Genetic Analysis of *Lactuca* Accessions with New Major Gene Resistance to Lettuce Downy Mildew

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ABSTRACT

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The short-lived effectiveness of major gene resistance in lettuce to downy mildew (*Bremia lactucae*) means that plant breeders are in constant need of new resistance genes. In this study, the genetics of resistance to *B. lactucae* in nine accessions of *Lactuca sativa* or *L. serriola* was analyzed using segregating F_2 populations. This analysis was aimed at establishing the number of resistance factors in these accessions, their phenotypic effects, and the mapping of these factors in linkage groups. The cultivar Mariska (*R*18) and the line CS-RL both carry a widely effective resistance factor, which is located in linkage group 1. In seven other accessions, eight novel resistance factors, dissimilar to recognized major genes, were detected and designated *R*23 to *R*30. Evidence was obtained for a new, fifth, linkage group, which contains resistance factors *R*23 and *R*25. Resistance factors *R*27, *R*28, and *R*29 could not be assigned to a linkage group. Many of the new resistance factors identified in this study provide resistance to races that are important in lettuce-growing areas. Therefore, they are a valuable addition to the set of resistance genes available to lettuce breeders.

Bremia lactucae Regel causes downy mildew, which results in yield losses in lettuce (*Lactuca sativa* L.) worldwide. Breeding for resistance to *B. lactucae* has exclusively been based on the use of genes that confer hypersensitivity (2). However, introduction of cultivars with these genes or gene combinations has quickly been followed by the appearance of new races of the fungus that can overcome the resistance (10).

The genetic interaction between *B. lactucae* and lettuce has been studied extensively (2,5,9) and a gene-for-gene relationship has been shown to exist. Dominant resistance genes (*Dm* genes) correspond with dominant avirulence genes (*Avr* genes). The combination of a *Dm* gene and a corresponding *Avr* gene results in an incompatible interaction, expressed as hypersensitivity of the host. *Dm* genes in *L. sativa* occur in clusters in the genome, and a monogenic segregation ratio is consistent with either a single gene or multiple tightly linked genes. Therefore, a new resistance factor is formally indicated as *R#*, until shown to be a single *Dm* gene (11). Evidence for a single *Dm* gene is a monofactorial segregation of resistance in the host and avirulence in the pathogen (5). To date, 22 *R* factors have been named (5). Except for *R*12 and *R*18, the genetics of avirulence in the pathogen has been studied for each of these factors. In total, 13 different *Dm* genes and corresponding *Avr* genes were detected (5). The 13 *Dm* genes occur in four linkage groups. The *Dm* genes in group one (*Dm* 1,2,3,6,14,15, and 16) and in group two (*Dm* 5/8 and 10) are tightly linked, whereas recombination between *Dm* genes in group three (*Dm* 4, 7, and 11) has never been found, indicating that these *Dm* genes may be allelic (5). *Dm*13 segregates independently from the other *Dm* genes and is the only gene assigned to group 4. No tight linkage between *Avr* genes has been found (5,7,9). *Dm* genes differ with respect to the level of resistance they confer (12). The level of resistance can also be affected by gene dosage (3) and temperature (12). Gene-dose effects of *Avr* genes have also been reported (9).

In a previous study, 1,789 *Lactuca* accessions were screened for new major genes for resistance to *B. lactucae,* and 46 accessions were identified with resistance phenotypes that could not be explained by recognized *Dm* genes (1). Nine of these accessions with resistance to races of *B. lactucae* that are important in the Netherlands (NL12, NL15, or NL16) were selected for a genetic analysis. The aim of this study was to determine the number of resistance factors in each accession, and for each resistance factor the phenotype and linkage group.

MATERIALS AND METHODS

Twenty races of *B. lactucae* were used. Fourteen races were isolated in the Netherlands from *L. sativa* (NL races, obtained from the Institute for Plant Protection [IPO-DLO], Wageningen), and six races were isolated from *L. serriola* in Czechoslovakia (13) (provided by A. Lebeda, Semo Vegetable Breeding Station, Smržice, Czech Republic). The races were maintained as previously published (1). Table 1 shows the virulence phenotypes of the races and the differential set of lettuce genotypes that were used for determining the virulence phenotypes.

The names and resistance phenotypes of the nine accessions that were analyzed are given in Table 2. Accessions were from the Centre for Genetic Resources (CGN), Wageningen, the Netherlands. The nine accessions included the cultivar Mariska (CGN10966), which was assigned *R*18 (4), and CS-RL (CGN11391), an F_{10} line from an interspecific cross between *L. serriola* and *L. sativa* 'Brun Hilde' (17), provided by A. Lebeda. The lettuce accessions were crossed with each other and with genotypes of the differential set. F_1 plants were checked morphologically to exclude plants resulting from self fertilization. F_2 populations were produced from individual F_1 plants.

