beet breeding line, it is possible that parts of its resistant chromosome IV are derived from sugar beet and not the original garden beet accession. This potential reduction in genetic variation on chromosome IV may have resulted in the linkage group being comparatively smaller than in PM-MAR and PM-LBT. The variation in resistance levels observed in the PM-GBT population (Fig. 1) could be due to incomplete penetrance of *Pm5*. Heterozygous individuals would need to be tested alongside homozygous resistant individuals to confirm this. With current data the resistance conditioned by *Pm5* can be described as partial. The proximal markers for *Pm4* and *Pm5* both mapped to within 2 cM of SNP marker MP0132. Therefore *Pm4* and *Pm5* could represent the same gene.

Pm6 appears to confer stronger resistance to powdery mildew than *Pm2*, *Pm4* and *Pm5*, although such comparisons are tentative since the PM-LBT population was evaluated for resistance in the field and the other populations were evaluated in a CE room. For each of the populations the subjectivity of the scoring method is expected to account for a proportion of the observed phenotypic variation, thereby reducing the apparent effect of the resistance genes. The proximal marker for *Pm6* was located 10.4 cM from the next marker on the linkage group and so there is scope for identifying a more tightly linked marker that would demonstrate even lower infection levels in resistant genotypes. The development of powdery mildew on most PM-LBT plants and the apparent loss of genetic resistance by 59 DAI suggest that *Pm6* confers a similar slow-mildewing phenotype to that described for *Pm1* (Lewellen and Schrandt 2001) whereby disease developed on mature leaves late in the season. In wheat, incomplete, non-race specific resistance retarding growth and reproduction of powdery mildew in adult plants has been termed 'slow-mildewing' (Shaner 1973), 'adult-plant resistance' (Gustafon and Shaner 1982) or 'partial resistance' (Hautea et al. 1987). Adult plant resistance in wheat is reportedly more durable than race-specific major gene resistance (Liu et al. 2001), widespread use of which has consistently led to the evolution of powdery mildew populations with matching virulence alleles (Tucker et al. 2006). There are no known virulence or physiologic races of sugar-beet powdery mildew, although contrasting descriptions of conidial development (e.g. Hull 1971; Mukhopadhyay and Russell 1979) suggest that different morphological variants may exist.

Resistance levels compared with sugar-beet cultivars

The original accessions of the PM-MAR and PM-GBT populations were found to be significantly more resistant to powdery mildew than three sugar-beet cultivars in a CE room evaluation. Both original accessions were segregating, suggesting that if marker screening was employed to select only resistant individuals, then the percentage of leaf area infected per plant would be even lower. Although the controlled environment may not be fully reflective of the field environment, we have previously shown that, in general, there is a significant correlation between glasshouse and field results for accessions tested in both environments (Spearman rank correlation $R = 0.64$; $P \leq 0.01$) (Luterbacher et al. 2004). The data suggest that the powdery mildew resistance levels of current sugar-beet cultivars can be significantly improved by introgression of the resistance genes from the sources studied here.

Future work and conclusions

Extensive field evaluations are required to determine the nature of resistance conferred by the five genes identified here. We have already made further crosses to sugar beet in order to study resistance in more advanced breeding material. Combining the different genes could result in further retardation of mildewing symptoms and perhaps complete resistance. Crosses between resistant plants of the three populations have been carried out to facilitate such studies. By pyramiding the resistance genes there is also excellent potential for achieving durable resistance to sugar-beet powdery mildew, obviating the requirement for chemical control. The molecular markers presented in this study are a valuable tool in achieving this.

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