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Linkage analysis of genes for resistance to downy mildew (*Bremia lactucae*) in lettuce (*Lactuca sativa*)

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Summary. The genetics of specific resistance was studied in F_2 populations which segregated for either one or two resistance genes. The resistance factors 1, 11 and 14 which had not previously been characterized genetically segregated as single dominant genes (Dm). Resistance was determined by three linkage groups; R 1/14, 2, 3, and 6 in the first, R 5/8, and 10 in the second and R 4, 7 and 11 in the third. Cultivars of lettuce commonly used in the differential series to detect virulence to R3 and R10, were demonstrated to carry two tightly linked resistance genes. Implications of this linkage arrangement to the manipulation and characterization of these resistance genes are discussed.

Key words: Host/parasite interaction – Specific resistance

Introduction

Disease resistance is one of the few agronomically important traits often inherited in a simple Mendelian manner. The possibility of cloning resistance genes and transforming susceptible plants lines to resistance therefore has attracted much interest. Compatibility and incompatibility in plant disease, however, is a consequence of complex interactions between host and parasite. Little is known of the genetic or molecular bases of these interactions and the molecular identification of a resistance gene is problematic. It is critically important to define the numbers and types of genes involved before molecular analyses are made. Gene-for-gene interactions have now been demonstrated over a taxonomically diverse range of diseases; however, few detailed studies have been made.

The *Lactuca sativa–Bremia lactucae* interaction (lettuce downy mildew) is now one of the genetically best characterized plant diseases. Crute and Johnson (1976) collated their observations and previously published data on interactions between lettuce cultivars and different *B. lactucae* isolates and proposed a gene-for-gene relationship between host and pathogen involving 10 different resistance genes. Since then seven additional R-factors have been identified (Johnson et al. 1978; Crute and Lebeda 1981, 1983; Crute, personal communication). Seven of the first 11 resistance factors identified (R2 to R6, R8 and R10) have been shown to segregate as single dominant genes and have been assigned the Dm gene designation (Johnson et al. 1977, 1978). R2, R3 and R6 seem to be tightly linked (Norwood and Crute 1980). Extensive genetic studies on other R factors had not been made. Linkage relationships of the resistance genes were not known as many genes had been identified following crosses to a limited number of cultivars which carried only one or no effective resistance genes.

Bremia lactucae is predominantly heterothallic (Michelmore and Ingram 1980; Michelmore and Ingram 1982). Studies on the genetic basis of specific virulence confirmed the gene-for-gene relationship; in most cases specific dominant genes for resistance in *L. sativa* were matched by specific dominant genes for avirulence in the pathogen (Norwood et al. 1983; Michelmore et al. 1984; Norwood and Crute 1984). These studies, however, posed several questions about the allelism and numbers of genes for resistance in *L. sativa*.

This study describes the inheritance of resistance factors 1, 7, 11 and 14 and further linkage relationships of the resistance genes. For clarity the R-prefix is used throughout this paper because the data suggest the assigned Dm-designations are not always justified.

Materials and methods

Some of the F_2 progenies of *L. sativa* were obtained from F. W. Zink (University of California, Davis). Other F_2 progenies were generated from crosses made in the glasshouse at the Department of Vegetable Crops at UC Davis. Segregation of morphological or allozyme polymorphisms or mildew resis-

tance was used to confirm the origin of putative F_2 populations.

Isolates of *B. lactucae* used in this study were selected from a large collection of isolates at UC Davis. These originated from Europe, Australia (imported under USDA license), or California (Durgan and Michelmore, unpublished). If the required virulence phenotypes were unavailable, crosses between isolates were made to obtain them as described by Michelmore et al. (1984). Isolates C82P24, C83M40, C83R19 and C83M47 were collected from California. Isolates SF3 and SF5 from Finland, and CG1 from Switzerland were obtained from I. R. Crute (NVRS, England). IMOs6b is a F_1 isolate from a cross between IM25R7 and CG1 (Michelmore et al. 1984). 24SF5n1 is a F_1 progeny isolate from a cross between C82P24 and SF5, and CEB10 is a F_1 progeny from a cross between IM25R7 and a Californian isolate.

Isolates were cultured on seedlings of cultivar 'Cobham Green' in which no specific R-factors have been detected. Suspensions of conidia were prepared by shaking cotyledons bearing asexual sporulation in distilled water. The spores were then pelletted in a low speed centrifuge and resuspended in distilled water (Michelmore and Crute 1983). Seedlings were inoculated by spraying to run-off with a spore suspension of 1×10^5 conidia ml^{-1}. All transactions were carried out in a sterile recycling airbench to effectively eliminate cross contamination between isolates. After inoculation the seedlings were incubated in sealed plastic boxes (GA-7, Magenta Corp., Chicago) in a growthroom at 15 °C and 14 h of light (300 uE/m^2/s) per day.

Resistance phenotypes of lettuce cultivars and virulence phenotypes of *B. lactucae* isolates were determined by observing a wide range of cultivar/isolate interactions (Michelmore and Crute 1983). Previously reported interactions between cultivars and European isolates were repeated to ensure the expected interaction phenotypes were observed.

