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Sources and genetic structure of a cluster of genes for resistance to three pathogens in lettuce

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Abstract The second largest cluster of resistance genes in lettuce contains at least two downy mildew resistance specificities, *Dm5/8* and *Dm10*, as well as *Tu*, providing resistance against turnip mosaic virus, and *plr*, a recessive gene conferring resistance against *Plasmopara lactucae-radicis*, a root infecting downy mildew. In the present paper four additional genetic markers have been added to this cluster, three RAPD markers and one RFLP marker, *CL1795*. *CL1795* is a member of a multigene family related to triose phosphate isomerase; other members of this family map to the other two major clusters of resistance genes in lettuce. Seven RAPD markers in the region were converted into sequence characterized amplified regions (SCARs) and used in the further analysis of the region and the mapping of *Dm10*. Three different segregating populations were used to map the four resistance genes relative to molecular markers. There were no significant differences in gene order or rate of recombination between the three crosses. This cluster of resistance genes spans 6.4 cM, with *Dm10* 1.2 cM from *Dm8*. Marker analysis of 20 cultivars confirmed multiple origins for *Dm5/8* specificity. Two different *Lactuca serriola* origins for the *Du5/8* specificity had previously been described and originally designated as either *Dm5* or *Dm8*. Some ancient cultivars also had the same specificity. Previously, due to lack of recombination in genetic analyses and the same resistance specificities, it was assumed that *Dm5* and *Dm8* were determined by the same gene. However, molecular marker analysis clearly identified genotypes characteristic of each source. Therefore, *Dm5/8* specificity is either ancient and widespread in *L. serriola* and some *L. sativa*, or else has arisen on multiple occasions as alleles at the same locus or at linked loci.

Key words Disease resistance · Lettuce · Downy mildew · Molecular markers · Genetic mapping

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Introduction

A major emphasis of lettuce breeding programs has been the introduction of genes for resistance to a variety of pathogens (Ryder 1986; Crute 1988). Most attention has been devoted to developing cultivars resistant to downy mildew, caused by the oömycete pathogen, *Bremia lactucae* (Crute 1987, 1992). There are at least 15 well-characterized dominant genes for resistance to downy mildew (*Dm*; Johnson et al. 1978; Farrara et al. 1987; Maisonneuve et al. 1994). Each *Dm* gene is matched by a specific avirulence gene in the pathogen in a gene-for-gene interaction (Crute and Johnson 1976; Ilott et al. 1989). Most of the resistance genes currently utilized originated either within cultivated *Lactuca sativa* or have been introgressed from *L. serriola*, the closest wild relative of lettuce (Crute 1987; Kesseli et al. 1991). Many additional sources of resistance have been identified (Farrara et al. 1987; Bonnier et al. 1994) and current breeding programs are introgressing resistance genes from *L. saligna* and *L. virosa* as well as *L. serriola*. When new resistances to downy mildew are first discovered they are designated R-factors; the *Dm* designation is only used after the resistance has been genetically characterized and single genes identified (Farrara et al. 1987).

Disease resistance genes in lettuce are organized in several distinct clusters. Three major clusters have been identified so far (Johnson et al. 1978; Hulbert and Michelmore 1985; Farrara et al. 1987; Kesseli et al. 1994). Genes for resistance to several parasites are present in two of the clusters. The largest cluster contains at least eight *Dm* genes (Farrara et al. 1987) plus a gene for root-aphid resistance (Crute and Dunn 1980); this cluster spans a genetic distance of 20 cM and a physical distance of at least 6 Mb (P. Anderson, R. Kesseli, and R. Michelmore, unpublished). Several additional R-factors have been mapped to this cluster (Bonnier et al. 1994). The second largest cluster contains at least two *Dm* genes (see below), together with *Tu*, providing resistance against turnip mosaic virus (Zink and Duffus 1973), and *plr*, conferring resistance to *Plasmopara lactucae-radicis* Stang. and Gilbn. root downy mildew (Vandemark et al. 1992). This cluster spans over 6.4 cM (Hulbert and Michelmore, 1985; Robbins et al. 1994, Kesseli et al. 1993). *R17*, a new resistance factor, is also loosely linked to this cluster (Maisonneuve et al. 1994). In addition, pedigree analysis indicated that a gene conditioning a non-lethal reaction to a virulent isolate of lettuce mosaic virus is also linked to this cluster (Zink et al. 1973). The third cluster contains three downy mildew resistance genes *Dm4*, *Dm7* and *Dm11* (Hulbert and Michelmore 1985). Not all resistance genes have been mapped to clusters; linkage to other resistance genes has not been shown for *Dm13*, *Ant1* (resistance to *Microdochium panattoniana*, O. Ochoa and R. Michelmore, unpublished) and *cor* (resistance to *Rhizomonas suberifaciens*; Brown and Michelmore 1988; Kesseli et al. 1994). We are currently mapping many of the known resistance genes in lettuce to gain a detailed understanding of their genetic organization.

Using molecular markers we have been developing a detailed genetic map of lettuce with emphasis on regions containing disease resistance genes. The genetic map currently comprises over 450 loci (Kesseli et al. 1994, and unpublished). The majority are restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP) markers. We have used near-isogenic lines (Paran et al. 1991), bulked segregant analysis (Michelmore et al. 1991), and deletion mutants (D. Lavelle, P. Okubara and R. Michelmore, unpublished) to map disease resistance loci and identify linked markers. Detailed knowledge of the genetic organization of each cluster will aid in the manipulation of resistance genes in breeding programs and in molecular studies of their function. The pedigrees of many lettuce cultivars are well documented. Dissection of these pedigrees using molecular markers allows the evolution of resistance gene clusters to be analyzed.

Resistance genes in the second largest cluster have come from several different origins. There were potentially at least three different sources for *Dm5/8*. This resistance specificity is documented as originating from *L. serriola* PI167150 (*Dm5*, Leeper et al. 1963), *L. serriola* PI91532 (*Dm8*, Jagger and Whitaker 1940; Whitaker et al. 1958; sometimes erroneously cited as originating from PI104854), or from old cultivars of *L. sativa* such as Sucrine and Bourguignonne Grosse Blonde d'Hiver (BGBH; Channon and Smith 1970; Norwood and Crute 1985). However, surveys of *B. lactucae* failed to identify any isolates that distinguished these different sources of resistance. Genetic analysis indicated that resistance mapped to the same locus (Hulbert and Michelmore 1985). Furthermore, genetic analysis in *B. lactucae* demonstrated that avirulence to these resistances co-segregated (Michelmore et al. 1984; Norwood and Crute 1984). Other avirulence genes in *B. lactucae* are not tightly linked (Ilott et al. 1989). Therefore, the co-segregation of specificity in both host and pathogen led to the conclusion that resistance was determined by a single gene that was designated *Dm5/8*. To distinguish the different sources of resistance for the purposes of this paper, the resistance from PI16750 is referred to as *Dm5*, resistance from PI91532 as *Dm8*, and resistance from cv Sucrine as *Dm5/8*. In addition, resistance that mapped to this cluster was identified in PI164937 and may have had the same specificity as *Dm8* (Zink 1973); however, this was not used as a source of resistance in breeding programs. The origins of *Dm10*, another *Dm* gene in this cluster, are unclear. It is present in old European latin and butterhead cultivars as well as in more modern California crisphead cultivars. *Tu* is present in the majority of lettuce genotypes tested. However, most cultivars derived from PI91532 (carrying *Dm8*) are susceptible to TuMV and *Dm8* has been shown to be linked in repulsion to *Tu* (Zink and Duffus 1969, 1970, 1974). The origin of the *plr* gene is unknown; *plr* is present in several old European butterhead cultivars (Vandemark et al. 1992; this paper).

