Theor Appl Genet (2002) 105:384–391
DOI 10.1007/s00122-002-0943-z

M. Jeuken · P. Lindhout

Lactuca saligna, a non-host for lettuce downy mildew (*Bremia lactucae*), harbors a new race-specific *Dm* gene and three QTLs for resistance

Received: 5 September 2001 / Accepted: 25 November 2001 / Published online: 14 June 2002

Abstract *Lactuca sativa* (lettuce) is susceptible to *Bremia lactucae* (downy mildew). In cultivated and wild *Lactuca* species, *Dm* genes have been identified that confer race-specific resistance. However, these genes were soon rendered ineffective by adaptation of the pathogen. *Lactuca saligna* (wild lettuce) is resistant to all downy mildew races and can be considered as a non-host. Therefore, *L. saligna* might be an alternative source for a more-durable resistance to downy mildew in lettuce. In order to analyze this resistance, we have developed an F_2 population based on a resistant *L. saligna* × susceptible *L. sativa* cross. This F_2 population was fingerprinted with AFLP markers and tested for resistance to two *Bremia* races NL14 and NL16. The F_2 population showed a wide and continuous range of resistance levels from completely resistant to completely susceptible. By comparison of disease tests, we observed a quantitative resistance against both *Bremia* races as well as a race-specific resistance to *Bremia* race NL16 and not to NL14. QTL mapping revealed a qualitative gene (*R39*) involved in the race-specific resistance and three QTLs (*RBQ1*, *RBQ2* and *RBQ3*) involved in the quantitative resistance. The qualitative gene *R39* is a dominant gene that gives nearly complete resistance to race NL16 in *L. saligna* CGN 5271 and therefore it showed features similar to *Dm* genes. The three QTLs explained 51% of the quantitative resistance against NL14, which indicated that probably only the major QTLs have been detected in this F_2 population. The perspectives for breeding for durable resistance are discussed.

Keywords Non-host resistance · *Lactuca saligna* · Lettuce · Downy mildew · QTL mapping

Communicated by F. Salamini

M. Jeuken (✉) · P. Lindhout
Laboratory of Plant Breeding, Wageningen University,
PO Box 386, 6709 PD Wageningen, The Netherlands
e-mail: marieke.jeuken@pv.dpw.wau.nl
Fax: +31 317 483457

Introduction

Several *Lactuca* species are host for the biotrophic oomycete *Bremia lactucae* (downy mildew; Lebeda and Syrovatko 1988). Because of major yield losses in lettuce (*Lactuca sativa*) cultivation due to downy mildew, lettuce breeders have put a large effort into obtaining resistance to this pathogen. In most lettuce cultivars *Dm* genes confer race-specific resistance to downy mildew (*B. lactucae*). The resistance of these *Dm* genes is controlled by single dominant genes that are matched by avirulence genes in *Bremia* in a gene-for-gene interaction, i.e. race-specificity. This results in an incompatible interaction associated with a hypersensitive response of the host (Crute and Johnson 1976). During the history of lettuce breeding 19 *Dm* genes have been identified and have been introgressed into commercial cultivars from cultivated germplasm sources or closely related species like *Lactuca serriola* (Landry et al. 1987; Crute 1992; Bonnier et al. 1994; Van Ettekoven and van der Arend 1999). The resistance of *Dm* genes is not durable since these genes become ineffective soon after their introduction as a result of rapid genetic adaptation of the pathogen (Crute 1992; Lebeda and Schwinn 1994; Reinink 1999). Two parameters play an important role in this high evolutionary potential of *Bremia* populations: (1) a mixed reproduction system (asexual and sexual), which can cause fast fixation of new virulence alleles or allele combinations arising from recombination and mutation (Crute 1992; McDonald and Linde 2002), and (2) new isolates are rapidly spread (= high gene flow) due to wind dispersal of spores. Since race-specific *Dm* genes are not durable, there is a need for an alternative race non-specific and durable resistance in lettuce breeding.

In a search for an alternative resistance, attempts have been made to exploit partial resistance in butterhead lettuce (Eenink 1981; Eenink and De Jong 1982; Eenink et al. 1982; Eenink et al. 1983). However, these attempts have not resulted in commercial breeding and the release of cultivars specifically bred for an increased level of partial resistance (Reinink 1999).

In addition to screening for resistance within the *L. sativa* species, the biodiversity for *Bremia* resistance has been surveyed in species closely related to *L. sativa*. This survey of four *Lactuca* species suggested that, of the *Lactuca* species that can be crossed with cultivated lettuce, only *Lactuca saligna* (52 accessions tested) is completely resistant to all *Bremia* races (20 races tested) and may be considered a non-host (Bonnier et al. 1992). This non-host status for *L. saligna* was already suggested earlier in small-scale experiments (Norwood 1981; Gustafsson 1989; Lebeda and Boukema 1991). At the histological level the *L. saligna* accessions varied in resistance symptoms involving the presence or absence of necrosis formation after *Bremia* inoculation (Lebeda and Reinink 1994). *L. saligna* accessions with *Bremia* resistance without necrosis formation are a very interesting source for alternative resistances.