Seedlings of F_2 progeny were grown on blotter paper moistened with Hewitt's solution (Hewitt 1952) in plastic boxes $11 \times 11 \times 4$ cm, 35 seedlings/box, in the growth room under conditions described above. Five seedlings of 'Cobham Green' were included in each box as susceptible checks. When the cotyledons were fully expanded (approx. 7–8 days after sowing) the seedlings were inoculated by spraying with spore suspensions, 1×10^5 conidia ml^{-1}, on two occasions, one day apart. When seedlings were inoculated simultaneously with two isolates, the concentration of spores from each isolate was 1×10^5 conidia ml^{-1}. Susceptible checks which escaped infection were observed very rarely (two occasions only); when this occurred, no data were recorded from the inoculation. The seedlings were observed 7 and 10 days after inoculation and scored as resistant or susceptible on the basis of the absence or presence of asexual sporulation which is easily visible as a white down of conidiophores on the cotyledons.

Results

Characterization of isolates of B. lactucae

All of the reactions were in agreement with the predictions of the gene-for-gene model postulated by Crute and Johnson (1976) or its modifications (Johnson et al. 1977, 1978; Norwood and Crute 1980; Crute and Lebeda 1981, 1983) except for minor changes (Tables 1 and 2). All isolates which were virulent on 'Mildura' also were virulent on 'Blondine' (R1) and 'Dandie' (R3). Attempts to create an isolate through sexual crosses which was virulent on 'Mildura' but not on 'Blondine' failed. Similar results have been obtained by Norwood and Crute (1984). As the resistance in 'Dandie' is conferred by R3, an additional R-factor (R1) must be postulated for 'Mildura', 'Solito' and those in the 'Kwiek' group (Table 1).

Inheritance of R1, R7, R11 and R14

Segregation of resistance in progenies of crosses between 'Blondine' (R1) and cultivars which are susceptible to SF5 closely fitted 3 : 1 ratios as expected for a single dominant gene (Table 3). The monogenic segregation of R1 was observed in progenies from several other crosses (Table 3). Zink (1978) also reported evidence of a single dominant gene in 'Blondine' which provided resistance to a Californian isolate. A good fit to the expected 3 : 1 ratio also was observed in progenies from crosses involving 'GL66' and two different cvs. where R7 alone was functioning. This confirms that 'GL66' has R7 which is conferred by a single dominant gene. Segregation ratios also indicated that R7 in 'Calmar' and 'Mesa 659' was determined by a single dominant gene. Resistance factor 11 from the breeding line HS and cv. 'Capitan' segregated as a single dominant gene when inoculated with each of six different isolates of *B. lactucae.* Similar results were obtained by Zink (1978) with crosses involving HS when inoculated with a Californian isolate and by Boulidard and Bannerot (1975) who inoculated progenies from HS with three different French isolates (D3, F1 and B2). Monogenic segregation of R14 was observed when segregating progenies from crosses with 'Liba' were inoculated with isolate C82P24 and when progenies from crosses with 'Valore' were inoculated with isolate C83M47. The gene segregating from these cultivars was assumed to be R14 because isolate C82P24 was virulent on R2, present in 'Liba', and isolate C83M47 was virulent on R2 and R4 which are present in 'Valore'.

Independent segregation of resistance genes

Independent segregation of different pairs of resistance genes was observed when F_2 populations were inoculated with isolates which allowed the simultaneous segregation of two resistance genes (Table 4). R7 in 'GL66' segregated independently of R1 and R3 in 'P. Blackpool' and 'Kwiek' and independently from R10 in 'Sucrine'. Independent segregation of R10 and R7 was also observed in progenies from the cross 'Sucrine' × 'Mesa 659'. Segregation ratios close to the expected 15 : 1 for two independent dominant genes were obtained from crosses involving 'Calmar' (R7 plus R8). R7 segregated as a single dominant gene independent of R1 in progenies from the crosses 'GL66' × 'Blondine'

Table 1. The interactions between lettuce cultivars and isolates of *B. Lactucae*

Cultivar	Isolate of *B. lactucae*										Postulated resistance factors
	C82P24	C83R19	C83M40	C83M47	SF3	SF5	CG1	IMOs6b	CEB10	24SF5nl	
'Cobham Green'; 'Lobjoits Cos'	+	+	+	+	+	+	+	+	+	+	0
'Blondine'	*	*	+	*	+	*	+	+	*	*	1=14+15?[a]
'Noran'; 'Amplus'; 'Knap'; 'Magiola'	*	*	*	+	*	+	*	*	*	*	2+4
'Mildura'	*	*	+	*	+	*	+	*	*	*	1+3
'Dandie'	+	+	+	+	+	*	+	*	*	+	3
'R4T57'	*	*	*	+	*	+	+	+	+	*	4
'Kwiek'; 'Proefutin's (P) Blackpool'; 'Kordaat'; 'Tonika'	*	*	*	*	*	*	+	*	*	*	1+3+4
'Valmaine'	+	+	*	+	+	*	*	*	+	+	5=8
'Sabine'	+	+	+	+	*	*	*	*	+	+	6
'Mesa 659'; 'Great Lakes' (GL)66	+	+	+	+	+	*	+	+	+	*	7
'Valverde'	+	+	*	+	+	*	*	*	+	+	8+?[a]
'Bourguignonne I'	*	*	*	*	*	*	*	*	+	*	8?+9
'Sucrine'	+	+	*	*	*	*	*	*	+	+	5=8+10
'Hilde × *L. serriola*' (HS); 'Capitan'	+	*	*	*	+	*	*	*	*	*	11
'Portato'	*	*	*	*	+	*	*	*	*	*	1+2+7
'Solito'	*	*	+	*	+	*	+	*	*	*	1+3+7
'Calmar'	+	+	*	+	+	*	*	*	+	*	7+8
'Hilde'	+	+	+	+	+	+	+	+	+	+	12
'Vanguard'	+	+	+	*	*	*	+	+	+?	+?	13
'Lednicky'	*	*	+	*	+	*	+	+	*	*	14=1
'Liba'	*	*	*	*	+	*	*	*	*	*	1=14+2
'Valore'	*	*	*	*	*	*	*	*	*	*	1=14+2+4
'Pennlake'	+	+	+	+	+	+	+	+	+	+	15