In the present paper we describe the genetic characterization of the sources of resistance genes in the second largest cluster and analyze the major lettuce pedigrees in which these genes have been selected. Marker analysis indicated that the different sources are distinct and identified recombination events during breeding programs. *Dm10* was mapped relative to *Dm8*. Segregation data from multiple F_2 populations were compared to study variation in recombination rates across the region.

Materials and methods

DNA manipulations and PCR

DNA was extracted from leaves using a modified CTAB protocol (Murray and Thompson 1980; Bernatzky and Tanksley 1986). The DNA was diluted to approximately 4–5 ng/μl in a modified TE buffer with reduced EDTA concentration (0.1 mM) and 20–25 ng was used in each 25-μl PCR reaction. Procedures for RAPD analysis were similar to Williams et al. (1990) and are described elsewhere (Michelmore et al. 1991; Paran et al. 1991). All RAPD primers were provided by Operon Technologies (Alameda, Calif.) All PCR reactions were performed in a Perkin Elmer Cetus 480 thermocycler. Conventional gel electrophoresis, Southern blotting and hybridization were done

according to standard protocols (Sambrook et al. 1989). Probes were labeled using random primer kits from Amersham (Arlington Heights, Ill.).

Generation of sequence characterized amplified region (SCAR) markers

Cloning and sequencing of polymorphic RAPD products were performed as described by Paran and Michelmore (1993). Extended primers were analyzed using the OLIGO 4.0 program (Rychlik and Rhoads 1989) before oligonucleotide synthesis, and their sequences optimized when needed. The thermal cycling program for amplification of SCAR markers was as described previously (Paran and Michelmore 1993). Annealing temperatures were optimized for each pair of primers as described.

Plant material

Leaves from at least ten individuals for each cultivar were pooled prior to DNA extraction for analysis of RAPD and SCAR genotypes. Ten to twenty individuals from each cultivar were screened for their reaction to *B. lactucae*, *P. lactucae-radicis* and TuMV as described previously (Farrara et al. 1987; Kesseli et al. 1993; Robbins et al. 1994).

Mapping of *Dm10*

A F_2 population from a cross between two crisphead cultivars, Salinas (*Dm7*, *Dm8* and *Dm13*) and E1 Toro (*Dm10*), was used to map *Dm10* relative to *Dm8*. DNA of 88 F_2 individuals was assayed for the genotypes at SCAR loci *SCS12*, *SCX03*, *SCD08* and *SCU16* and RAPD locus $OPL08_{630}$, the only PCR-based markers that were polymorphic in this cross. *SCS12* (in *cis* with *Dm8*) and *SCU16* (in *cis* with *Dm10*) could be assayed together since they both have an annealing temperature of 68 °C; *SCD08* (in *cis* with *Dm8*) and *SCX03* (in *cis* with *Dm10*) were also assayed together as they have an annealing temperature of 60 °C. More than 20 individuals of each F_3 family were screened for resistance to *B. lactucae* as described previously (Farrara et al. 1987). Recombinant families were assayed twice. *Dm8* was assayed using isolate CG1 of *B. lactucae* that recognizes *Dm2*, *Dm5/8*, *Dm6*, *Dm11*, and *Dm14*. *Dm10* was assayed using isolate SF3, that recognizes *Dm4*, *Dm6*, *Dm10* and *Dm16*, as well as isolate C83M47, that recognizes *Dm1*, *Dm10*, *Dm11*, and *Dm15*.

Genetic analysis

Segregation data were analyzed using MAPMAKER (Lander et al. 1987) to determine gene orders and to calculate multipoint genetic distances. Recombination frequencies were converted to genetic distances using the Kosambi mapping function. To compare maps derived from different F_2 populations, all data sets were reanalyzed using MAPMAKER 3.0. Datasets were tested for heterogeneity using the $G_{\text{heterogeneity}}$ function of GMendel 3.0 (Holloway and Knapp 1993).

Results

New markers linked to the *Dm5/8* region

Three new RAPD markers, $OPG06_{1350}$, $OPN13_{400}$, and $OPV01_{1000}$, were identified as linked to *Dm8* during the mapping of arbitrary RAPD polymorphisms using our basic Calmar × Kordaat mapping population (Kesseli et al. 1994; P. Okubara, unpublished). These markers were placed precisely relative to *Dm8*, and other markers in the region, using recombinants from 202 additional F_2 individuals of the same population (Fig. 1).

A new RFLP marker linked to *Dm8* was detected by cDNA clone pCL1795. This RFLP probe hybridizes to sequences related to triose phosphate isomerase (TPI) and detects a multigene family in lettuce, some members of which had been mapped to the two other clusters of *Dm* genes as well as elsewhere in the genome (Paran et al. 1992). Furthermore, there seemed to be a correlation between *Dm8* and a RFLP detected by pCL1795 in our survey of lettuce cultivars (Kesseli et al. 1991). Therefore, we predicted that a member of this multigene family was also linked to the *Dm5/8* region. To test this hypothesis, we used bulked segregant analysis (Michelmore et al. 1991) to screen a range of endonucleases for their ability to detect RFLPs linked to *Dm8*. pCL1795 was hybridized to Southern blots of pooled DNA samples of either 11 homozygous-resistant (*Dm8Dm8*) or 12 homozygous-susceptible (*dm8dm8*) F_2 plants that had been digested with 1 of 12 restriction enzymes: *Acc*I, *Bam*HI, *Cla*I, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dII, *Hin*dIII, *Kpn*I, *Pvu*I, *Sac*I, *Xba*I. As controls, we used similarly bulked DNA samples for the *Dm1,3* and *Dm4,7* clusters; RFLPs linked to *Dm8* would not be polymorphic between the bulks for the other two clusters. Polymorphisms between the *Dm1,3* bulks were found using *Acc*I, *Hin*dIII and *Eco*RI digests, confirming results obtained by Paran et al. (1992). No polymorphisms were ob-

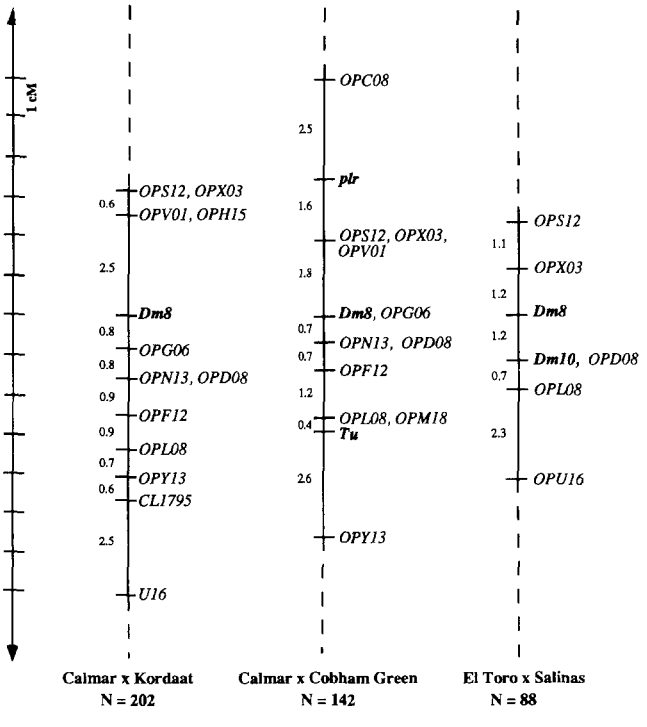

Fig. 1 Genetic maps of the *Dm5/8* region derived from analyses of three crosses of *Lactuca sativa*. The number of F_2 individuals analyzed (*N*) is shown below each map. The genetic distances in cM are shown. The maps are aligned on the position of *Dm8*. The Calmar × Cobham Green was derived from data described in Kesseli *et al.* (1993) Robbins *et al.* (1994).