Very little is known about the genetics of resistance in non-host species (Heath 2001; Kamoun 2001). It remains unclear whether the phenomenon "non-host resistance" comprises one or several defense mechanisms explained by known or new types of resistance. Therefore, a study on the resistance of *L. saligna* to *Bremia* may reveal new insights into the "non-host" defense mechanisms of plants. In this study we investigated the genetics and specificity of *Bremia* resistance in *L. saligna*.

Materials and methods

Plant material and genotype (linkage) analysis

An F_2 population was generated from the cross of *L. saligna* CGN 5271 as a resistant female parent with *L. sativa* cv "Olof", a butterhead cultivar as the male parent. Olof is considered to be a generally susceptible cultivar for all European *Bremia* races and devoid of effective *Dm* genes. The F_2 population was derived from a single F_1 plant and consisted of 126 individuals. Plant material and DNA samples were prepared as described in Jeuken et al. (2001).

All 126 F_2 plants have been fingerprinted by AFLP analysis for the construction of a genetic linkage map. This map consisted of 488 markers on nine linkage groups covering 854 cM (Jeuken et al. 2001). All F_2 plants were selfed and the F_3 seed was collected. F_3 plants from selected lines were grown in a randomized block design.

Bremia disease tests

F_2 population

Bremia was maintained in plastic boxes on seedlings of susceptible cultivars. The inoculum for disease tests was prepared by washing sporulating seedlings in tap water. The spore concentration was adjusted to 3×10^5 spores per ml.

Disease tests were performed on mature plants with two *Bremia* races. For each *Bremia* race two tests were done on leaf discs of 9 and 10 week-old plants (i.e. first and second test). For each disease test four leaf discs of 17 mm in diameter were taken from full-grown leaves of each F_2 plant and placed upside down on filter paper moistened with water in a plastic box of 40 × 25 × 8 cm. The four leaf discs of each F_2 plant were placed per pair randomly in two replicates in the box. As controls at least six leaf discs of the susceptible parent *L. sativa* Olof and at least four leaf discs of the resistant parent *L. saligna* CGN 5271 were included in each box, which contained in total 198 leaf discs. Transparent plastic lids covered the boxes. Growth conditions were a photoactive period of 16 h and a constant temperature of 15 °C. The leaf discs were inoculated by spraying with a spore suspension. To minimize the risk of escapes, a second inoculation was performed the day after the first inoculation. After inoculation the leaf discs were incubated in the dark for 12 h. Ten days after inoculation, the leaf discs were assessed for sporulation. Leaf discs were scored for infection severity according to the following classes: class 0: no sporulation, class 1: 1–25% of leaf disc area sporulates; class 2: 26–50%; class 3: 50–75% and class 4: 75–100% (Lebeda and Reinink 1991; Lebeda and Pink 1998). Per disease test the infection severity score of an F_2 plant was calculated as the average of the observations of the four leaf discs. A low or high infection severity was interpreted as resistance or susceptibility, respectively. Per disease test, an analysis of variance was carried out to calculate environmental variance and the wide-sense heritability (H^2). $H^2 = S^2_g/S^2_g + S^2_e$, in which S^2_g is the variance between genotypes and S^2_e is the variance within genotypes based on four observations per genotype.

F_3 lines

A disease test with *Bremia* race NL16 was performed for eight F_3 lines on mature plants of 11-weeks old. The *Bremia* disease test was performed as described above, except that from each F_3 plant six leaf discs were taken and placed per pair in three replications randomly in the box. Furthermore, *Bremia* infection severity was scored on leaf discs distributed as the percentage of leaf area covered with sporulation, instead of scoring the infection severity in classes. To make comparisons with the F_2 disease test results, the plant and line averages of the F_3 observations were transformed to infection severity class units.

QTL mapping

The software program MapQTL 4.0 (Van Ooijen and Maliepaard 1996) was used to perform the Kruskal–Wallis test, the Interval Mapping method and the Restricted MQM Mapping method on each data set of the four *Bremia* disease tests of the F_2 population. The Kruskal–Wallis test is a non-parametric test in which no assumptions are being made for the probability distributions of the quantitative trait (after fitting the QTL genotype). In Interval Mapping and MQM Mapping the distributions are assumed to be normal. The complete DNA marker data set of 488 markers was used in the Kruskal–Wallis test and Interval Mapping. A subset of 182 markers, spaced approximately 5 to 15 cM, was used as a framework map for the restricted MQM Mapping method. These markers were chosen on the basis of maximal information content and genome coverage. A data set of a *Bremia* disease test consisted of the average infection severity score per F_2 plant based on observations on four leaf discs.