+ = compatible; * = incompatible interaction
?[a] R factor has yet to be confirmed, see text

Table 2. Virulence phenotypes of isolates of *B. lactucae* used in this study

Isolate	Virulence to resistance factor														
	1	2[a]	3	4	5	6	7	8	9[b]	10[c]	11	12	13	14	15
C82P24	*	+	+	*	+	+	+	+	*	+	+	+	+	*	+
C83R19	*	+	+	*	+	+	+	+	*	+	*	+	+	*	+
C83M40	+	*	+	*	*	+	+	*	*	+	*	+	+	+	+
C83M47	*	+	+	+	+	+	+	+	*	*	*	+	+	*	+
SF3[d]	+	+	+	*	+	*	+	+	*	*	+	+	+	+	+
SF5[d]	*	+	*	+	*	*	*	*	*	*	*	+	+	*	+
CG1	+	*	+	+	*	*	+	*	*	+	*	+	+	+	+
IMOs6b	+	*	*	+	*	*	+	*	*	+	*	+	+	+	+
CEB10	*	*	*	+	+	+	+	+	+	+	*	+	+	*	+
24SF5nl	*	+	+	*	+	+	*	+	*	+	*	+	+	*	+

+ = virulent; * = avirulent
[a] Virulence on R2 was determined by using F_2 populations segregating for R2 and R4 ('Amplus' or 'Noran' × 'GL66') when the isolate was avirulent on R4
[b] Virulence on R9 was determined by reaction on 'Bourguignone' and may not be valid if this cultivar carries R8
[c] Virulence on R10 was determined using leaf disks from F_2 progeny of the cross 'GL66' × 'Sucrine' when the isolate was avirulent on R5
[d] First characterized by Osara and Crute (1981)

Table 3. Segregation of individual downy mildew resistance factors 1, 2, 4, 7, 11 and 14 in F_2 populations of *L. sativa*

Cultivars crossed	Isolate of *B. lactucae*	No. of F_2 seedlings resistant : susceptible	$\chi^2_{3:1}$	R-factors assumed effective in each parent
'GL66' × 'Amplus'	C82P24	201 : 59	0.74	−×4
'GL66' × 'Noran'	C82P24	109 : 40	0.27	−×4
'GL66' × 'Noran'	C83R19	171 : 46	1.67	−×4
'GL66' × 'Tonika'	C83M40	164 : 65	1.40	−×4
'Blondine' × 'Noran'	SF5	240 : 74	0.34	1×−
'Blondine' × 'Amplus'	SF5	180 : 58	0.05	1×−
'GL66' × 'Portato'	C82P24	188 : 69	0.47	−×1
'GL66' × 'Solito'	C82P24	231 : 72	0.25	−×1
'Calmar' × 'Solito'	C82P24	155 : 59	0.75	−×1
'GL66' × 'Noran'	SF5	294 : 101	0.07	7×−
'GL66' × 'Amplus'	SF5	362 : 122	0.01	7×−
'Calmar' × 'Sucrine'	24SF5nl	160 : 69	3.32	7×−
'Mesa 659' × 'Sucrine'	24SF5nl	142 : 63	3.59	7×−
'Solito' × 'HS'	CG1	184 : 57	0.23	−×11
'Capitan' × 'Mesa 659'	CG1	177 : 52	0.64	11×−
'Capitan' × 'Mesa 659'	C83M40	133 : 50	0.53	11×−
'Noran' × 'HS'	SF5	125 : 36	0.60	−×11
'GL66' × 'HS'	C83R19	179 : 75	2.78	−×11
'Capitan' × 'Mesa 659'	CEB10	149 : 57	0.78	11×−
'Capitan' × 'Mesa 659'	C83M47	168 : 63	0.64	11×−
'GL66' × 'Liba'	C82P24	226 : 74	0.02	−×14
'Calmar' × 'Liba'	C82P24	164 : 44	1.64	−×14
'GL66' × 'Valore'	C83M47	219 : 65	0.68	−×14
'GL66' × 'Valore'	CG1	198 : 54	1.71	−×2
'GL66' × 'Liba'	C83M40	205 : 70	0.03	−×2