served between the *Dm4,7* bulks for any of the digests; RFLPs detected by pCL1795 that are linked to *Dm4* and *Dm7* have only been identified using a different population (Paran et al. 1992). RFLPs were detected between the *Dm8* bulks in digests with *Acc*I and *Kpn*I; a band was present in the *dm8dm8* bulked DNA sample that was absent in the *Dm8Dm8* bulk (Fig. 2). To confirm linkage, pCL1795 was hybridized to DNA of the eight susceptible and eight resistant F_2 individuals that had been used to construct the *Dm8* bulks; pCL1795 hybridized to a unique *Acc*I fragment only in the susceptible individuals; no recombinants were observed among these 16 individuals. The polymorphic 2.5-kb fragment detected in the *Acc*I digest was designated $CL1795_{A25}$ and mapped precisely using nine recombinants between $OPU16_{360}$ and $OPX03_{1250}$. $CL1795_{A25}$ mapped between $OPY13_{800}$ and $OPU16_{360}$ (Fig. 1). This provides further evidence that the *Dm* genes in the different clusters have arisen by genome duplication.

Generation of SCAR markers

Seven RAPD loci were converted into SCAR loci: $OPS12_{1250}$, $OPX03_{1250}$, $OPG06_{1350}$, $OPN13_{400}$, $OPD08_{680}$, $OPF12_{1340}$ and $OPU16_{360}$. A variety of primer lengths (20–25 nucleotides) and annealing temperatures (60–68 °C) were used (Table 1). The initial pair of primers for *SCF12* amplified the same-sized fragment from both parents; however, when primers that started five or eight bases inside the sequence of the RAPD primer were used, a band was amplified only from Calmar resulting in a dominant polymorphism. The other SCAR primers also amplified bands from only one parent, two from Kordaat and four from Calmar, and were therefore dominant markers. No co-dominant SCAR loci were found such as those previously identified as linked to the *Dm1,3* region (Paran and Michelmore 1993). We were not successful in designing functional SCAR primers for $OPY13_{800}$; three different primers were tried but no amplification was obtained in either combination over a range of annealing temperatures. Primers derived from $OPC08_{370}$ amplified the same-sized band in both Calmar and Kordaat; digestion of these amplification products with 30 restriction enzymes did not reveal a polymorphism between these cultivars.

The cloned RAPD fragments were tested as hybridization probes. To determine which fragments represented single or low-copy genomic sequences, an initial screen was performed using reverse genomic Southern blots. All the cloned RAPD fragments were transferred to a membrane and hybridized to labeled genomic lettuce DNA. The whole cloned fragment could be used as a probe on genomic Southern blots only when there was a faint, or absent, hybridization signal after overnight autoradiography. When there was detectable hybridization in the reverse genomic blots, subfragments of the cloned RAPD sequences were generated using each of eight endonuclease digests and tested for low-copy sequences as above. Only clones of $OPU16_{360}$, $OPN13_{400}$ and $OPC08_{370}$ seemed to be comprised entirely of low-copy genomic DNA in reverse genomic blots. Low-copy subfragments were identified for $OPX03_{1250}$, $OPG06_{1350}$ and $OPF12_{1340}$, but not for $OPS12_{1250}$, $OPD08_{680}$, $OPY13_{800}$. Between two to seven bands were observed when subfragments of $OPX03_{1250}$ and $OPF12_{1340}$ were tested as probes on Southern blots with total genomic DNA digested with *Eco*RI, *Eco*RV, *Hin*dIII and *Bam*HI. A polymorphic band was identified for both of these probes in each of the *Eco*RI, *Eco*RV and *Hin*dIII digests of Calmar and Kordaat; the remainder of the bands were monomorphic. For $OPN13_{400}$ and a subfragment of $OPG06_{1350}$ tested on genomic Southern blots, only non-polymorphic bands were observed in all digests with significant smears due to weak hybridization to many fragments. As none of the cloned fragments identified single RFLP loci, they could not be used as hybridization probes in this study. Therefore, nearly all the analyzes were made using PCR-based markers; the only hybridization probes used, pCL877 and pCL1795, were identified in an earlier screen of random cDNA clones.

Fig. 2 Bulked segregant analysis with pCL1795 detecting linkage of members of the TPI multigene family to two clusters of resistance genes. Bulked DNA samples of homozygous-resistant (*R*) or -susceptible (*S*) individuals for each of the three major clusters of *Dm* genes (*Dm1,3*; *Dm5/8*; *Dm4*) were digested with *Acc*I and hybridized to pCL1795 (see text). Fragments that are polymorphic between the bulks are arrowed

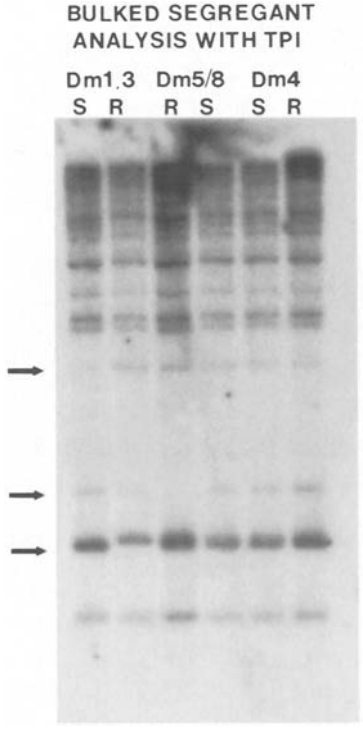

Table 1 SCAR (Sequence Characterized Amplified Region) markers in the *Dm5/8* region. SCAR markers are listed according to their order on the genetic map (Fig. 1)