Resistance tests were carried out in plastic boxes with translucent plastic covers measuring 31 × 46 × 8 cm. Each race was maintained on seedlings of a lettuce cultivar with resistance to most of the other races. Seedlings were inoculated when the cotyledons were fully expanded. Ten days after inoculation the spore-bearing seedlings were washed in water, and spores were collected.

F_2 populations were tested as seedlings. In each box, 500 F_2 seeds and 50 seeds of the susceptible cultivar Cobham Green were laid out on filter paper moistened with a nutrient solution (1). The parents of the F_2 populations were included in one additional box per test, together with the genotypes of the differential set. After sowing, the boxes were closed and incubated at 2 C

for 24 h to overcome seed dormancy. The boxes were then transferred to a growth room with a photoperiod of 10 h per 24 h and a constant temperature inside the boxes of 15 C. Seedlings were inoculated 5 to 8 days later, when the cotyledons were fully expanded, by spraying a spore suspension (5×10^4 spores/ml) until runoff. One to 3 days after the first inoculation, the seedlings were inoculated a second time to reduce the chance of escapes. After each inoculation, 12 to 20 h of darkness was provided. Sporulation was scored visually 8 to 12 days after the second inoculation. Individual seedlings were scored either as resistant or as susceptible, depending on the absence or presence of visible sporophores.

Parents and genotypes of the differential set were tested on 20 seedlings and scored as presented in Tables 1 and 2.

For each accession, first the number of independently segregating resistance factors was established. The resistance phenotypes of the accessions (Table 2) indicated against which races at least one effective resistance factor was present. When accessions showed a mixed or incompletely compatible reaction with a particular race, the accession was considered to carry no resistance gene for that race. It was known that resistance in both Mariska (*R*18) (4) and CS-RL (A. Lebeda, *personal communication*) is inherited monofactorially. The number of resistance factors for the other seven accessions was determined by testing an F_2 from a cross with Cobham Green with all races that gave an incompatible reaction with the tested accession. Cultivar Cobham Green was susceptible to all races used in this analysis and was previously considered to be universally susceptible but was later found to carry resistance to races of *B. lactucae* isolated from *L. serriola* (13). The resistance of Cobham Green has not further been characterized and is indicated as *R*?.

After establishing the numbers of effective resistance factors per combination of accession and race, the resistance phenotype of the individual resistance factors was determined. When the segregation indicated the presence of more than one factor, the resistance phenotypes of individual resistance factors were compared with those of recognized *Dm* genes or *R* factors (Table 1). The linkage group to which the resistance factors belong were determined by analyzing F_2 populations from crosses between the tested accessions and genotypes with known *Dm* genes in known linkage groups.

RESULTS

Mariska and CS-RL. The resistance of Mariska and CS-RL to the races NL1, NL3, NL12, NL13, NL15, NL16, and 1/82 is inherited as a dominant allele at a single locus (Table 3). The segregation of the resistance factors in Mariska (*R*18) and in

TABLE 1. Virulence phenotypes of races of *Bremia lactucae* and resistance phenotypes of the differential set of lettuce genotypes used in the present study[a]

Cultivar or line	*Dm* gene or *R* factor	Linkage group	Races from *Lactuca sativa* (NL code)														Races from *L. serriola*					
			1	2	3	4	5	6	7	10	11	12	13	14	15	16	26/81	27/81	1/82	2/82	3/82	4/82
Cobham Green	*R*?[b]	Unknown	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	−	+
Lednicky	*Dm*1	1	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	−	−
UCDM2	*Dm*2	1	+	+	−	+	−	+	+	+	−	−	−	(+)	+	+	−	−	+	−	−	+
Dandy	*Dm*3	1	−	+	−	−	+	−	+	+	−	−	+	+	+	+	−	−	−	−	−	−
R4T57D	*Dm*4	3	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	−	+	−	−	+
Valmaine	*Dm*5/8	2	−	+	+	+	−	+	−	+	+	+	+	+	+	+	−	−	−	−	−	−
Sabine	*Dm*6	1	−	+	+	+	(−)	(+)	+	+	+	+	+	+	−	+	−	−	−	−	−	−
Mesa 659	*Dm*7+13	3,4	−	−	+	+	+	−	+	+	+	+	+	−	+	+	+	+	−	+	(+)	−
	*Dm*7[c]	3	−	−	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	(+)	+
UCDM10	*Dm*10	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
Capitan	*Dm*11	3	−	−	−	−	−	+	(−)	(−)	−	+	+	+	+	+	(+)	(−)	−	−	−	+
Hilde	*R*12	Unknown	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	(−)	−	+
Pennlake	*Dm*13	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	(−)	−	−
UCDM14	*Dm*14	1	+	+	+	+	−	+	+	+	+	+	+	+	−	−	(+)	+	−	−	−	−
PIVT 1309	*Dm*15	1	(−)	−	+	−	+	(−)	(−)	(+)	+	+	+	−	−	−	+	+	−	+	+	−
LSE/18	*Dm*16	1	−	−	−	−	−	−	−	−	+	+	−	−	−	+	+	+	+	+	+	+