The criterion for detecting a QTL was set by a significance level of 0.005 in the Kruskal–Wallis test. For the Restricted MQM method a LOD threshold of 3.7 was used (Van Ooijen 1999). In this paper the QTL mapping results are presented on the F_2 population with two adjusted scores, which were based on disease test results of corresponding F_3 lines.

Results

Bremia disease tests on the F_2 population

The two *Bremia* races NL14 and NL16 were used in disease tests. NL16 was chosen since it was the most common and complex race on lettuce in Europe. Race NL14 was chosen as a contrasting race. Race NL14 and NL16 are virulent on cultivars with *Dm1*, *Dm2*, *Dm3*, *Dm4*, *Dm5/8*, *Dm6*, *Dm10*, *Dm11*, *Dm13*, *Dm14* and *Dm16* (Table 1). NL14 and NL16 have no avirulence factors that interact with *Dm15*, *R17*, *R18*, *R36*, *R37* and *R38*

Table 1 Differential set of lettuce genotypes used to characterize two isolates of *B. lactucae* for virulence spectrum. "+" indicates a compatible/susceptible reaction. "–" indicates an incompatible/resistant reaction. [a]These lines were used as controls during maintenance and propagation of *Bremia* races

Cultivar/line	*Dm* gene/*R*-factor	*Bremia* NL14	*Bremia* NL16
Olof	None	+	+
Lednicky	1	+	+
UcDm2	2	+	+
Dandy	3	+	+
R4T57	4	+	+
Valmaine	5/8	+	+
Sabine	6	+	+
Reskia	3 and 7	–	+
UCDm10	10	+	+
Fila	2 and 11	+	+
Norden	3 and 11	+	+
Pennlake	13	+	+
UcDm14[a]	14	+	–
Spiky[a]	14	+	–
PIVT1309	15	–	–
Strada[a]	16	–	+
Luxor[a]	2 and 16	–	+
Mariska	18	–	–

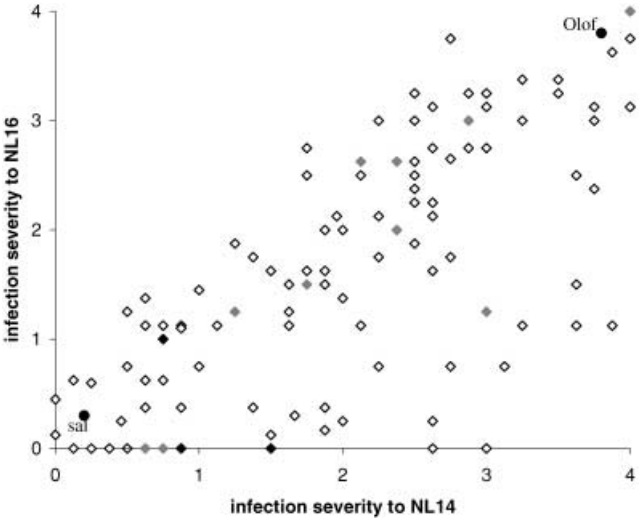


Fig. 1 Comparison of the F_2 disease tests against *Bremia* races NL14 and NL16. The infection severity scores (diamond symbol) are expressed as the average class number per F_2 plant based on observations on maximally eight leaf discs in two experiments. *L. sativa* Olof is the susceptible control (27 and 36 observations for NL14 and NL16) and *L. saligna* CGN 5271 is the resistant control (17 and 24 observations; black dot symbols). Infection severity scores: class 0: no sporulation, class 1: 1–25% of leaf disc area sporulates, class 2: 26–50%, class 3: 50–75% and class 4: 75–100%. The infection severity scores represented by two and three F_2 plants are shown by grey and black diamonds

(according to convention for *Bremia* resistance nomenclature; Van Ettekoven and Van der Arend 1999).

The F_2 population showed a wide and continuous range in infection severity scores from completely uninfected (scale value 0) to completely infected (scale value 4) in tests with both *Bremia* races. The susceptible parent *L. sativa* Olof showed an average infection severity score of 3.8 against NL14 and 3.8 against NL16. The resistant parent *L. saligna* CGN 5271 showed an average infection severity score of 0.2 against NL14 and 0.3 against NL16. If sporulation occurred on the *L. saligna* parent, it was only seen at the cutting edge of the leaf disc. The score on this resistant reference accession never increased more than 5% of the leaf area (class 1 on the disease class scale). The heritability was 0.86 in the first test and 0.80 for the second test with NL16 and in the two tests with NL14 0.82 and 0.74 respectively. These high heritabilities imply a high genetic variation and a small error in these disease tests on this F_2 population.