SCAR[a] primers	Sequence[b]	#n[c]	Annealing[d] temperature	Band size[e]	C/K[f]	Polymorphism[g]	High[h] copy	Internal low-copy[i] sequence as probe
SCC08A	GGTGGAGACATAGGTGGTTATTTA	25	60, 65 °C	370	K	No polym.	No	Not tested
SCC08B	GGTGCTGAAACATTTCAAAATCTT	24						
SCS12A	CTGGGTGAGTAGGTGCTGTGAGTG	24	68 °C	1250	C	Dominant	Yes	Not found
SCS12B	CTGGGTGAGTGTGTAGTTACTTTC	24						
SCX03A	TGGCGCAGTGTAAGGGTTGAG	21	60 °C	1250	K	Dominant	Yes	*Hin*dIII/*Hae*II; 550 bp; Dominant
SCX03B	TGGCGCAGTGGAAATGCAAAT	21						
SCG06A	GTGCCTAACCTCACACTCACCAT	23	65 °C	1350	C	Dominant	Yes	*Msp*I/*Hae*II; 300 bp; Not polymorphic
SCG06B	GTGCCTAACCTAAATATGCGACAG	24						
SCN13A	AGCGTCACTCGAAGGGTTTAGG	22	60 °C	185*	C	Dominant	No	Not polymorphic
SCN13B	ATTTCAAACTGCAAGTGAACTACG	24						
SCD08A	GTGTGCCCCACAATTACCTATATC	24	60 °C	680	C	Dominant	Yes	Not found
SCD08B	GTGTGCCCCAGTATGCGGGTGATG	24						
SCF12A	ACGGTACCAGTGACGAGGAGATTC	24	68 °C	1340	C	Dominant	Yes	Yes; *Hin*dIII/*Taq*I; 740 bp; Dominant
SCF12D	GGAAACTCGACCCCAAAGAT	20						
SCF12C	GAGGAGATTCGCAGGATGAT	20	68 °C	1340	C	Dominant	Yes	Yes; *Hin*dIII/*Taq*I; 740 bp; Dominant
SCF12D	GGAAACTCGACCCCAAAGAT	20						
SCF12A	ACGGTACCAGTGACGAGGAGATTC	24	68 °C	1340	C	No polym.	Yes	
SCF12B	ACGGTACCAGGTCAATTGGAAACT	24						
SCF12B	ACGGTACCAGGTCAATTGGAAACT	24	68 °C	1340	C	No polym.	Yes	
SCF12C	GAGGAGATTCGCAGGATGAT	20						
SCY13A	GGGTCTCGGTAGAAGGCTACTTTA	25	50–68 °C	800**	C	No ampl.	Yes	Not found
SCY13B	GGGTCTCGGTACCTATCTATTGAT	24						
SCY13A	GGGTCTCGGTAGAAGGCTACTTTA	25	50–68 °C	800**	C	No ampl.	Yes	Not found
SCY13C	ACCTATCTATTGATGACCTGAGAA	24						
SCU16A	CTGCGCTGGACGAAAAAAATGGTA	24	68 °C	360	K	Dominant	No	Not tested
SCU16B	CTGCGCTGGATCATGAAGATGATG	24						

[a] The names of the primers used to amplify the SCAR marker shown by the first five characters
[b] The sequence of each SCAR primer; the sequence of the progenitor RAPD primer is underlined if it was included in the SCAR primer
[c] The number of nucleotides in each primer
[d] The annealing temperature used to detect polymorphism between Calmar and Kordaat; when no polymorphism was detected or no amplification occurred, the range of temperatures tested is shown
[e] The approximate size of the fragment amplified by the SCAR and RAPD primers *: size of the original RAPD band is 400 bp; **: size of the RAPD-band
[f] Whether the dominant RAPD polymorphism is amplified from Calmar or Kordaat
[g] The nature of the polymorphism (no polym. = no polymorphism detected; no ampl. = no amplification)
[h] Whether or not the SCAR-fragment contained sequences that had a high-copy number in the genome
[i] Whether parts of the amplified fragment could be identified that consisted of single- or low-copy sequences and the enzymes used to identify the low-copy fragment within the amplified sequence and the size of the band after digestion

Mapping *Dm10*

Dm10 was mapped relative to *Dm8* and SCAR markers *SCS12*, *SCX03*, *SCD08* and *SCU16*, as well as RAPD marker $OPL08_{630}$, using an F_2 population derived from El Toro × Salinas. This cross was selected as it segregated for both *Dm8* and *Dm10* as well as for five molecular markers distributed through the region. DNA from 88 F_2 individuals was analyzed to determine the genotypes of the SCAR loci. Eighty-eight F_3 families were screened with isolates of *B. lactucae* diagnostic for *Dm8* and *Dm10*. Ten F_2 individuals were homozygous for the parental multilocus genotype of Salinas, 19 were homozygous for the parental multilocus genotype of El Toro, 52 were heterozygous at all loci, and seven were recombinant. The genotypes were determined for individual F_3 plants of two critical families to determine the heterozygosity of *SCD08* and $OPL08_{630}$. This placed *Dm10* between *Dm8* and $OPL08_{630}$, 1.2 cM from *Dm8* and co-segregating with *SCD08* (Fig. 1).

Comparison of the *Dm5/8* region in different segregating populations

Three different populations have been used to map four resistance genes in the *Dm5/8* region relative to molecular markers: *Dm8* was mapped using 202 F_2 individuals of Calmar × Kordaat (Landry et al. 1987; Kesseli et al. 1994, and unpublished data), *Dm8*, *Tu* and *plr* were mapped using 142 F_2 individuals of Calmar × Cobham Green (Kesseli et al. 1993; Robbins et al. 1994), and *Dm8*

and *Dm10* were mapped using 88 F_2 individuals of El Toro × Salinas (this paper). To compare the genetic maps derived from the three different F_2 populations, we reanalyzed the data using MAPMAKER. The maps were compared using the $G_{heterogeneity}$ function of GMendel version 3.0 (Holloway and Knapp 1993) to test for significant differences between the datasets. No significant heterogeneity in either gene order or recombination distance was detected. Therefore, we attempted to construct a consensus map using Joinmap (Stam 1993). However, the resulting gene order was clearly inconsistent with the position of recombination events and therefore a meaningful consensus map could not be generated.

Marker analysis of the *Dm5/8* region

The genotypes for disease-resistance loci *Dm5*, *Dm8*, *Dm5/8*, *Dm10*, *plr* and *Tu*, RFLP locus *CL877* (previously mapped to this region; Landry et al. 1987), SCAR loci *SCS12*, *SCX03*, *SCG06*, *SCN13*, *SCD08*, *SCF12*, and *SCU16*, and RAPD loci $OPL08_{630}$, $OPV01_{1000}$, and $OPY13_{800}$, were determined for 20 lettuce cultivars (Table 2). These cultivars were selected to represent the diversity of resistance-gene combinations in the *Dm5/8* region. Calmar, Salinas, Avoncrisp (crisphead cultivars), Avondefiance, and Diana (butterhead cultivars) all contain *Dm8* derived from *L. serriola* PI91532 (Jagger and Whitaker 1940; Welch et al. 1965; Dawson 1976; Ryder 1979; Crute 1987). Great Lakes and Mesa 659 contain *Tu* and no known *Dm* gene in this cluster; they were derived from Imperial D which was the other parent in the original cross with *L. serriola* PI91532 (Fig. 3; Bohn and Whitaker 1951; Thompson and Ryder 1961). Valmaine contains *Dm5* in combination with *Tu* from *L. serriola* PI167150 (Leeper et al. 1963). Old European cultivars Sucrine (Latin type) and Bourguignonne Grosse Blonde d'Hiver (BGBH, butterhead type) contain *Dm5/8* in combination with *Dm10* (Crute and Johnson 1976; Norwood and Crute 1984, 1985). Vanguard, El Toro (Californian crisphead cultivars) and Kinemontepas (old European butterhead cultivar) contain *Dm10* in the absence of *Dm5/8*. Kordaat, Cobham Green, Blondine, May King, Hilde, Mildura (all European butterhead cultivars) and Gallega (Latin type) have neither *Dm5/8* nor *Dm10*; however, they all contain *Tu* and Cobham Green, Mildura and May King contain both *Tu* and *plr* (Zink and Duffus 1974; Vandemark et al. 1992). Data from our disease screens were consistent with the reactions reported in the literature.