[a]Interactions were scored as −, incompatible (no sporulation); (−), incompletely incompatible (only a few sporophores); (+), incompletely compatible (reduced sporulation on at least 80% of the seedlings); or +, compatible (profuse sporulation on at least 80% of the seedlings).
[b]Cobham Green was previously considered to be universally susceptible but later was found to carry resistance (indicated as *R*?) to races of *B. lactucae* isolated from *L. serriola* (13).
[c]Because no genotype is available with only *Dm*7, the resistance phenotype of *Dm*7 is induced from the resistance phenotypes of Mesa 659 (*Dm*7 + *Dm*13) and Pennlake (*Dm*13) and information from Lebeda (14).

TABLE 2. *Lactuca* accessions that were analyzed genetically and their interactions with 14 races of *Bremia lactucae* isolated from *L. sativa* and six races isolated from *L. serriola*[a]

Accession	Races from *L. sativa* (NL code)														Races from *L. serriola*					
	1	2	3	4	5	6	7	10	11	12	13	14	15	16	26/81	27/81	1/82	2/82	3/82	4/82
L. sativa 'Mariska'	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
L. serriola × *L. sativa*																				
CS-RL	−	−	−	(−)	−	(−)	−	−	−	−	−	−	−	−	(−)	(−)	−	(−)	(−)	−
L. serriola[b]																				
CGN5153[s]	−	−	−	−	+	−	+	+	+	+	(+)	−	−	±	+	+	−	+	+	−
CGN14255	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	+	−
CGN14256	(−)	+	−	−	−	−	−	(−)	−	−	−	−	−	−	+	+	−	+	+	−
CGN14270	−	(−)	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	+	−
CGN14280	−	(−)	−	−	−	−	−	(−)	−	±	(−)	−	−	−	+	+	−	(+)	+	−
PI491178	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	+	−
PI491229	+	±	+	(−)	(+)	+	−	(+)	+	+	+	±	+	−	+	+	+	+	+	+

[a]Interactions were scored as −, incompatible (no sporulation); (−), incompletely incompatible (only a few sporophores); (+), incompletely compatible (reduced sporulation on at least 80% of the seedlings); +, compatible (profuse sporulation on at least 80% of the seedlings); or ±, mixed reaction (profuse sporulation on less than 80% of the seedlings).
[b]CGN5153[s] represents selected plants from a heterogeneous accession.

TABLE 3. Reaction of F_2 plants from crosses of lettuce downy mildew differentials and several *Lactuca* accessions after inoculation with races of *Bremia lactucae*