The Pearson correlation coefficient between two tests with the same race was 0.75 and 0.80 for NL14 and NL16 respectively. Similar results appeared for tests with two *Bremia* races. To present the results of the tests with different *Bremia* races, we averaged the infection severity scores of F_2 plants from two tests with the same race. In this way, the number of missing values of plant and/or leaf discs observations was minimized. Because of the high correlation between disease tests with the same race, it was not necessary to make corrections to standardize the two tests before averaging. The results of the tests with different *Bremia* races were compared for the detection of a possible race-specificity of the resistance (Fig. 1). Two trends were observed in this comparison. Firstly, the largest group of plants was scattered around the diagonal, indicating that their resistance level against NL14 and NL16 was very similar. Secondly, a small group of plants had a high NL16 resistance and low NL14 resistance, but an opposite group with a low resistance to NL16 and a high resistance to NL14 was not present. These results suggested mainly race non-specific effects, however also some plants with specific resistance to NL16.

QTL mapping

As a wide and continuous range of infection severity scores in F_2 plants was observed, the conclusion was drawn that the resistance was (at least partly) a quantitative trait. To dissect and map the underlying genes, we performed QTL mapping on each data set of each disease test separately. This revealed four QTLs in the F_2 population (Table 2). The disease test results of the F_2 population corresponded well with the detected QTLs, except for five F_2 plants. Their disease test results seemed not to agree well with the average disease test result of all F_2 plants with the same genotype at the specific locus of a detected QTL. To check whether the result of the disease test or the genotyping result for this F_2 plant was incorrect, we tested the F_3 line in a disease test with race NL16. F_3 seeds were available from three out of five F_2 plants. An ambiguous infection severity score of the F_2 parent was adjusted by the average of infection severity scores of the resistant F_3 plants if there was a difference of one unit (1.0) or more on the infection severity class scale (0–4) between the F_2

Table 2 Detected QTLS for *Bremia* resistance in an F_2 population from *L. saligna* × *L. sativa* QTLs were identified if the threshold of the Kruskal–Wallis test or the Restricted MQM mapping was exceeded. QTLs are presented per *Bremia* race from the disease test with the highest LOD score

QTL name	Chromosome number and QTL interval[f]	*Bremia* race	Kruskal–Wallis test[a] significance	Restricted MQM mapping fit dominance yes[b]		
				Peak LOD Score[d]	Exp%	Add
R39	9, 3 cM	NL16	xxxx	18[c]	54	2.1
RBQ1	7,59–65 cM	NL16	xxx[e]	3.8[e]	24[e]	0.9[e]
		NL14	xxxx	7.5	26	0.7
RBQ2	1,15–21 cM	NL16	x	3.6[c]	12	0.5
		NL14	x	3.1	13	0.5
RBQ3	9,32–41 cM	NL16	xxx	5.3	23	0.5
		NL14	xx	3.9[c]	12	0.3

[a] The Kruskal–Wallis test *P*-value = significance level. x = 0.005, threshold significance level, xx = 0.001, xxx = 0.0005, xxxx = 0.0001
[b] Exp% = proportion of the explained phenotypic variance. Add = additive effect of the resistance allele,
[c] Use of cofactors at LOD peaks,
[d] Italic numbers represent LOD values below threshold,
[e] These data were obtained by QTL mapping on 71 F_2 plants with susceptibility alleles for *R39*,
[f] This QTL interval covers a chromosome region harboring markers that show a Kruskal–Wallis test *P*-value > 0.005

Table 3 The infection severity scores of F_2 parents and F_3 lines based on the QTL allele composition. F_3 line-infection severity scores are estimated from maximally 72 observations (12 plants × 6 observations) and the F_2 parent resistance value is estimated from maximally eight observations (1 plant × 4 observations × 2 tests). QTL genotype: a = homozygous *L. sativa*, h = heterozyguous, b = homozygous *L. saligna*, c = b or h, d = a or h not distinguishable with dominant markers, u = unknown. * = average based on resistant plants only