The marker analysis indicates at least two distinct derivations for *Dm5/8* specificity (Table 2). All five cul-

Table 2 Genotypes of selected lettuce cultivars at loci in the *Dm5/8* region. Lettuce types are indicated by c, crisphead; b, butterhead; r, romaine and i, latin. *S12*, *X03*, *G06*, *N13*, *D08*, *F12* and *U16* are SCAR loci; *V01*, *L08* and *Y13* are RAPD loci; *877* is an RFLP locus; *plr*, *Dm5*, *Dm8*, *Dm5/8*, *Dm10* and *Tu* are disease resistance loci. + implies presence of a resistance gene or a RAPD or RFLP locus; − implies absence of a resistance gene or RAPD or RFLP locus (note: this is the reverse of some other conventions where + indicates sporulation of the pathogen and therefore absence of the resistance gene). n.t., not tested. Gene order was derived from those shown in Fig. 1. As not all loci segregated in all three crosses, some local gene orders are ambiguous

Cultivar	Type	*plr*	*S12*	*X03*	*V01*	*Dm5/8*	*G06*	*N13*	*Dm10*	*D08*	*F12*	*L08*	*Tu*	*877*	*Y13*	*U16*
Dm8 (PI91532)																
Calmar	c	−	+	−	−	+	+	+	−	+	+	+	−	+	+	−
Salinas	c	−	+	−	−	+	+	+	−	+	+	+	−	+	+	−
Avoncrisp	c	−	+	−	−	+	+	+	−	+	+	−	+	−	−	−
Avondefiance	b	n.t.	+	−	−	+	+	+	−	+	+	−	+	−	−	−
Diana	b	−	+	−	−	+	+	+	−	+	+	−	+	−	−	−
Dm5 (PI167150)																
Valmaine	r	−	−	+	−	+	−	+	−	−	+	−	+	−	+	+
Dm5/8 + *Dm10*																
Sucrine	I	+	−	+	−	+	−	+	+	−	+	−	+	−	−	+
BGBH	b	+	−	+	−	+	−	+	+	−	+	−	+	−	−	+
Dm10																
Vanguard	c	−	−	+	−	−	+	+	+	−	+	−	+	−	+	+
El Toro	c	−	−	+	−	−	+	+	+	−	+	−	+	n.t	+	+
Kinemontepas	b	+	−	+	+	−	+	−	+	−	+	−	+	n.t.	+	+
No *Dm5/8*, no *Dm10*																
Great Lakes	c	n.t.	−	+	−	−	+	+	−	−	+	−	+	−	−	−
Mesa 659	c	−	−	+	−	−	+	+	−	−	+	−	+	−	−	−
Gallega	I	n.t.	−	+	−	−	+	+	−	−	+	−	+	−	−	−
Blondine	b	−	−	+	+	−	+	−	−	−	−	−	+	−	+	+
May King	b	+	−	+	+	−	+	−	−	−	−	−	+	−	−	+
Kordaat	b	−	−	+	+	−	−	−	−	−	−	−	+	−	−	+
Cobham Green	b	+	−	+	+	−	−	−	−	−	−	−	+	−	−	−
Hilde	b	−	−	+	+	−	−	−	−	−	−	−	n.t.	−	−	−
Mildura	b	+	−	+	+	−	−	−	−	−	−	−	n.t.	−	−	−

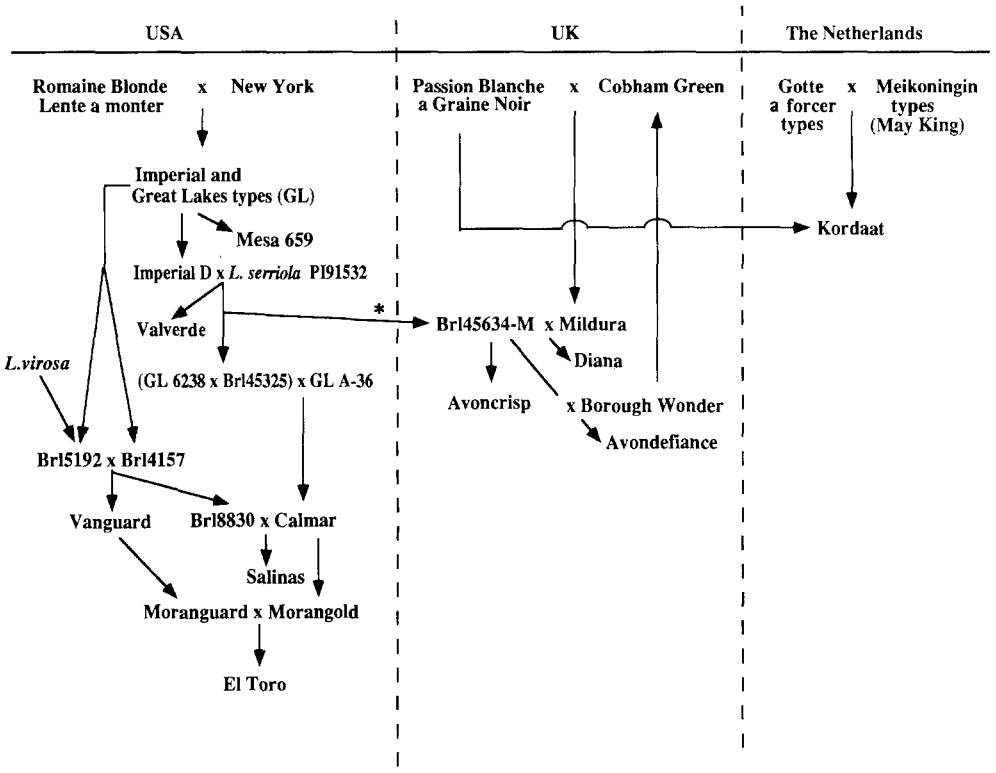

Fig. 3 Interrelationships of cultivars analyzed in this study (Jagger and Whitaker 1940; Bohn and Whitaker 1951; Leeper and Whitaker 1959; Thompson and Ryder 1961; Welch et al. 1965; Dawson 1976; Ryder 1979; Crute 1987). Several cultivars (Valmaine, Kinemontepas, Gallega, Blondine, Hilde, Sucrine, BGBH) are present in Table 2 but are not included in this diagram because their interrelationships are unknown. * location of the recombination event resulting in the association of *Dm8* with *Tu*

tivars that have *Dm8* from *L. serriola* PI91532 have similar genotypes. They share dominant alleles at *SCS12*, *SCG06*, *SCN13*, *SCD08*, *SCF12* and *Dm8*. Unfortunately, PI91532 is no longer available for analysis. The marker genotype in PI91532 for the region was presumably as in Calmar and Salinas since both these crisphead cultivars retain the association of *Dm8* with TuMV susceptibility found in PI91532 (Zink and Duffus 1969). Avondefiance, Avoncrisp and Diana have a genotype that is a hybrid between those of Calmar and Mesa 659. This indicates that a fortuitous recombination event occurred in the interval between *SCD08* and $OPL08_{630}$ sometime in the complex pedigree between PI91532 × Imperial D and the generation of the related breeding lines USDA 45325 and 45634-M (Welch et al. 1965). This broke the association of *Dm8* with TuMV susceptibility and resulted in the transmission of *Dm8* and *Tu* to Avondefiance, Avoncrisp and Diana (Fig. 3). Valmaine, the only cultivar carrying *Dm5* derived from *L. serriola* PI167150 that we tested, differs from Calmar at 7 out of the 11 molecular-marker loci (Table 2). Therefore, *Dm5* seems to have been introgressed into *L. sativa* independently from *Dm8*. Sucrine has the same genotype as BGBH; it differs from Valmaine at 3 out of the 15 loci tested (Table 2). However, the dissimilar loci (*plr*, *Dm10*, and *OPY13*) are distributed throughout the region and therefore these two old cultivars may represent a third derivation of the *Dm5/8* specificity.