Parents[a]	Race	No. of F_2 plants[b] S	No. of F_2 plants[b] R	Expected ratio[c]	χ^2	P[d]	Escape frequency (%) in 50 seedlings of Cobham Green
Mariska × Cobham Green	NL1	110	388	1:3	2.25	0.15	0
	NL3	104	396	1:3	4.70	0.03	0
	NL12	115	383	1:3	0.97	0.35	8
	NL13	117	381	1:3	0.60	0.46	0
	NL15	115	385	1:3	1.07	0.32	0
	NL16	118	381	1:3	0.49	0.49	0
	1/82	106	394	1:3	3.85	0.05	0
Mariska × Lednicky	NL3	0	496	$1{:}4r^{-2}-1$	...	...	0
Mariska × Valmaine	1/82	27	473	1:15	0.62	0.45	0
Mariska × Capitan	NL1	26	473	1:15	0.92	0.36	0
Mariska × Pennlake	1/82	25	475	1:15	1.33	0.25	0
CS-RL × Cobham Green	NL1	169	590	1:3	3.03	0.09	n.t.
	NL3	194	541	1:3	0.76	0.41	n.t.
	NL12	113	384	1:3	1.36	0.25	2
	NL13	121	371	1:3	0.04	0.86	0
	NL15	101	393	1:3	5.47	0.02	0
	1/82	106	392	1:3	3.67	0.06	0
CS-RL × R4T57D	NL16	109	391	1:3	2.73	0.10	0
CS-RL × Lednicky	NL3	0	499	$1{:}4r^{-2}-1$	...	...	0
CS-RL × Valmaine	1/82	29	470	1:15	0.16	0.70	0
CS-RL × Capitan	NL1	38	456	1:15	1.75	0.20	0
CS-RL × Pennlake	1/82	37	460	1:15	1.21	0.28	0
CS-RL × Mariska	NL13	0	497	$1{:}4r^{-2}-1$	...	...	4
	NL15	0	499	$1{:}4r^{-2}-1$	...	...	6
	NL16	0	498	$1{:}4r^{-2}-1$	...	...	0
Cobham Green × CGN5153[s]	NL1	80	420	1:3	21.60	<0.001	0
	NL2	43	456	1:15	4.77	0.03	6
	NL3	88	410	1:3	14.27	<0.001	0
	NL4	142	358	1:3	3.08	0.08	0
	NL6	158	340	1:3	12.02	<0.001	0
	NL14	44	454	1:15	5.68	0.02	0
	NL15	77	423	1:3	24.58	<0.001	0
	1/82	126	369	1:3	0.05	0.84	0
	4/82	124	376	1:3	0.01	>0.90	0
Sabine × CGNN5153[s]	NL15	25	474	1:15	0.94	0.36	0
Mesa 659 × CGN5153[s]	NL14	0	499	$1{:}16r^{-2}-1$	...	...	0
Pennlake × CGN5153[s]	1/82	16	483	1:15	7.89	0.005	2
CGN5153[s] × CGN14255	NL4	0	500	$1{:}16r^{-2}-1$	...	...	0
Cobham Green × CGN14255	NL1	31	467	1:15	0.00	1.00	0
	NL2	106	394	1:3	3.85	0.05	16
	NL3	25	474	1:15	1.31	0.25	20
	NL4	30	470	1:15	0.05	0.84	0
	NL5	50	448	1:15	12.21	<0.001	0
	NL6	48	451	1:15	9.67	<0.001	0
	NL7	34	463	1:15	0.30	0.61	2
	NL10	40	459	1:15	2.66	0.11	0
	NL11	38	462	1:15	1.56	0.22	0
	NL12	35	464	1:15	0.50	0.49	0
	NL13	35	450	1:15	0.77	0.41	0
	NL14	33	465	1:15	0.12	0.74	0
	NL15	48	452	1:15	9.58	<0.001	0
	NL16	44	456	1:15	5.55	0.02	0
	1/82	32	465	1:15	0.03	0.88	0
	4/82	35	465	1:15	0.48	0.49	0
Dandy × CGN14255	1/82	8	489	1:63	0.01	>0.90	0
Valmaine × CGN14255	1/82	6	494	1:63	0.43	0.51	0
R4T57D × CGN14255	NL13	0	497	$1{:}16r^{-2}-1$	...	...	4
Capitan × CGN14255	NL2	15	485	1:15	9.01	<0.001	18
Pennlake × CGN14255	1/82	9	488	1:63	0.20	0.68	4
Cobham Green × CGN14256	NL1	135	364	1:3	1.12	0.31	0
	NL3	151	346	1:3	7.68	0.006	0
	NL4	149	351	1:3	6.14	0.01	0
	NL5	229	271	1:3	115.37	<0.001	0
	NL6	227	272	1:3	111.74	<0.001	0
	NL7	152	348	1:3	7.78	0.005	6
	NL10	158	341	1:3	11.82	<0.001	0
	NL11	123	377	1:3	0.04	0.86	2
	NL12	143	357	1:3	3.46	0.07	0

(*continued on next page*)

[a] The female parent in the cross is always listed first. CGN5153[s] represents selected plants from a heterogeneous accession.
[b] Number of F_2 seedlings showing either susceptible (S) or resistant (R) reactions to *Bremia lactucae*.
[c] Ratio of susceptible to resistant plants. With independently segregating factors, this ratio is 1:3, 1:15, or 1:63, respectively, when 1, 2, or 3 dominant resistance factors are segregating. When the parents of the cross both have a resistance gene in the same linkage group, the expected ratio is $1{:}(4{\cdot}r^{-2}-1)$, in which r is the recombination frequency between the two genes.
[d] P = Probability of a greater value due to chance alone. In linked resistance genes, the expected ratio of susceptible to resistant seedlings (and thus P) is unknown.