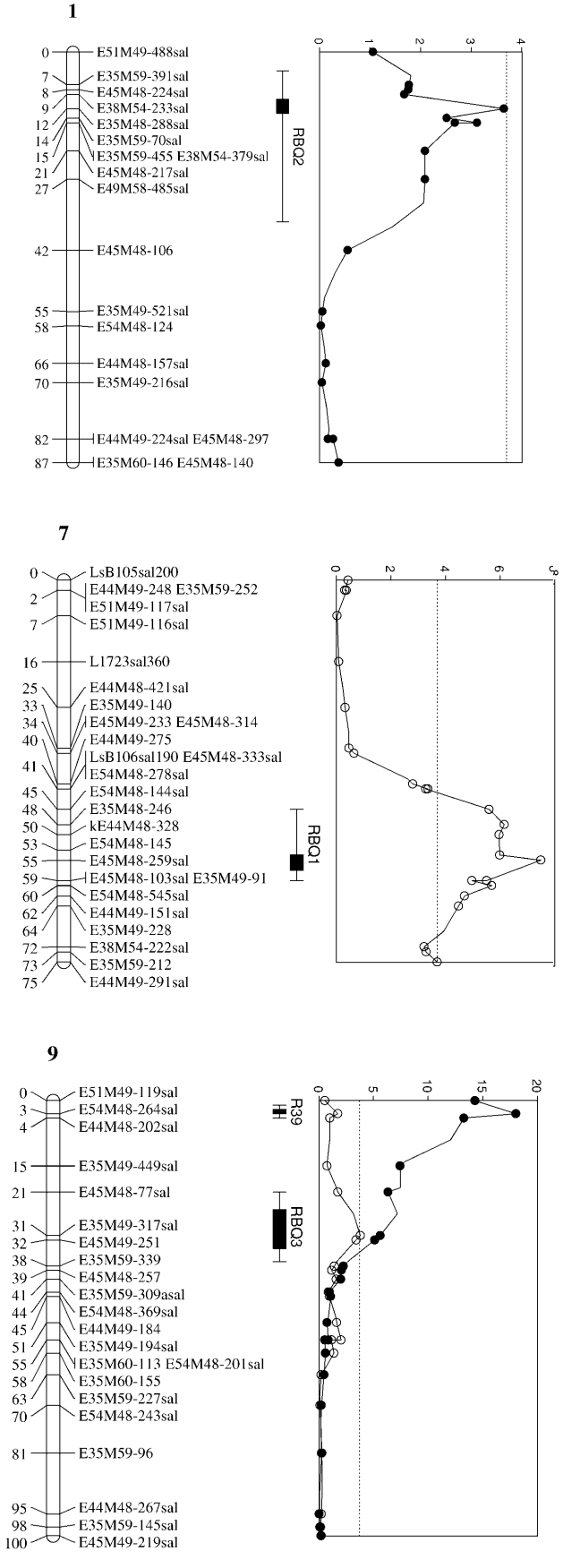
F_2 plant number, F_3 line number	QTL genotype of F_2				Average infection severity score against *Bremia* NL16			
	R39	*RBQ1*	*RBQ2*	*RBQ3*	Expected in F_2 plant based on additive effect (Table 2)	Observed in F_2 parent and expected for F_3 line	Observed in F_3 line	# Of tested F_3 plants
Experiment to check F_2 plants with ambiguous disease results								
61	h	a	a	a	3.8–2.1=1.7[e]	1.5	0*	11
123	c	a	a	h	3.8–2.1=1.7[e]	3.7	3.5	12
38	h	a	u	h	3.8–2.1=1.7[e]	2.5	0.4*	12
Experiment for verification of QTLs								
113	a	b	b	d	3.8–(2*0.9)–(2*0.5) = 1.0 or d = h then 1.0–0.5 = 0.5	1.0	0.5	12
34	a	a	h	a	3.8–0.5 = 3.3	2.0	1.8	11
90	a	a	h	a	3.8–0.5 = 3.3	3.3	2.7	12
35	a	a	a	a	3.8	4.0	2.4	7
107	a	a	b	d	3.8–(2*0.5) = 2.8 or d = h then 2.8–0.5 = 2.3	1.6	0.7	11
Controls						F_2 disease tests	F_3 disease tests	
L. sativa	a	a	a	a		3.8	3.9	
L. saligna	b	b	b	b		0.3	0.2	

[e] The average resistance level of 47 plants with one *R39* allele was 0.9 and for 71 plants with no *R39* allele it was 3.0. Therefore, 0.9 would be a more realistic expected resistance level than 1.7

and the F_3 data. Disease test results of two out of three F_2 plants (38 and 61) have been adjusted based on disease test results of corresponding F_3 lines (Table 3).

In general, the detected QTLs were identified as being significant in both tests with the same *Bremia* race. However, sometimes a QTL just below the significance level was observed, except for one QTL (*RBQ1)*, which was not detected in the second disease test with race NL16 (on 10 week-old plants).

The resistance against *Bremia* NL16 was mainly explained by one QTL on the top of Chromosome 9 with a LOD score of 18 and an explained variance of 54% (Table 2 and Fig. 2). This QTL was not identified with the *Bremia* disease test data sets of race NL14.



The top of Chromosome 9 has a distorted segregation ratio with an excess of *L. sativa* alleles (Jeuken et al. 2001). At the QTL locus, 71 plants were homozygous for the *L. sativa* alleles, 47 plants were heterozygous and no plants were homozygous for the *L. saligna* alleles. For eight plants it was not possible to distinguish whether they were heterozygous or homozygous for *L. saligna* alleles because the informative AFLP marker, linked to the QTL, was not scored codominantly. The plants, that were homozygous for the *L. sativa* alleles of the QTL locus, had an average infection severity score of 3.0 (based on first NL16 disease test data), while the plants heterozygous at this locus showed an average infection severity score of 0.9 (based on first NL16 disease test data). The effect of one allele of this gene was therefore high as it decreased infection severity by 2.1 units on the infection severity scale. This large effect and its race-specific nature, suggested that this "QTL" represents a qualitative race-specific resistance gene similar to *Dm* genes. At the locus of this gene no *Bremia* resistance gene has been mapped before (see Discussion). We propose to designate this new gene *R39*, which follows the previously cited *R38* gene (Van Ettekoven and van der Arend 1999).