The number of possible derivations for genotypes lacking *Dm5/8* resistance is less clear. The crisphead cultivars tested had similar genotypes reflecting their common ancestry. One progenitor of El Toro was Vanguard and they both have the same genotype. The El Toro/Vanguard genotype is similar to that of Mesa 659 (*Dm10* plus two terminal loci differ; Table 2); Vanguard and Mesa 659 share progenitors. There are six genotypes among the seven European butterheads that lack *Dm5/8*; however, several have similar genotypes. Mildura and Cobham Green have identical genotypes; the former was the product of a backcross program with the latter as the recurrent parent (Dawson 1976). May King and Kordaat are related and have 13 out of 15 alleles in common. May King and Cobham Green share 13 out of 15 alleles; however, they have no documented progenitors in common and May King is a short-day (forcing) type and Cobham Green is a day-neutral type. Cobham Green, a dark green selection out of Borough Wonder, and Blondine represent old butterhead pedigrees; each has a distinct genotype. Kinemontepas, another old butterhead cultivar, is the most distinct having three to six alleles different from the other butterheads. Gallega, a Latin type, has yet another genotype. Therefore, the molecular-marker data indicate at least five derivations for the region with a *dm5/8* allele.

There may also be multiple derivations for *Dm10* specificity. The American crisphead cultivars, Vanguard and El Toro, have *Dm10* but are not known to be related to the European butterheads, Kinemontepas and BGBH, or to the latin type Sucrine. This is supported by the distinct molecular-marker genotypes. The majority of the region in Vanguard and El Toro is identical to Great Lakes except for *Dm10* in the middle, and two markers at one end, of the region studied. Similarly, Sucrine and Valmaine are identical except for two markers close to each end of the region studied and at *Dm10* in the center. Interestingly, Sucrine and BGBH have identical genotypes; both are ancient cultivars whose interrelationship is unknown. In contrast, the

genotypes of the two butterheads with *Dm10*, BGBH and Kinemontepas, are different.

Discussion

Cluster of diverse resistance genes

We have used molecular markers to analyze the second largest cluster of resistance genes so far identified in lettuce. This cluster is now known to contain at least four genes for resistance to two fungi and a virus and spans 6.4 cM; another resistance gene (*R17*) is loosely linked (Maisonneuve et al. 1994). There may also be a gene for resistance to lettuce mosaic virus in this region (Zink et al. 1973). The majority of the breeding effort in relation to this region has been to introduce downy mildew resistance (*Dm5* or *Dm8*) into American romaine and crisphead cultivars where it remained effective for over 10 years (Crute 1987). The other resistance genes have been co-inherited or lost from the breeding programs due to their linkage in coupling or repulsion with *Dm8*. Most cultivars are resistant to TuMV except those selected to include *Dm8*. A fortuitous recombination event produced *Dm8* in coupling with *Tu* and resulted in the generation of cultivars in Britain with both resistance genes.

The variety of genotypes that we detected for the *Dm5/8* region in European butterhead types, reflected our inclusion of diverse old cultivars for this study. In addition, there has been little conscious selection for the *Dm5/8* region in these cultivars as *Dm5/8* specificity proved only briefly effective against European isolates of *B. lactucae* (Crute 1987). The root downy mildew caused by *P. lactucae-radicis* has only been recognized recently and very locally in the USA (Stanghellini et al. 1990). Resistance to *P. lactucae-radicis* conferred by *plr* seems to have been maintained without selection in the European butterhead cultivars.

Multiple derivations of *Dm5/8* specificity

We had previously concluded that *Dm5/8* resistances were determined by the same gene since they exhibited the same reaction to a wide range of isolates of downy mildew and their progeny (Michelmore et al. 1984; Norwood and Crute 1984). Furthermore, no susceptible recombinants were detected in crosses between Calmar or Valverde and Valmaine or Sucrine (Hulbert and Michelmore 1985). We still have not identified an isolate that distinguishes these different sources of resistance; however, as we had assumed that they were the same gene, we have not always included all three sources in all our isolate surveys. Genetic studies on *B. lactucae* have continued to indicate co-segregation of avirulence to these cultivars (Ilott et al. 1989). The original segregation data for the three sources of resistance clearly provide strong evidence of linkage; however, as the resistances were in repulsion, the test for allelism was weak (Hulbert and Michelmore 1985; see below).

Our molecular marker data now strongly suggest that there were at least two, and probably three, distinct derivations for *Dm5/8* specificity. The region containing resistance derived from *L. serriola* PI91532, is clearly different from all the other genotypes. Several markers have mutually exclusive patterns between those cultivars with *Dm8* compared with all other lines. The relationship between resistance derived from PI167150 in Valmaine and resistance in Sucrine is unclear. Their pedigrees suggest no common progenitor; however, their marker genotypes are similar although not identical. The marker data therefore suggests that *Dm5/8* specificity is either ancient and widespread in *L. serriola* and some *L. sativa*, or else has arisen several times as alleles at the same locus or at linked loci.

Characteristics of markers in the region

We converted the RAPD markers in the region to SCAR markers to increase their reliability and allow multiplex analysis. It was possible to clone all the RAPD fragments that we tried except $OPH15_{1800}$ ($OPV01_{1000}$ and $OPL08_{630}$ became available late in the study and we did not attempt to clone these fragments). $OPH15_{1800}$ is a large fragment that amplifies poorly and is difficult to score; it was therefore not used in the cultivar survey as a RAPD marker. The program OLIGO was a good predictor of primer performance. In most cases SCAR primers could be made as extensions of the progenitor RAPD sequences (Table 1). However, in the case of *SCF12* it was necessary to use sequences initernal to the RAPD sequence; for $OPC08_{370}$ and $OPY13_{800}$ this approach was not successful. Initially, we tested all SCARs at a 60 °C annealing temperature; however, this frequently resulted in multiple amplification products and therefore higher temperatures had to be employed for many of the markers (Table 1). The different optimal temperatures restricted the combinations that could be multiplexed.

SCAR primer pairs were generated for nine RAPD loci in the *Dm5/8* region, of which seven turned out to amplify dominant markers between Calmar and Kordaat at the optimal temperature; two primer pairs for $OPY13_{800}$ did not amplify at all and *SCC08* amplified a monomorphic band from Calmar and Kordaat. No co-dominant markers were identified. This is in contrast to the *Dm1,3* and *Dm4,7* clusters (Paran and Michelmore 1993), where eight RAPD markers were converted into five co-dominant SCAR markers. The seven molecular markers from *SCG06* to $OPY13_{800}$ beside *Dm8* all amplify bands from the Calmar genotype that are lacking in many of the other genotypes. This is suggestive of a distinct region being introgressed from *L. serriola* PI91532. The lack of co-dominant SCAR markers for Calmar × Kordaat could result from se-

quence divergence at the priming site or hemizygosity between these cultivars. However, recombination between all markers was detected in the mapping populations and co-segregating blocks of markers were not observed; therefore, if there is hemizygosity it must be of limited length.