and 'Noran' × 'Portato'. R7 from 'GL66' also segregated independently from R14 in 'Rapide' and 'Ancora'. Progenies derived from cross between cultivars susceptible to isolate C83M40 with cultivars carrying R2 and R4 all exhibited two factor segregation when inoculated with this isolate. Independent assortment of R2 with R8 from 'Calmar' and R11 from 'HS' was also observed. R8 in 'Calmar' segregated independently of R11 in HS, R3 in 'Kordaat', and R4 in 'P. Blackpool' and 'Kordaat'. R4, in the breeding line 'R4T57' also segregated independently of R5 in 'Valmaine', which in turn segregated independently of R6 in 'Sabine'. R4 in 'R4T57', 'Amplus' and 'Noran' segregated independently of R1 in 'Blondine'. R1 and R4 segregated independently from each other in progenies from crosses with 'P. Blackpool', 'Kwiek' and 'Tonika'. R4 also segregated independently of R14 in 'Valore', 'Rapide' and 'Ancora'.

Linkage between resistance factors 1, 2, 3 and 14

Isolate CEB10 permitted the expression of both R1 and R2, but not R4, in progenies derived from crosses between 'Blondine' (R1) with 'Amplus' or 'Noran' (R2 + R4). The observed segregation of resistance was significantly different from the expected 15 : 1 ratio for two independent genes. This suggested tight linkage between R1 and R2 (Table 5). Further evidence for linkage between these genes was obtained when resistance from 'GL66' × 'Portato' did not segregate as expected for two dominant genes. The absence of susceptible recombinants when progenies from the cross 'Blondine' × 'Portato' were inoculated with SF5 indicates that 'Portato' has R1, in addition to R2.

When F_2 progenies from crosses between 'Blondine' and 'Kwiek', 'P. Blackpool', or 'Solito' were inoculated with isolates (SF5 and C82P24) avirulent on R1, no segregation was observed (Table 5). This suggests that these cultivars carry R1 in addition to R3. The segregation of R1 and R3 in progenies from the crosses 'Magiola' × 'P. Blackpool', 'GL66' × 'Kwiek', 'Calmar' × 'Solito' and 'Lobjoits Cos' × 'Mildura' when inoculated with isolate SF5 or isolate CEB10 fit monogenic more closely than digenic segregation ratios. This indicated that R1 and R3 are closely linked. There was a slight excess of resistant plants in each F_2 progeny from that expected for a 3 : 1 ratio. To demonstrate R1 and R3 were separate genes, recombinant progeny from the cross 'Lobjoits Cos' × 'Mildura' were selected which carried R3 but not R1. The seedlings were first inoculated with isolate IMOs6b (virulent on R1 but not R3);

Table 4. Digenic segregation of downy mildew resistance in F_2 populations of *Lactuca sativa*

Cultivars crossed	Isolate of *B. lactucae*	No. of F_2 seedlings resistant : susceptible	$\chi^2_{15:1}$	R-factors assumed effective in each parent
'GL66' × 'P. Blackpool'	SF5	231 : 16	0.02	7×1+3
'GL66' × 'Kwiek'	SF5	217 : 12	0.40	7×1+3
'GL66' × 'Sucrine'	SF5	306 : 29	3.31	7×10
'Sucrine' × 'Mesa 659'	SF5	470 : 34	0.21	10×7
'Calmar' × 'Amplus'	SF5	277 : 19	0.01	7+8×–
'GL66' × 'Blondine'	SF5	230 : 19	0.81	7×1
'Noran' × 'Portato'	SF5	246 : 12	1.13	–×1+7
'GL66' × 'Ancora'	SF5	289 : 18	0.08	7×14
'GL66' × 'Rapide'	SF5	151 : 8	0.40	7×14
'GL66' × 'Rapide'	C83M40	178 : 11	0.06	–×2+4
'GL66' × 'Noran'	C83M40	171 : 14	0.55	–×2+4
'GL66' × 'Ancora'	C83M40	209 : 18	1.09	–×2+4
'GL66' × 'Amplus'	C83M40	126 : 10	0.28	–×2+4
'Blondine' × 'Noran'	C83M40	134 : 11	0.44	–×2+4
'Blondine' × 'Amplus'	C83M40	143 : 11	0.21	–×2+4
'Calmar' × 'Amplus'	CG1	270 : 15	0.47	8×2
'Calmar' × 'Knap'	CG1	249 : 14	0.39	8×2
'Calmar' × 'Portato'	C83M40	170 : 10	0.15	8×2
'Portato' × 'HS'	C83M40	336 : 26	0.54	2×11
'Calmar' × 'HS'	C83M40	328 : 22	0.00	8×11
'Calmar' × 'Kordaat'	IMOs6b	257 : 17	0.00	8×3
'Calmar' × 'P. Blackpool'	C83M40	238 : 16	0.00	8×4
'Calmar' × 'Kordaat'	C83M40	224 : 11	0.99	8×4
'R4T57' × 'Valmaine'	C83M40	386 : 21	0.83	4×5
'Sabine' × 'Valmaine'	CG1	210 : 12	0.27	6×5
'Blondine' × 'R4T57'	C82P24	146 : 10	0.01	1×4
'Blondine' × 'Amplus'	C82P24	168 : 12	0.05	1×4
'Blondine' × 'Noran'	C82P24	168 : 9	0.41	1×4
'GL66' × 'P. Blackpool'	C82P24	309 : 14	2.02	–×1+4
'GL66' × 'Kwiek'	C82P24	287 : 21	0.17	–×1+4
'GL66' × 'Tonika'	C82P24	185 : 12	0.01	–×1+4
'GL66' × 'Valore'	C82P24	181 : 14	0.29	–×4+14
'GL66' × 'Rapide'	C82P24	149 : 9	0.08	–×4+14
'GL66' × 'Ancora'	C82P24	339 : 11	0.30	–×4+14