The three other QTLs had smaller effects on the infection severity level against both races and were designated *RBQ1*, *RBQ2* and *RBQ3* (*R*esistance to *B*remia *Q*TL) (Table 2). *RBQ1* gave resistance against NL14 and also showed resistance against NL16 with a LOD value of 3.6 just below the threshold (3.7). Because *R39* explained a large part of the resistance to NL16, we selected 71 F_2 plants with susceptible alleles for *R39* and repeated QTL mapping. Now *RBQ1* was identified as being significant (Table 2). *RBQ2* was identified against NL14 and NL16 with the Kruskal–Wallis test, but its LOD score in the Restricted MQM Mapping was just below the significance level. *RBQ3* was positioned approximately 28 cM away from the *R39* locus. This made it difficult in the NL16 data sets to distinguish it from *R39* and to define it as a separate QTL. An extra peak in the LOD profile was observed with a LOD score of 5 (Fig. 2).

Verification of QTLs by five F_3 lines

We performed a disease test on F_3 lines to verify QTLs detected in the F_2 population. Therefore, we used marker-

Fig. 2 The position of QTLs for *Bremia* resistance are shown beside the marker linkage maps of chromosomes 1, 7 and 9, which are derived from the F_2 mapping population *L. saligna* × *L. sativa* (Jeuken et al. 2001). Map positions are given in cM. Bars indicate the QTL interval in which the inner bar shows a one LOD support confidence interval and the outer bar shows a two LOD support confidence interval. Graphs near the chromosomes show the QTL likelihood profile based on restricted MQM mapping with the use of cofactors. Lines with solid circles are based on results of a disease test with *Bremia* race NL16. Lines with open circles are based on results of a disease test with *Bremia* race NL14. The LOD threshold value of 3.7 is shown as a dotted line

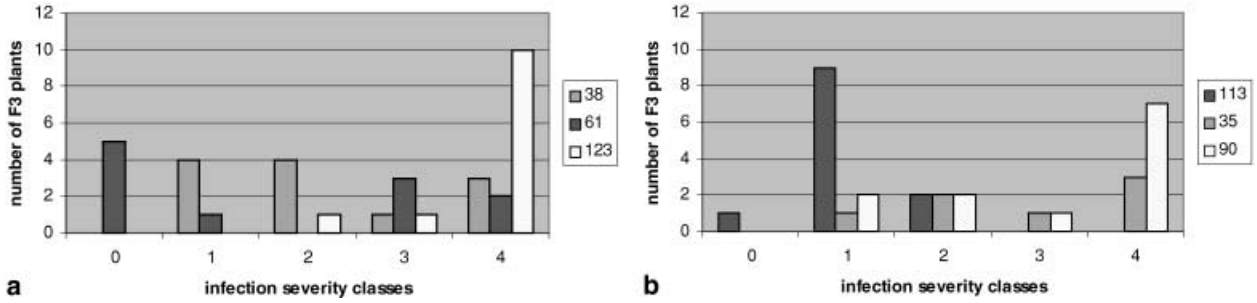


Fig. 3 Frequency distribution of F_3 lines according to infection severity classes against *Bremia* NL16. Plants, scored in classes from 0 to 4, are interpreted as in a range from completely resistant to completely susceptible plants. For explanation see legends Table 3

assisted selection to select for F_2 plants with none, one or two QTLs preferably in a homozygous state. The resistance alleles of a QTL were considered to be present if all scored markers in the QTL interval (Table 2) supported the homozygous *L. saligna* state of that locus. Similarly, susceptibility alleles were selected by markers in the homozygous *L. sativa* state in this interval. In total five F_3 lines, consisting of at least seven plants, were used for the *Bremia* disease test with race NL16 for QTL verification. Four F_3 lines were selected for presence of QTLs based on flanking markers and they indeed showed resistance levels (Table 3, Fig. 3b). Segregation of resistance was observed in F_3 lines from F_2 plants with a heterozygous QTL, like plants 34 and 90. Remarkably, F_3 line 35 was selected for susceptibility and lack of QTLs, but still three out of seven plants were intermediate resistant with an infection severity score of 1 to 2.

In conclusion, resistant plants can be selected on the genotype level by marker-assisted selection.

Discussion

Disease tests on F_2 population

Based on the fact that the variance in observations on the four leaf discs per F_2 plant was low and the heritability high, we conclude that the disease tests were accurate. Furthermore, the majority of the disease test results of the F_3 lines were in agreement with the F_2 parent infection severity scores, indicating a good repeatability. With these four data sets of infection severity scores of F_2 plants, the same QTLs were found in all performed tests (of MapQTL 4.0), which confirms the reliable detection of QTLs.