TABLE 3. (*continued from preceding page*)

Parents[a]	Race	No. of F_2 plants[b] S	No. of F_2 plants[b] R	Expected ratio[c]	χ^2	P[d]	Escape frequency (%) in 50 seedlings of Cobham Green
Cobham Green × CGN14256	NL13	136	343	1:3	2.94	0.09	0
	NL14	153	346	1:3	8.53	<0.005	0
	NL15	143	356	1:3	3.56	0.06	0
	NL16	135	365	1:3	1.07	0.32	0
	1/82	41	455	1:15	3.44	0.07	0
	4/82	43	457	1:15	4.71	0.03	0
Dandy × CGN14256	NL1	28	470	1:15	0.33	0.59	2
Valmaine × CGN14256	NL1	32	465	1:15	0.03	0.88	0
R4T57D × CGN14256	NL13	0	471	$1{:}4r^{-2}-1$	...	...	2
Pennlake × CGN14256	1/82	0	499	$1{:}16r^{-2}-1$	...	...	2
Cobham Green × CGN14270	NL1	45	452	1:15	6.67	0.01	8
	NL2	135	361	1:3	1.30	0.25	4
	NL3	21	471	1:15	3.30	0.07	8
	NL4	33	467	1:15	0.10	0.75	0
	NL5	132	367	1:3	0.56	0.47	0
	NL6	166	331	1:3	18.70	<0.001	0
	NL7	43	455	1:15	4.83	0.04	0
	NL10	69	431	1:15	48.64	<0.001	0
	NL11	38	460	1:15	1.62	0.22	0
	NL12	37	462	1:15	1.16	0.30	0
	NL13	48	411	1:15	13.87	<0.001	4
	NL14	24	475	1:15	1.77	0.20	2
	NL15	41	458	1:15	3.29	0.07	0
	NL16	55	445	1:15	19.25	<0.001	2
	1/82	134	361	1:3	1.13	0.30	0
	4/82	103	394	1:3	4.85	0.03	0
Dandy × CGN14270	NL1	5	494	1:63	1.02	0.34	2
Valmaine × CGN14270	NL1	11	486	1:63	1.37	0.24	0
R4T57D × CGN14270	NL13	0	483	$1{:}16r^{-2}-1$	...	...	16
Pennlake × CGN14270	1/82	22	476	1:15	2.85	0.09	4
CGN14270 × CGN14255	NL2	38	462	1:15	1.56	0.22	4
Cobham Green × CGN14280	NL1	161	338	1:3	14.04	<0.001	0
	NL2	212	286	1:3	81.99	<0.001	6
	NL3	99	399	1:3	6.96	0.009	0
	NL4	141	359	1:3	2.73	0.10	0
	NL5	71	426	1:15	54.78	<0.001	0
	NL6	357	139	1:3	538.75	<0.001	0
	NL7	207	297	1:3	71.72	<0.001	0
	NL10	257	243	1:3	185.86	<0.001	0
	NL11	246	253	1:3	157.13	<0.001	0
	NL12	489	11	1:3	1,466.35	<0.001	0
	NL13	201	285	1:3	69.36	<0.001	0
	NL14	160	339	1:3	13.28	<0.001	0
	NL15	105	395	1:3	4.27	0.04	0
	NL16	44	454	1:15	5.68	0.02	0
	1/82	290	208	1:3	293.34	<0.001	0
	4/82	172	327	1:3	23.86	<0.001	0
Dandy × CGN14280	NL1	42	456	1:15	4.05	0.05	2
Valmaine × CGN14280	NL1	42	456	1:15	4.05	0.05	0
R4T57D × CGN14280	NL13	3	492	$1{:}4r^{-2}-1$	...	...	4
Pennlake × CGN14280	1/82	61	438	1:15	30.40	<0.001	0
CGN14270 × CGN14280	NL2	49	451	1:15	10.75	<0.001	4
CGN14280 × CGN14256	NL3	0	498	$1{:}4r^{-2}-1$	...	...	20
R4T57D × PI491178	NL1	87	411	1:3	15.06	<0.001	0
	NL3	79	418	1:3	21.97	<0.001	2
	NL4	105	387	1:3	3.51	0.06	0
	NL5	39	460	1:15	2.09	0.17	0
	NL6	101	399	1:3	6.14	0.01	0
	NL7	120	380	1:3	0.26	0.64	0
	NL10	120	378	1:3	0.22	0.66	0
	NL11	108	391	1:3	3.00	0.09	0
	NL12	111	387	1:3	1.95	0.18	0
	NL13	0	496	$1{:}4r^{-2}-1$	...	...	4
	NL14	99	398	1:3	6.84	0.009	0
	NL15	96	403	1:3	8.83	<0.005	0
	NL16	107	393	1:3	3.46	0.07	0
	1/82	114	383	1:3	1.13	0.30	0
	4/82	101	399	1:3	6.14	0.01	0
Valmaine × PI491178	NL1	19	473	1:15	4.79	0.03	4
	NL13	111	375	1:3	1.21	0.28	0
Sabine × PI491178	NL1	27	469	1:15	0.55	0.47	0
Pennlake × PI491178	1/82	31	467	1:15	0.00	1.00	0
CGN14256 × PI491178	NL1	0	489	$1{:}4r^{-2}-1$	...	...	0
Cobham Green × PI491229	NL4	126	374	1:3	0.01	>0.90	0
	NL7	114	386	1:3	1.29	0.26	0
	NL16	112	387	1:3	1.74	0.20	0
Mariska × PI491229	NL16	0	500	$1{:}4r^{-2}-1$	...	...	0
PI491229 × CGN14255	NL16	8	491	1:63	0.01	>0.90	0