the seedlings which were susceptible (83 seedlings out of 304) and thus did not carry R3, were removed. The remaining 221 seedlings were inoculated with isolate C83M47 (virulent on R3 but not R1) and 13 were found to be susceptible and therefore carried R3 but not R1. This confirms the linkage between R1 and R3 because 13 out of 221 progeny carrying R3 but not R1 is significantly less than the expected 1/4 for two independent genes.

R2 and R3 also seemed to be tightly linked (Table 5). F_2 progenies from cultivars 'Solito', 'P. Blackpool' and 'Kordaat' were crossed with cultivars 'Noran', 'Magiola' and 'Liba'. When progenies were inoculated with isolate IMOs6b which allows the phenotypic expression of both R2 and R3, no susceptible recombinants were observed.

Similarly no recombination was observed between R1 and R14 in F_2 progenies from crosses between 'Liba' and 'Mildura', 'Kordaat' and 'Solito' or in progenies from 'Blondine' crossed with 'Ancora'. This suggested that R1 is linked, allelic or identical to R14. No isolate tested could differentiate between the resistance of 'Blondine' (R1) and 'Lednicky' (R14). Furthermore, virulence on R1 segregated in parallel to virulence on R14 in 20 progeny isolates from a cross between isolates C82P24 and SF3. R1 and R14 therefore are either tightly linked in the host and virulence to these R-factors is also tightly linked in the pathogen or, as is more likely, R1 and R14 are determined by the same allele. 'Blondine' may have an additional resistance gene which is effective against some Czechoslovakian isolates (CS3 to CS7 and CS10; Crute and Lebeda 1983).

Isolate CEB10 allows the expression of both R14 and R2 in the cvs. 'Liba' and 'Valore'. Digenic segregation was not observed, however, when progenies from

Table 5. Cosegregation of downy mildew resistance factors 1, 2, 3 and 14 in F_2 populations of *L. sativa*

Cultivars crossed	Isolate of *B. lactucae*	No. of F_2 seedlings resistant : susceptible	$\chi^2_{3:1}$	R-factors assumed effective in each parent
'Blondine' × 'Amplus'	CEB10	682 : 2		1×2
'Blondine' × 'Noran'	CEB10	997 : 0		1×2
'GL66' × 'Portato'	CEB10	235 : 66	1.52	−×1+2
'Blondine' × 'Portato'	SF5	450 : 0		1×1+2
'Blondine' × 'Kwiek'	SF5	631 : 0		1×1+3
'Blondine' × 'P. Blackpool'	SF5	554 : 0		1×1+3
'Blondine' × 'Solito'	C82P24	547 : 0		1×1
'Magiola' × 'P. Blackpool'	SF5	215 : 61	1.24	−×1+3
'GL66' × 'Kwiek'	CEB10	134 : 28	5.14*	−×1+3
'Calmar' × 'Solito'	CEB10	179 : 51	0.98	−×1+3
'Logjoits Cos' × 'Mildura'	CEB10	190 : 46	3.82	−×1+3
'Solito × Noran'	IMOs6b	275 : 0		3×2
'Magiola' × 'P. Blackpool'	IMOs6b	248 : 0		2×3
'Noran' × 'Kordaat'	IMOs6b	215 : 0		2×3
'Solito' × 'Liba'	IMOs6b	257 : 0		3×2
'Liba' × 'Kordaat'	IMOs6b	431 : 0		2×3
'Liba' × 'Kordaat'	C83M47	380 : 0		14×1
'Liba' × 'Mildura'	C83M47	442 : 0		14×1
'Solito' × 'Liba'	C82P24	394 : 0		1×14
'Solito' × 'Liba'	C83M47	425 : 0		1×14
'Blondine' × 'Ancora'	SF5	337 : 0		1×14
'Calmar' × 'Liba'	CEB10	223 : 61	1.88	−×2+14
'GL66' × 'Valore'	CEB10	138 : 48	0.07	−×2+14
'GL66' × 'Valore'	CG1&C83M47	322 : 127	2.58	−×2+14
'Noran' × 'Valore'	CG1	595 : 0		2×2+1

* Probability of a larger χ^2 value is less than 0.05

crosses with 'Liba' or 'Valore' with cultivars susceptible to CEB10 were inoculated with this isolate. This indicated that the two genes are linked. Linkage was confirmed by simultaneous inoculations. When progenies from the cross 'GL66' × 'Valore' were inoculated simultaneously with isolates CG1 (detected only R2) and C83M47, (detected only R14; Table 3) segregation ratios again fit a 3 : 1 ratio instead of an expected 9 : 7 ratio for two independent, dominant genes (Table 5). The presence of two genes was confirmed by sequential inoculation of seedlings of the same F_2 population. After inoculation with C83M47, 70 seedlings out of 253 were susceptible (lacking R14) and were removed; the remaining 183 seedlings were then inoculated with isolate CG1 and 16 were susceptible (lacked R2). Therefore, R2 and R14 are linked genes rather than alleles of a single gene.