A new race-specific resistance gene *R39*

From QTL mapping on data sets of disease tests with NL16, it was shown that *R39* on Chromosome 9 explained most of the resistance. In the F_2 population one allele of *R39* already had a very large effect on the infection severity score of 2.1 units. In F_3 line 61 one allele of *R39* even explained an average infection severity difference of 3.0 units between the groups of susceptible and resistant F_3 plants. This almost equals the difference between *L. sativa* Olof and *L. saligna*. The large effect and the race-specificity of *R39* suggests that it is a qualitative gene (*Dm* gene), which interacts with *Bremia* in a gene-for-gene relationship based on the hypersensitive response. Evidence for the hypersensitive response was found in a histological study in which *L. saligna* 5271 showed necroses upon inoculation with NL16 (Sedlarova et al. 1999). Resistance to race NL16 and susceptibility for NL14 is only observed for the known resistance gene *Dm*14 (Table 1). However, *R39* is probably not *Dm*14 because in another histological study it was shown that the resistance of *L. saligna* CGN 5271 (harbouring *R39*) against race NL5 (avirulent on *Dm*14) was **without** necrosis (Lebeda and Reinink 1994). In addition, *Dm*14 was mapped by using the Calmar × Kordaat map on Chromosome 2 in a resistance cluster with *Dm*3 (Kesseli et al. 1994). This was not consistent with the map position of *R39* on Chromosome 9 at 3 cM. *R39* was also not mapped on the positions of three other known *Dm* gene resistance clusters (Kesseli et al. 1994). More information on the resistance spectrum of *R39* will be obtained by disease tests with a differential set of *Bremia* races.

The result of a *Dm*-like gene was not completely unexpected as in histological studies the observation of necroses formation on *L. saligna* accessions after *Bremia* inoculation could have been a consequence of a hypersensitive response of a potential *Dm* gene (Lebeda and Reinink 1994). Associations between hypersensitive responses and non-host resistances were seen for other oomycetes like *Phytophthora infestans* (Vleeshouwers et al. 2000) and other pathogens like *Cladosporium fulvum* (Laugé et al. 2000). Though even if *R39* is associated with the hypersensitive response, this does not proof that *R39* contributes to the non-host resistance of *L. saligna*. Also it is not clear why *Bremia*, after the speciation of a common *Lactuca* ancestor into the *L. serriola/L. sativa* cluster and the *L. saligna* cluster, still contained virulence genes that could break through *R39* in *L. saligna*, while *L. saligna* is a non-host for *Bremia*.

Three QTLs for Bremia resistance

The three detected QTLs (*RBQ1, RBQ2* and *RBQ3*) all were effective against both races. The detection of these QTLs from tests with race NL16 was sometimes less sensitive compared to tests with NL14. This was due to the

nearly complete resistance of *R39* to NL16, which in fact reduced the population size for QTL mapping to the number of F_2 plants without *R39*. However, *RBQ2* and *RBQ3* were still detected using data of all 126 F_2 plants. *RBQ1* was detected when QTL mapping was performed on all the 71 F_2 plants that were homozygous for the susceptibility alleles of *R39*. From the three identified QTLs, *RBQ1* had the largest effect. The three QTLs together explained 51% of the quantitative resistance to NL14. As the calculated environmental variance was low this meant that probably not all QTLs for resistance have been detected yet. The major part of the unexplained variance may be due to minor QTLs that did not reach the detection level or too strong epistatic interactions, which cannot be detected by the QTL mapping methods used. Another factor, which may have hampered the detection of QTLs, could have been the presence of local extreme distorted segregation ratios against wild parent alleles in the F_2 population. The possibility of undetected QTLs in the F_2 population was supported by the results of the expected susceptible F_3 line 35, in which some plants occurred with unexpectedly low infection severity scores.

We assumed that this quantitative resistance, which implied non-race specificity and was explained partly by these three QTLs, could be the main reason for the non-host status of *L. saligna* CGN 5271.

General conclusion

We have been able to perform a first molecular genetical analysis of the resistance of *L. saligna* to *Bremia*. It was concluded that the dissection of the resistance has been successful as four loci were detected that harbor genes for *Bremia* resistance. This study revealed that the resistance of *L. saligna* seemed to be explained by a combination of a qualitative race-specific resistance and a quantitative resistance, which was a race non-specific, proposed non-host resistance. From the breeders' perspective the localization of a quantitative resistance was exiting, as it seemed an alternative resistance to *Bremia* that is non-race specific and more durable than *Dm* genes. Molecular markers flanking the QTLs will facilitate the breeding for this quantitative resistance.

Acknowledgements We thank P. Stam and R.E. Niks for critically reading the manuscript and their advisory support in this study. We also thank Nickerson Zwaen BV for providing the F_2 seeds.