CS-RL in F_2 populations from crosses with genotypes with known *Dm* genes shows that both factors are located in linkage group 1. No susceptible plant was found in a total of 1,494 F_2 seedlings from a cross between CS-RL and Mariska, which indicates that the resistance factors in both accessions are allelic or tightly linked. The results for both accessions were identical: none of the races used is virulent on Mariska or CS-RL (Table 2). However, according to Lebeda and Blok (17) CS-RL is resistant to the races CS2 and CS8, whereas Mariska is not. This means that if CS-RL also carries *R*18, the resistance to the races CS2 and CS8 must be conferred by an additional resistance factor. It is, on the other hand, also possible that CS-RL carries a resistance factor in linkage group 1 that is dissimilar to *R*18. Further studies using races CS2 and CS8 should distinguish between these two options.

CGN5153^s carries at least two resistance factors (Table 3), which are either unlinked or weakly linked. The resistance phenotype of CGN5153^s excludes the presence of all known *Dm* genes except *Dm*7. In the F_2(Mesa 659 [*Dm*7+13] × CGN5153^s) no susceptible seedlings were detected with NL14. Therefore CGN5153^s probably carries *Dm*7. Because of its resistance phenotype, CGN5153^s must also carry another resistance factor. We propose to designate this resistance factor *R*23. The resistance phenotype of *R*23 was induced from information about the resistance phenotype of *Dm*7 and the number of effective resistance factors per combination of F_2 and race. *R*23 provides resistance to races NL2, NL3, NL4, NL14, NL15, 1/82, and 4/82. The cross between CGN5153^s and CGN14255 showed that *R*23 is closely linked to a resistance factor in CGN14255.

CGN14255 carries at least two independent resistance factors (Table 3). The resistance phenotypes of both factors cannot be explained by recognized *Dm* genes, and we propose to name these factors *R*24 and *R*25. *R*24 provides resistance to all races used, except NL2, 26/81, 27/81, 2/82, and 3/82 (Tables 2 and 3). The second factor, *R*25, has the same resistance phenotype as *R*24, except that it also provides resistance to NL2.

Using race NL13, it was shown that one of both resistance factors is closely linked to *Dm*4 (cross with *R*4T57D) and thus must be located in linkage group 3. The factor in linkage group 3 must be *R*24, because the results of the F_2 (Capitan [*Dm*11] × CGN14255) and race NL2 showed that *R*25 is not or only weakly linked to *Dm*11 and thus *R*25 cannot be in linkage group 3. Tests with race 1/82 revealed that both factors segregated independently from *Dm*3 (cross with Dandy; linkage group 1), *Dm*5/8 (cross with Valmaine; linkage group 2), and *Dm*13 (cross with Pennlake; linkage group 4). Therefore, *R*25 must be located in a fifth linkage group. Because *R*23 was not located in linkage group 3, and because of the linkage to the resistance in CGN14255, *R*23 must also be located in linkage group 5.

CGN14256 also carries at least two resistance factors (Table 3). The numbers of effective resistance factors per race could be explained by a combination of *R*24 and a factor providing resistance to 1/82 and 4/82, which we propose to name *R*26. Resistance to NL13 is linked to *Dm*4 (cross with *R*4T57D; linkage group 3). These results strongly suggest that CGN14256 carries *R*24. *R*esistance to race 1/82 was linked to *Dm*13 (cross with Pennlake) and thus *R*26 is located in linkage group 4.