Cosegregation of resistance factors 5, 8 and 10

No susceptible recombinants were detected in over 3,000 progenies of crosses between 'Valmaine' (R5) with 'Valverde' and 'Calmar' (R8), after inoculation with isolates CG1 or C83M40 (Table 6). These R-factors are therefore either tightly linked genes, alleles of a single gene or identical.

When F_2 progenies of 'Sucrine' crossed with 'Calmar' or 'GL66', were inoculated with isolate SF3 (virulent on R5 and R8), monofactorial segregation of R10 was observed. No susceptible seedlings were observed, however, when F_2 progenies from crosses between 'Sucrine' with 'Calmar' (R8) or 'Valmaine' (R5) were inoculated with isolates which are avirulent on R5 and R8 (isolates C83M40, CG1 or SF5). Two explanations are possible: (i) R5 (=R8) and R10 are tightly linked genes or alleles of a single gene or (ii) 'Sucrine' has R5 as well as R10. Resistance in F_2 progenies from the cross 'GL66' × 'Sucrine' segregated monogenically when inoculated with isolate C83M40; therefore, if 'Sucrine' has both R5 and R10, isolate C83M40 probably is virulent on R10 and resistance to C83M40 in 'Sucrine' is conferred by R5. Thus, monogenic segregation from inoculations with isolates SF3 or C83M40 supports both hypotheses. If 'Sucrine' has both genes but they are independent, an F_2 population of 'GL66' × 'Sucrine' should segregate 9 : 7, resistant : susceptible, when inoculated simultaneously with SF3 and C83M40. If 'Sucrine' has only R10, or has both but they are

Table 6. Cosegregation of downy mildew resistance factors 5, 8 and 10 in F_2 populations of *L. sativa*

Cultivars crossed	Isolate of *B. lactucae*	No. of F_2 seedlings resistant : susceptible	$\chi^2_{3:1}$ [a]	R-factors assumed effective in each parent
'Valverde' × 'Valmaine'	CG1	739 : 0		8×5
'Valverde' × 'Valmaine'	C83M40	1,303 : 0		8×5
'Calmar' × 'Valmaine'	CG1	342 : 0		8×5
'Calmar' × 'Valmaine'	C83M40	964 : 0		8×5
'Calmar' × 'Sucrine'	CG1	505 : 0		8×5
'Calmar' × 'Sucrine'	C83M40	634 : 0		8×5
'Sucrine' × 'Valmaine'	SF5	730 : 0		5+10×5
'Sucrine' × 'Valmaine'	C83M40	590 : 0		5×5
'Calmar' × 'Sucrine'	SF3	273 : 96	0.20	−×10
'GL66' × 'Sucrine'	SF3	225 : 83	0.62	−×10
'GL66' × 'Sucrine'	C83M40	429 : 147	0.08	−×5
'GL66' × 'Sucrine'	C83M40&SF3	425 : 178	6.57*	−×5+10
'Sucrine' × 'Valmaine'	SF3	530 : 221	7.851**	10×−

[a] Probability of a larger χ^2 value is less than 0.05 (*) or 0.01 (**)

Table 7. Cosegregation of downy mildew resistance factors 4, 7 and 11 in F_2 populations of *L. sativa*

Cultivars crossed	Isolate of *B. lactucae*	No. of F_2 seedlings resistant : susceptible	R-factors assumed effective in each parent
'Tonika' × 'HS'	C83M40	1,100 : 0	4×11
'Noran' × 'HS'	C83M40	1,139 : 0	2+4×11
'Noran' × 'HS'	C83R19	345 : 0	4×11
'Capitan' × 'Mesa 659'	SF5	926 : 0	11×7
'Capitan' × 'Mesa 659'	24SF5nl	762 : 0	11×7
'GL66' × 'HS'	SF5	693 : 0	7×11
'Calmar' × 'Amplus'	24SF5nl	815 : 0	7×4
'GL66' × 'Noran'	24SF5nl	863 : 0	7×4

tightly linked, a 3 : 1 ratio would be expected. The observed ratio of 2.4 : 1 (425 : 178) is significantly different from a 9 : 7 ratio ($P < 0.001$) or a 3 : 1 ratio ($P < 0.01$). This result is consistent with the hypothesis that 'Sucrine' has two resistance genes determining R5 and R10 and the two genes are linked with a low frequency of recombination. To test this hypothesis, 618 F_2 seedlings from 'GL66' × 'Sucrine' were inoculated with isolate SF3. After the susceptible seedlings were removed (177 out of 618), the resistant seedlings were inoculated with isolate C83M40. Fourteen of the remaining 441 were found to be susceptible to isolate C83M40, indicating that they had R10, but not R5. This was later confirmed by inoculating leaf discs of the same plants with each of the two isolates. Fourteen out of 441 is significantly less than the 1 : 4 ratio expected for independent segregation of R5 and R10 ($P < 0.001$). 'Sucrine', therefore, has two resistance genes determining R5 and R10 and these two genes are linked.