The SCAR fragments for the *Dm5/8* region did not provide useful hybridization probes. The majority contained high-copy sequences. Although some subfragments were lower copy, no single-copy fragments were identified. Therefore, co-dominant hybridization probes specific for the region could not be developed. Of the two RFLP markers, *CL877* and *CL1795*, neither has a single-banded phenotype. *CL877* has two or three bands (Kesseli et al. 1991); *CL1795* is a multigene family with members that map to several regions of the genome including the other clusters of resistance genes (Paran et al. 1992). The lack of single-copy probes for any locus in the region prevented long-range restriction mapping to gain an estimate of the physical size of the region.

Genetic characteristics of region

In all three segregation analyses, the markers are spaced fairly evenly over the map with no major changes in either distance or gene order (Fig. 1). Similarity between the maps for Calmar × Kordaat and Calmar × Cobham Green was expected since Kordaat and Cobham Green only differ at terminal loci (Table 2). El Toro × Salinas (same genotype as Calmar) represents a cross between two of the most dissimilar genotypes identified (Table 2); however, the resulting map is still similar to the previous two. This lack of variation implies that there are no significant differences in heterozygosity or hemizygosity between the parental genotypes involved in these crosses.

The segregation data described in this paper is mostly consistent with previous genetic analyses. The gene order in the region is slightly different than that published previously (Kesseli et al. 1994) as the current data is based on a greater number of recombinants. Previous genetic analysis to map *Dm10* was based solely on the segregation of disease-resistance genes. Analysis of *Dm5/8* and *Dm10* in *cis* in Sucrine × Mesa 659 and Sucrine × Amplus indicated that these two genes were 4.2 ± 0.8 cM apart (T. Nakahara and R. Michelmore, unpublished). No recombinants were detected between *Dm10* and *Dm5* in 3018 F_2 progeny of Vanguard × Valmaine indicating that these two genes are within 6 cM (T. Nakahara and R. Michelmore, unpublished). In the current analysis, *Dm8* and *Dm10* were shown to be only 1.2 cM apart. Both *Dm5* and *Dm5/8* remain to be mapped precisely and placed relative to molecular markers.

It is still not clear whether *Dm5*, *Dm8* and *Dm5/8* are separate loci, the same allele in different genetic backgrounds, or different alleles with the same specificity at a single locus. In a previous study, no susceptible recombinants were obtained in 3348 F_2 individuals from Calmar or Valverde × Valmaine; however, because these are dominant loci in repulsion, the resolution of the analysis was poor and *Dm5* and *Dm8* could still be up to 6 cM apart ($P = 0.05$). Additionally, no recombinants were detected among 1140 and 1320 F_2 individuals from Sucrine × Valmaine and Calmar × Sucrine respectively; therefore, *Dm5/8* could be even further apart from *Dm5* and *Dm8* (Hulbert and Michelmore 1985).

In contrast to the segregation data, the cultivar analysis detected little evidence of recombination within the *Dm5/8* region. Only one clear recombination event had occurred over many generations of breeding involving the introgression of *Dm8*. The other resistance genes in the region in most cases parallel the origins of the *Dm5/8* specificity. Salinas (*Dm8*) and El Toro (*Dm10*) are the result of similar crosses between Calmar (*Dm8*) and Vanguard types (*Dm10*) and exhibit exactly the genotype of one parent or the other: Salinas is the same as Calmar and El Toro is the same as Vanguard. The one clear recombination event occurred between *SCD08* and $OPL08_{630}$ in the complex pedigree leading to breeding line 45634-M. The resulting genotype was then inherited without further recombination in subsequent breeding programs.

Our current analysis provides little indication as to the derivation of *Dm10*. Several cultivars differ for *Dm10* but not for flanking markers; whether this is indicative of the spontaneous generation of *Dm10* or of recombination events on either side is unclear. Also, allelism of the *Dm10* specificity in Sucrine and Vanguard remains to be tested.

Comparison to the *Dm1,3* region

There are several similarities and dissimilarities between the *Dm5/8* region and the other well-characterized cluster of resistance genes in the *Dm1,3* region of lettuce. Both clusters contain multiple genetically distinct loci for resistance to downy mildew as well as other pathogens. Both contain TPI-related sequences. The distribution of markers reflecting the pattern of recombination differed between the clusters. Many RAPD markers were found that amplified from the region around *Dm3* which strictly co-segregated with *Dm5*; hemizygosity was hypothesized as the reason for this lack of recombination (P. Anderson, unpublished). In contrast, no clusters of markers were identified in the *Dm5/8* region in all three segregating populations analyzed and therefore extensive differences in heterozygosity or hemizygosity are lacking between these parents.

Implications of the study

There are clearly multiple derivations for *Dm5/8* specificity and possibly also for *Dm10*. Therefore, they should be considered at least as potentially different alleles until it is shown otherwise. In isolate surveys, cultivars repre-

senting the three sources of *Dm5/8* and the two sources of *Dm10* should be included to increase the possibility of identifying an isolate of *B. lactucae* that can distinguish the resistance genes from different sources. *Dm5/8* specificity should be characterized at the molecular level from all three sources to determine whether the same resistance specificity has evolved independently several times. There is precedence for this. Diverse species are capable of recognizing bacteria carrying the same avirulence gene; therefore; functionally homologous genes for resistance exist in different species (Whalen et al. 1988).

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References

Bernatzky ZR, Tanksley SD (1986) Genetics of actin-related sequences in tomato. Theor Appl Genet 72:314–321

Bohn GW, Whitaker TW (1951) Recently introduced varieties of head lettuce and methods used in their development. US Dept Agric Circ No 881:1–27

Bonnier FJM, Reinink K, Groenwold R (1994) Genetic analysis of *Lactuca* accessions with major gene resistance to lettuce downy mildew. Phytopathology 84:462–468

Brown PR, Michelmore (1988) The genetics of corky root resistance in lettuce. Phytopathology 78:1145–1150

Channon AG, Smith Y (1970) Further studies on races of *Bremia lactucae* Regel. Hort Res 10:14–19

Crute IR (1987) The geographical distribution and frequency of virulence determinants in *Bremia lactucae*: relationships between genetic control and host selection. In: Wolfe MS, Caten CE (eds) Populations of plant pathogens: their dynamics and genetics. Blackwell Scientific Publications, Oxford, pp 193–212

Crute IR (1988) The impact of breeding on pest and disease control in lettuce. Asp Appl Biol 17:305–312

Crute IR (1992) The role of resistance breeding in the integrated control of downy mildew (*Bremia lactucae*) in protected lettuce. Euphytica 63:95–102

Crute IR, Dunn JA (1980) An association between resistance to root aphid (*Pemphigus bursarius* L.) and downy mildew (*Bremia lactucae* Regel) in lettuce. Euphytica 29:483–488