CGN14270 carries at least two independent resistance factors (Table 3). The numbers of resistance factors effective per race suggest the presence of *R*24 and a new resistance factor, which we propose to name *R*27. If the first factor is indeed *R*24, *R*27 provides resistance to the races NL1, NL2, NL3, NL4, NL7, NL10, NL11, NL12, NL13, NL14, NL15, and NL16. A linkage to *Dm*4 (cross with *R*4T57D; linkage group 3) was observed with race NL13, strongly suggesting that CGN14270 carries *R*24. Because of the independent segregation of the two factors, *R*27 is not located in linkage group 3. A test with NL2 showed no linkage between *R*27 and factor *R*25 in CGN14255 (linkage group 5), and tests with NL1 revealed no linkage to *Dm*3 (cross with Dandy; linkage group 1) and *Dm*5/8 (cross with Valmaine; linkage group 2). Therefore, *R*27 must be located either in linkage group 4 or in a sixth linkage group. Linkage between *R*27 and *Dm*13 (linkage group 4) could not be studied, because *Dm*13 was not effective against the races to which *R*27 provided resistance.

CGN14280 carries at least two resistance factors (Table 3). Possibly the expression of resistance to NL12 was very incomplete. If so, CGN14280 could carry *R*24 or *R*25. With NL13, a linkage to *Dm*4 (cross with *R*4T57D; linkage group 3) was observed and with NL3 a linkage to a resistance factor in CGN14256 was seen. Therefore CGN14280 probably carries *R*24. In nearly all cases, the number of susceptible plants in the F_2 populations was above expectations for a mono- or difactorial segregation. This could be explained if the heterozygous expression of *R*24 was incomplete in CGN14280.

The other resistance factor, which we propose to name *R*28, provides resistance to NL2, NL5, and NL16. Possibly, *R*28 also provides resistance to NL3 and NL15, because with these races the number of susceptible seedlings in the F_2 (Cobham Green × CGN14280) was lower than expected for a monofactorial segregation, resulting in an estimated number of effective resistance factors significantly above one. A test with NL2 detected no linkage of *R*28 to *R*27 (linkage group 4 or 6). Assuming that the excess of susceptible F_2 plants in the cross Cobham Green × CGN14280 was due to incomplete expression, *R*28 inherits independently from *R*24 (linkage group 3). The linkage group to which *R*28 belongs could not be clarified.

PI491178 carries at least two resistance factors (Table 3). The number of factors effective per race could be explained by the presence of *R*24 and another factor that provides resistance to at least NL5. Table 3 shows that the factor providing resistance to NL13 is linked to *Dm*4 (linkage group 3), and the factor providing resistance to NL1 is linked to a resistance factor in CGN14256. These results are in agreement with the hypothesis that PI491178 carries *R*24. We propose to name the other resistance factor *R*29. With NL5, an independent segregation of two factors was found and therefore *R*29 is not located in the same linkage group as *R*24 (group 3). The linkage group for *R*29 was not clarified.

PI491229 probably carries one resistance factor (Table 3). Its resistance phenotype (Table 2) excludes the presence of all recognized *Dm* genes. The resistance factor, which we propose to name *R*30, provides resistance to NL4, NL7, and NL16. It could also provide resistance to NL2, NL5, NL10, or NL14, but in that case the expression of *R*30 is incomplete for these races in PI491229. A linkage to *R*18 (cross with Mariska; linkage group 1) was found with NL16. Therefore, *R*30 must be located in linkage group 1.

DISCUSSION

The analyses showed that the cultivar Mariska (*R*18) and the line CS-RL both carry a widely effective resistance factor in linkage group 1. In seven other accessions, eight novel resistance factors were detected and were named *R*23 to *R*30. Apart from the resistance factors identified in this study, many others are still expected to be found. Bonnier et al (1) identified 46 accessions with a resistance phenotype that could not be attributed to recognized *Dm* genes. Only nine of the 46 accessions have been analyzed here. Furthermore, considerable numbers of *L. serriola* accessions have not yet been studied for their resistance phenotype or have been studied with only a few races. Even accessions with the same resistance phenotype may carry different resistance factors. Accessions CGN14255 and CGN14270 were chosen because they represented a larger group of accessions with resistance to all races used, except for the races 26/81, 27/81, 2/82, and 3/82. Accessions CGN14256 and PI491178 represented a group with resistance to all races used, except for NL2, 26/81, 27/81, 2/82, and 3/82. Surprisingly, in both combinations the two accessions with the same resistance phenotype differed for the resistance factors they carried. Therefore, more new resistance factors are not only expected in accessions with a new resistance phenotype that have not yet been tested but can also be found in accessions with similar resistance phenotypes as tested in this study.