A significant excess of susceptible progenies were observed when seedlings from the cross 'Sucrine' × 'Valmaine' were inoculated with isolate SF3 (allowing the expression of R10). Progeny tests of F_2 plants are currently being made. Estimates of the frequency of recombination between R5 and R10 cannot be made without further examination of this phenomenon.

Cosegregation of resistance factors 4, 7 and 11

Individually, R4, R7 and R11 segregated as single dominant genes (Table 3). No segregation was observed, however, between R4 and R7, R4 and R11, and R7 and R11 in large numbers of progenies (Table 7). These factors are therefore either tightly linked genes or alleles at a single locus.

Discussion

The DM resistance factors R1 to R8, R10, R11 and R14 are conferred by single dominant genes arranged in three linkage groups: R1, 2, 3, 6, and 14 in one group; R5, 8 and 10 in the second; and R4, 7 and 11 in the third. Resistance factors 5 and 8, as well as R1 and R14, however, may be conferred by the same alleles. Resistance factors 12 and 15 could not be detected by the isolates available in this study and segregation of R9 could not be examined in detail because the seedlot of 'Bourguigonne' proved to be lacking R9. R13 in 'Vanguard' has only been recently identified.

These linkage groups are consistent with previously published data. Evidence for independent segregation of R2 and R11 (Boulidard and Bannerot 1975), R7 with R8 (Johnson et al. 1978) R4 with R6 and R6 with R8 (Norwood and Crute 1980) have been reported previously. R2 and R3 were shown to be tightly linked but different genes (Norwood and Crute 1980); R6 was shown to be in the same linkage group. Boulidard and Bannerot (1975) found no susceptible recombinants when progenies from crosses between cultivars carrying R7 and HS (R11) were inoculated with isolates allowing the expression of both genes.

In the past, the genetic nature of DM resistance often has been studied in crosses to a susceptible cultivar; few if any crosses and inoculations were made to determine linkage to other R-factors. This has resulted in redundant designation of R-factors as *Dm* genes. No evidence exists that several R-factors are actually different genes occurring at different loci. R4, R7 and R11 factors may be alleles at a single locus. Recombinants were observed between some R-gene combinations in the other two linkage groups. Recombinants have been observed in F_2 progenies cosegregating for R1 with R3, R2 with R3, R2 with R14, R5 with R10 and possibly R1 with R2. R1 and R14, however, may be the same allele. Similarly, no recombinants were observed in progeny of crosses between 'Valmaine' (R5) and 'Valverde' (R8). R5 and R8 were overcome simultaneously in Texas when a new pathotype appeared (Sleeth and Leeper 1966). In crosses between *B. lactucae* isolates, monogenic segregation of virulence to R5 and R8 also indicated that the resistance in these two cultivars may be governed by the same allele (Michelmore et al. 1984; Norwood and Crute 1984). Isolates of *B. lactucae* have been identified (CS6 and CS7), however, which are virulent on 'Valmaine' but not 'Valverde' (Lebeda 1984). The simplest hypothesis to accommodate these observations is that 'Valmaine' and 'Valverde' both carry R5, but 'Valverde' also carries another resistance factor which is rarely effective. To test this hypothesis, the Czechoslovakian isolates should be tested on a segregating population from crosses with 'Valverde' to try to identify an isolate, or combination of isolates, which will detect both of these genes.

Segregation data for virulence to R11 in progenies from crosses of *B. lactucae* isolates suggested, on the basis of a gene-for-gene interaction, that this resistance factor is controlled by two genes (Michelmore et al. 1984; Norwood and Crute 1984). The data presented above (Table 3), however, are inconsistent with this hypothesis because monogenic segregation was observed in F_2 progenies segregating for R11 after inoculation with six different *B. lactucae* isolates. Furthermore, one of these isolates was CG1 which should detect both genes according to the proposed virulence genotype (Michelmore et al. 1984). If R11 is controlled by two genes, therefore, they must be tightly linked.

The process of mapping and naming novel resistance factors would be greatly simplified if these genes continued to occur in one of the three linkage groups. A line containing an unmapped resistance gene could be crossed to a tester cultivar possessing three resistance genes, one in each of the three linkage groups. The relationship of the unmapped resistance gene could then be determined from a single F_2 population by testing with only three isolates of *B. lactucae.* All three isolates should be avirulent on the cultivar with the unmapped resistance factor and each of the isolates should be avirulent on a different gene in the tester cultivar, but virulent on the other two. Cultivars such as 'Diana' (R3, R7, R8) and 'Avoncrisp' (R6, R7, R8) could be useful as such tester cultivars.

The grouped distribution of DM resistance genes is of consequence to their use in breeding programs. Some R-factor combinations will be more difficult to obtain in a single inbred line than others. By far the majority of the cultivars with multiple sources of resistance have genes from different linkage groups (Lebeda 1984). Further pyramiding of resistance genes will be severely hampered unless novel resistance genes map to new loci. This is in contrast to the ability of pathogen isolates to exhibit virulence to many or all R factors simultaneously with no apparent decrease in fitness (Dixon and Wright 1978; Wellving and Crute 1978; Michelmore and Ingram 1980).