Crute IR, Johnson AG (1976) The genetic relationship between races of *Bremia lactucae* and cultivars of *Lactuca sativa*. Ann Appl Biol 83:125–137

Dawson PR (1976) Development of some English lettuce varieties resistant to downy mildew, *Bremia lactucae*. Ann Appl Biol 86:49–61

Farrara BF, Ilott TW, Michelmore RW (1987) Genetic analysis of factors for resistance to downy mildew (*Bremia lactucae*) in species of lettuce (*Lactuca sativa* and *L. serriola*) Plant Pathol 36:499–514

Holloway JL, Knapp SJ (1993) GMendel 3.0 Users Manual. Oregon State University, Corvallis, USA

Hulbert SH, Michelmore RW (1985) Linkage analysis of genes for resistance to downy mildew (*Bremia lactucae*) in lettuce (*Lactuca sativa*). Theor Appl Genet 70:520–528

Ilott TW, Hulbert SH, Michelmore RW (1989) Genetic analysis of the gene-for-gene interaction between lettuce (*Lactuca sativa*) and *Bremia lactucae*. Phytopathology 79:888–897

Jagger IC, Whitaker TW (1940) The inheritance of immunity from mildew (*Bremia lactucae*) in lettuce. Phytopathology 30:427–433

Johnson AG, Laxton SA, Crute IR, Gordon PLL, Norwood JM (1978) Further work on race-specific resistance in lettuce (*Lactuca sativa*) to downy mildew (*Bremia lactucae*). Ann Appl Biol 89:257–264

Kesseli RV, Ochoa O, Michelmore R (1991) Variation at RFLP loci in *Lactuca* ssp. and the origin of cultivated lettuce (*L. sativa*). Genome 34:430–436

Kesseli R, Witsenboer H, Stanghellini M, Vandemark G, Michelmore R (1993) Recessive resistance to *Plasmopara lactucae-radicis* maps by bulked segregant analysis to a cluster of dominant disease-resistance genes in lettuce. Mol Plant-Microbe Interact 6:722–728

Kesseli RV, Paran I, Michelmore RW (1994) Analysis of a detailed genetic linkage map of *Lactuca sativa* (Lettuce) constructed from RFLP and RAPD markers. Genetic 136:1435–1446

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Landry BS, Kesseli RV, Farrara B, Michelmore RW (1987) A genetic map of lettuce (*Lactuca sativa* L.) with restriction fragment length polmorphism, isozyme, disease-resistance and morphological markers. Genetics 116:331–337

Leeper PW, Whitaker TW (1959) Valverde, a new downy mildew immune lettuce variety. Texas A and M Univ Agr Exp Sta L-440

Leeper PW, Thompson RC, Whitaker TW (1963) Valmaine, a new downy mildew immune romaine lettuce variety. Texas A and M Univ Agr Exp Sta L-610

Maisonneuve B, Bellec Y, Anderson P, Michelmore RW (1994) Rapid mapping of two genes for resistance to downy mildew from *Lactuca serriola* to existing clusters of resistance genes. Theor Appl Genet 89:96–104

Michelmore RW, Norwood JM, Ingram DS, Crute IR, Nicholson P (1984) The inheritance of virulence in *Bremia lactucae* to match resistance factors 3,4,5,6,8,9,10 and 11 in lettuce (*Lactuca sativa*). Plant Pathol 33:301–315

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Murray MG, Thompson WF (1980) Rapid isolation of high-molecular-weight plant DNA. Nucleic Acids Res 8:4321–4325

Norwood JM, Crute IR (1984) The genetic control and expression of specificity in *Bremia lactucae* (lettuce downy mildew). Plant Pathol 33:385–400

Norwood JM, Crute IR (1985) Race-specific resistance to lettuce downy mildew (*Bremia lactucae*) in the lettuce cultivar Bourguignonne Grosse Blonde d'Hiver. Ann Appl Biol 106: 595–599

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993

Paran I, Kesseli RV, Michelmore RW (1991) Identification of RFLP and RAPD markers to downy mildew resistance genes in lettuce with near-isogenic lines. Genome 34:1021–1027

Paran I, Kesseli RV, Michelmore RW (1992) Recent amplification of triose phosphate isomerase-related sequences in lettuce. Genome 35:627–635

Robbins MA, Witsenboer H, Michelmore RW, Laliberte J-F, Fortin MG (1994) Genetic mapping of turnip mosaic virus resistance in *Lactuca sativa*. Theor Appl Genet 89:583–589

Rychlik W, Rhoads RE(1989) A computer program for choosing optimal oligonucleotides for filter hybridization, sequencing and in vitro amplification of DNA. Nucleic Acids Res 17:8543–8551

Ryder E (1979) 'Salinas' lettuce. Hort Sci 14:283–284

Ryder EJ (1986) Lettuce breeding. In: Breeding Vegetable Crops. In: Bassett MJ (ed) AVI Publishing Co Westport, pp 433–474

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Stam P (1993) Construction of integrated linkage maps by means of a new computer package: Joinmap. Plant Jour 3:739–744

Stanghellini ME, Adaskaveg JE, Rasmussen SL (1990) Pathogenesis of *Plasmopara lactucae-radicis*, a systemic root pathogen of cultivated lettuce. Plant Dis 74:173–178

Thompson RC, Ryder EJ (1961) Descriptions and pedigrees of nine varieties of lettuce. Techn Bull US Dept Agric 1244:1–19

Vandemark GJ, Stanghellini ME, Rasmussen SL, Michelmore RW (1992) Inheritance of resistance in lettuce to *Plasmopara lactucae-radicis*. Phytopathoglogy 82:272–274

Welch JE, Grogan RG, Zink FW, Kihara GM, Kimble KA (1965) Calmar, a new lettuce variety resistant to downy mildew. Calif Agric 19(8):3–4

Whalen MC, Stall RE, Staskawicz BJ (1988) Characterization of a gene from a tomato pathogen determining hypersensitive resistance in a non-host species and genetic analysis of this resistance in bean. Proc Natl Acad Sci USA 85:6743–6747

Whitaker TW, Bohn GW, Welch JE, Grogan RG (1958) History and development of head lettuce resistant to downy mildew. Am Soc Hort Sci 72:410–416

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Zink FW (1973) Inheritance of resistance to downy mildew (*Bremia lactucae* Reg.) in lettuce. J Am Soc Hort Sci 98 : 293–296

Zink FW, Duffus JE (1969) Relationship of turnip mosaic virus susceptibility and downy mildew (*Bremia lactucae*) resistance in lettuce. J Am Soc Hort Sci 94:403–407

Zink FW, Duffus JE (1970) Linkage of turnip mosaic virus susceptibility and downy mildew (*Bremia lactucae*) resistance. J Am Soc Hort Sci 95:420–422

Zink FW, Duffus JE (1973) Inheritance and linkage of turnip mosaic virus and downy mildew (*Bremia lactucae*) reaction in *Lactuca serriola*. J Am Soc Hort Sci 98:49–51

Zink FW, Duffus JE (1974) Reaction of downy mildew-resistant lettuce cultivars to infection by turnip mosaic virus. Phytopathology 65:243–245

Zink FW, Duffus JE, Kimble KA (1973) Relationship of a non-lethal reaction to a virulent isolate of lettuce mosaic virus and turnip mosaic susceptibility in lettuce. Am Soc Hort Sci 98:41–45