The interpretation of the results was based on the minimum

number of independently segregating factors necessary to explain the data. In principle, however, it is possible that more factors were segregating, each of them effective against a complementary set of races. Since many of the newly recognized factors provide resistance to races that are important in lettuce-growing areas in Europe, such as NL12, NL15, and NL16, they are very useful in breeding for resistance to *B. lactucae*. Two of these resistance factors, *R*23 and *R*25 were located in a new, fifth linkage group. The linkage groups of *R*27, *R*28, and *R*29 have not yet been determined. New linkage groups provide more possibilities for combining resistance genes. Until now only linkage groups 1, 2, and 3 contained genes effective against NL races.

Accession CGN14256 carries a factor closely linked to *Dm*13 that was named *R*26. This accession was susceptible to the races 26/81, 27/81, 2/82, and 3/82 (Table 2). According to Lebeda (14) these races have no virulence to *Dm*13, because they are avirulent on Pennlake (*Dm*13). However, in our experiments these races were virulent on Mesa 659, which carries *Dm*7 and *Dm*13 (17). Since no recombinants were found between *R*26 and *Dm*13, it seems likely that the resistance phenotype of *Dm*13, as given in Table 1, is incorrect. Based on our results, it seems likely that *Dm*13 is identical to *R*26 and does not provide resistance to 26/81, 27/81, 2/82, and 3/82. In addition to *Dm*13, Pennlake carries a resistance factor effective against these four races. This could be *R*12 or the factor found in Cobham Green (*R*?). Farrara et al (5) identified *Dm*13 as a single resistance gene. As they did not use the *B. lactucae* isolates from *L. serriola* used in this study, their results do not disagree with the proposed correction of the resistance phenotype of *Dm*13.

In several F_2 populations, the segregation ratios found deviated significantly from the ratios expected for one, two, or three independent resistance factors. Such deviations could have several causes. First, deviating segregation ratios could be due to genetic modifications of resistance or susceptibility. Each seedling bearing sporophores was scored susceptible. In some cases, seedlings showed only little sporulation, sometimes accompanied by necrosis. If incomplete susceptibility occurs, the number of resistant seedlings is overestimated, but if incomplete resistance occurs, the number of resistant seedlings is underestimated. Incomplete susceptibility or incomplete resistance is dependent on the expression of *Dm* genes and *Avr* genes (18,20), gene-dose effects of *Dm* genes and *Avr* genes (8), the effect of modifier genes in both host and races (9), or the occurrence of partial resistance caused by non-race-specific genes. Second, escapes from infection could cause overestimation of the number of resistant plants. Seedlings from the susceptible control showed in some cases less than 100% sporulation, but even if a susceptible control does show 100% sporulation, the absence of escapes in the F_2 is not guaranteed, as the frequency of escapes can depend on the level of partial resistance of the genotypes tested. Third, linkage between resistance factors would also lead to deviating segregation ratios. When two resistance factors are linked, the expected segregation ratio lies between a mono- and a difactorial inheritance. However, for races avirulent to both resistance factors, this intermediate ratio should not be race-dependent. Fourthly, selection in the gametophytic or sporophytic phase could also lead to deviating segregation ratios. A resistance factor might be linked to a fitness-related factor. Selection can then cause overestimation or underestimation of the number of resistant plants.

Farrara et al (5) and Ilott et al (9) stressed the importance of a complementary genetic analysis in both the host and the pathogen. As we did not make a genetic analysis in the pathogen, information on corresponding *Avr* genes in the pathogen is lacking. Therefore, it is not possible to conclude whether the new resistance factors detected are single *Dm* genes or combinations of linked *Dm* genes. Until a complementary study of the corresponding *Avr* genes in *B. lactucae* shows how many *Avr* genes are segregating, the term *R* factor should be used.

Since virulence to all new resistance factors (except *R*18 and the factor in CS-RL) was already present in the set of races used, resistance based on these factors is not expected to be more durable than resistance based on previously used *Dm* genes and *R* factors. Therefore, strategies should be used to increase the durability of the effects of the resistance factors. One strategy could be the accumulation of several effective resistance genes in one cultivar (5). Strategies may also be based on the use of resistance genes that vary in time or space (21). However, these strategies may meet practical drawbacks. First, as long as growers are free to choose their cultivars, the diversification of the use of resistance genes will be hard to achieve. Second, even if this diversification were obtained, it would be unlikely that durable protection could be achieved, owing to the variability of the pathogen and the ease with which populations of *B. lactucae* adapt to resistance genes used in lettuce breeding (15). Therefore, research on non-race-specific resistance (16,19) should continue. However, cultivars with an acceptable level of non-race-specific resistance have not been developed yet, and consequently new race-specific genes for resistance to *B. lactucae* remain important for lettuce breeding. The new resistance factors described in this paper may help to bridge the gap until a more durable form of resistance to *B. lactucae* is available.

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