References

Bonnier FJM, Reinink K, Groenwold R (1992) New sources of major gene resistance in *Lactuca* to *Bremia lactucae*. Euphytica 61:203–211

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

Crute IR (1992) From breeding to cloning (and back again?): a case study with lettuce downy mildew. Annu Rev Phytopathol 30:485–506

Crute IR, Johnson AG (1976) The genetic relationship between races of *Bremia lactucae* and cultivars of *Lactuca sativa*. Ann Appl Biol 89:476–474

Eenink AH (1981) Partial resistance in lettuce downy mildew (*Bremia lactucae*). 1. Search for partially resistant genotypes and the influence of certain plant characters and environments on the resistance level. Euphytica 30:619–628

Eenink AH, de Jong CJ (1982) Partial resistance in lettuce downy mildew (*Bremia lactucae*). 3. Correspondence between resistance levels of cotyledons and leaf discs and resistance of adult plants. Euphytica 31:761–770

Eenink AH, Bijker W, den Toom A (1982) Partial resistance in lettuce downy mildew (*Bremia lactucae*). 2. Differential interactions between plant genotypes and fungus races and the relationship of latent period, infection frequency and number of infected leaves with the level of partial resistance. Euphytica 31:73–83

Eenink AH, Groenwold R, Bijker W (1983) Partial resistance in lettuce downy mildew (*Bremia lactucae*). 4. Resistance after natural, semi-artificial and artificial infestation and examples of mutual interference of resistance levels. Euphytica 32:139–149

Gustafsson I (1989) Potential sources of resistance to lettuce downy mildew (*Bremia lactucae*) in different *Lactuca* species. Euphytica 40:227–232

Heath MC (2001) Non-host resistance to plant pathogens: nonspecific defense or the result of specific recognition events? Physiol Mol Plant Pathol 58:53–54

Jeuken M, van Wijk R, Peleman P, Lindhout P (2001) An integrated interspecific AFLP map of lettuce (*Lactuca*) based on two *L. sativa* ×*L. saligna* F_2 populations. Theor Appl Genet 103:638–647

Kamoun S (2001) Nonhost resistance to *Phytophthora*: novel prospects for a classical problem. Curr Opin Plant Biol 4:295–300

Kesseli RV, Paran I, Michelmore RW (1994) Analysis of a detailed genetic linkage map of *Lactuca sativa* (Lettuce) constructed from RFLP and RADP markers Genetics 136:1434–1446

LaugéR, Goodwin PH, de Wit PJGM, Joosten MHA (2000) Specific HR-associated recognition of secreted proteins from *Cladosporium fulvum* occurs in both host and non-host plants, Plant J 23:735–745

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

Lebeda A, Boukema IW (1991) Further investigation of the specificity of interactions between wild *Lactuca* spp. and *Bremia lactucae* isolates from *Lactuca serriola*. J of Phyt 133:57–64

Lebeda A, Pink DAC (1998) Histological aspects of the response of wild *Lactuca* spp. and their hybrids, with *L. sativa* to lettuce downy mildew (*Bremia lactucae*). Plant Pathol 47:723–736

Lebeda A, Reinink K (1991) Variation in the early development of *Bremia lactucae* on lettuce cultivars with different levels of field resistance. Plant Pathol 40:232–237

Lebeda A, Reinink K (1994) Histological characterisation of resistance in *Lactuca saligna* to lettuce downy mildew (*Bremia lactucae*), Physiol Mol Plant Pathol 44:125–139

Lebeda A, Schwinn FJ (1994) The downy mildews – an overview of recent research progress. J Plant Dis Protec 101:225–254

Lebeda A, Syrovatko P (1988) Specificity of *Bremia lactucae* Isolates from *Lactuca sativa* and some Asteraceae plants. Acta Phytopathol Entomol Hungarica 23:39–48

McDonald BA, Linde C (2002) Pathogen population genetics and the durability of disease resistance. Euphytica (in press)

Norwood JM, Crute IR, Lebeda A (1981) The location and characteristics of novel sources of resistance to *Bremia lactucae* Regel (downy mildew) in wild *Lactuca* species. Euphytica 30:659–668

Reinink K (1999) Lettuce resistance breeding. In: Proc EUCARPIA Meeting on Leafy Vegetables, Olomouc Czech Republic 1999, pp 139–147

Sedlarova M, Lebeda A, Binarova P (1999) Reorganization of the microtubular cytoskeleton in *Lactuca* spp. cells during early stages of infection by lettuce downy mildew (*Bremia lactucae*). In: Proc EUCARPIA Meeting on Leafy Vegetables, Olomouc Czech Republic 1999, pp 199–204

Van Ettekoven K, van der Arend AJM (1999) Identification and denomination of “new” races of *Bremia lactucae*. In: Proc EUCARPIA Meeting on Leafy Vegetables, Olomouc Czech Republic 1999, pp 171–181

Van Ooyen JW (1999) LOD significance thresholds for QTL analysis in experimental populations of diploid species. Heredity 83:613–624

Van Ooyen JW, Maliepaard C (1996) MapQTL version 4.0: software for the calculation of QTL positions on genetic maps. CPRO-DLO, Wageningen, The Netherlands

Vleeshouwers VGAA, van Dooijeweert W, Govers F, Kamoun S, Colon LT (2000) The hypersensitive response is associated with host and nonhost resistance to *Phytophthora infestans*. Planta 210:853–864