The data also provide support for the hypothesis that localized genomic distribution of resistance genes is a general phenomenon. Linkage analyses of resistance genes rarely have been made due to the complexities of multiple disease interactions. Resistance in lettuce to root aphid is tightly linked to dowy mildew R6 (Crute and Dunn 1980) in linkage group 1 and resistance to turnip mosaic virus is linked in repulsion to R8 (Zink and Duffus 1970) in linkage group 2. In barley, there are 30 alleles for resistance to *Erysiphe graminis* f. sp. *hordei* at the *M1a* locus (Griese 1981) and there are 14 alleles for resistance to *Puccinia sorghi* at the *Rpl* locus in maize which is closely linked to resistance to *P. polysori* (Hooker and Saxena 1971). In flax, the 29 alleles determining resistance to *Melampsora lini* are clustered in 5 loci (Shepherd and Mayo 1972). The functional and evolutionary significance of such dis-

tributions may be revealed when these genes are cloned and characterized at the molecular level. The present study has defined genotypes for further analysis.

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References

Boulidard L, Bannerot H (1975) Laitue. Rep Stat Genet d'amelioration Plant, Versailles for 1972–1974, pp M1–M18

Crute IR, Dunn JA (1980) An association between resistance to root aphid (*Pemphigus bursarius* L.) and downy mildew (*Bremia lactucae*) 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

Crute IR, Lebeda A (1981) Evidence for a race-specific resistance factor in some lettuce (*Lactuca sativa* L.) cultivars previously considered to be universally susceptible to *Bremia lactucae* Regel. Theor Appl Genet 60:185–189

Crute IR, Lebeda A (1983) Two new specific resistance factors to *Bremia lactucae* identified in cultivars of lettuce. Ann Appl Biol 102:128–129

Dixon GR, Wright IR (1978) Frequency and geographical distribution of specific virulence factors in *Bremia lactucae* populations in England from 1973 to 1975. Ann Appl Biol 88:278–294

Flor HH (1956) The complementary genetic systems in flax and flax rust. Adv Genet 8:29–54

Griese H (1981) Powdery mildew resistance genes in the *Ml-a* and *Ml-k* regions on barley chromosome 5. Hereditas 95: 51–62

Hewitt EJ (1952) Sand and water culture methods used in the study of plant nutrition. Techn Commun 22, Commonwealth Bureau Hortic Plantation Crops, East Malling, Maidstone, Kent

Hooker AL, Saxena KMS (1971) Genetics of disease resistance in plants. Annu Rev Genet 5:407–424

Johnson AG, Crute IR, Gordon PL (1977) The genetics of race specific resistance in lettuce (*Lactuca sativa*) to downy mildew (*Bremia lactucae*). Ann Appl Biol 89:257–264

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

Lebeda A (1984) Race-specific factors of resistance to *Bremia lactucae* in the world assortment of lettuce. Sci Hortic 22:23–32

Michelmore RW, Crute IR (1983): A method for determining the virulence phenotypes of isolates of *Bremia lactucae.* Trans Br Mycol Soc 79:542–546

Michelmore RW, Ingram DS (1980) Heterothallism in *Bremia lactucae.* Trans Br Mycol Soc 75:47–56

Michelmore RW, Ingram DS (1982) Secondary homothallism in *Bremia lactucae.* Trans Br Mycol Soc 78:1–9

Michelmore RW, Norwood JM, Ingram DS, Crute IR, Nicholson P (1984) Further studies on the inheritance of virulence in *Bremia lactucae:* Specific virulence to match resistance factors 3, 4, 5, 6, 8, 9, 10 and 11 in lettuce (*Lactuca sativa*). Plant Pathol 33:301–317

Norwood JM, Crute IR (1980) Linkage between genes for resistance to downy mildew (*Bremia lactucae*) in lettuce. Ann Appl Biol 94:127–135

Norwood JM, Crute IR (1984) The genetic control of avirulence in *Bremia lactucae.* Plant Pathol 33:385–401

Norwood JM, Michelmore RW, Crute IR, Ingram DS (1983) The inheritance of specific virulence in *Bremia lactucae* (Downy mildew) to match resistance factors 1, 2, 4, 6 and 11 in the *Lactuca sativa* (lettuce). Plant Pathol 32:177–186

Osara K, Crute IR (1981) Variation for specific virulence in the Finnish *Bremia lactucae* populations. Ann Agric Fenn 20:198–209

Shepherd KW, Mayo GME (1972) Genes conferring specific plant disease resistance. Science 175:375–380

Smith B, Leeper PW (1966) Mildew resistant lettuce susceptible to a new physiologic race of *Bremia lactucae* in south Texas. Plant Dis Rep 50:460

Wellving A, Crute IR (1978) The virulence characteristics of *Bremia lactucae* populations present in Sweden from 1971 to 1976. Ann Appl Biol 89:251–256

Zink FW (1978) Search for resistance to downy mildew and the nature of inheritance of resistance. Calif Iceberg Lettuce Res Prog Annu Rep 1977–1978

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