Plant Pathology (2014) **63**, 344–353 Doi: 10.1111/ppa.12087

Evaluation and QTL mapping of resistance to powdery mildew in lettuce

I. Simko*, G. Rauscher†, R. G. Sideman‡, J. D. McCreight and R. J. Hayes

United States Department of Agriculture, Agricultural Research Service, U.S. Agricultural Research Station, 1636 E. Alisal St, Salinas, CA, 93905, USA

Lettuce (*Lactuca sativa*) is the major leafy vegetable that is susceptible to powdery mildew disease under greenhouse and field conditions. Quantitative trait loci (QTLs) for resistance to powdery mildew under greenhouse conditions were mapped in an interspecific population derived from a cross between susceptible *L. sativa* cultivar Salinas and the highly susceptible *L. serriola* accession UC96US23. Four significant QTLs were detected on linkage groups LG 1 (*pm-1.1*), LG 2 (*pm-2.1* and *pm-2.2*) and LG 7 (*pm-7.1*), each explaining between 35 to 42% of the phenotypic variation. The four QTLs are not located in the documented hotspots of lettuce resistance genes. Alleles for the disease resistance at the four QTLs originated from both parents (two from each), demonstrating that even highly susceptible accessions may provide alleles for resistance to powdery mildew. These QTLs appeared to operate during limited periods of time. Results of the field trials with $F_{2:3}$ and $F_{3:4}$ families derived from a Soraya (moderately resistant) × Salinas cross demonstrated effective transfer of resistance to powdery mildew in this material. An integrated rating approach was used to estimate relative levels of resistance in 80 cultivars and accessions tested in a total of 23 field and greenhouse experiments. Generally, very low resistance was observed in crisphead-type lettuces, while the highest relative resistance was recorded in leaf and butterhead types. Comparison of two disease assessment methods (percentage rating and the area under the disease progress steps, AUDPS) for detection of QTLs shows that the two approaches complement each other.

Keywords: aggregate-ranking, area under the disease progress steps, field resistance, *Golovinomyces cichoracearum*, greenhouse resistance, *Lactuca sativa*

Introduction

Powdery mildew of lettuce is caused by the fungus *Golovinomyces cichoracearum sensu stricto* (formerly *Erysiphe cichoracearum*; Braun, 1987; Lebeda & Mieslerová, 2011). The disease is usually more severe on plants grown in warm climates or under greenhouse conditions in the absence of standing water on the leaf surface. The fungus may develop on both leaf surfaces, producing white, powdery spores. Affected leaves become slightly yellow, then brown, and eventually die, resulting in a lower quality of product. Heavily infected plants grow slower and produce lower yield.

Most lettuce cultivars are susceptible to powdery mildew (Dixon, 1981; Lebeda *et al.*, 2007, 2012; Lebeda & Mieslerová, 2011). Monogenic dominant resistance to the disease was reported in crisphead cultivar Imperial 850 (Whitaker & Pryor, 1941). Resistance and moderate resistance were reported in some butterhead cultivars (Lebeda, 1985b) and several wild species (Lebeda, 1985a, 1994). Of the nine wild *Lactuca* species (*L. aculeata*, *L. dentata*, *L. perennis*, *L. saligna*, *L. serriola*, *L. tatarica*, *L. tenerrima*, *L. viminea* and *L. virosa*) that were tested for natural infection with *G. cichoracearum*, *L. serriola* was the most susceptible. The accessions of *L. saligna* showed variable levels of resistance, whilst the lowest levels of infection were observed on *L. virosa*, *L. viminea*, *L. tenerrima* and *L. tatarica* (Lebeda, 1985a, 1994). Results of studies with *Lactuca* species show the presence of different physiological races of powdery mildew, and race-specific interaction between the pathogen and accessions of *L. sativa*, *L. serriola* and *L. saligna* (Lebeda & Mieslerová, 2011; Lebeda *et al.*, 2012). However, development of the system for classification of pathogen races has started only recently (Lebeda & Mieslerová, 2011; Lebeda *et al.*, 2012).

Little is known about the genetics of lettuce resistance to powdery mildew. The objectives of the present study were to: (i) determine the genetic architecture of resistance in lettuce grown under greenhouse conditions, (ii) investigate inheritance of lettuce resistance to powdery mildew in field conditions, (iii) identify cultivars with overall high levels of resistance to natural infection of powdery mildew, and (iv) compare disease assessment

*E-mail: ivan.simko@ars.usda.gov
†Present address: Agricultural Biotechnology, DuPont Pioneer, Wilmington, DE 19880, USA
‡Present address: Department of Biological Sciences, University of New Hampshire, Durham, NH 03824, USA

Published online 28 June 2013

Plant Pathology

methods (percentage rating and the area under the disease progress steps, AUDPS) for detection of quantitative trait loci (QTLs).

Materials and methods

Plant material

The interspecific mapping population of Salinas × UC96US23 (Johnson *et al.*, 2000) was selected for the study because the parents differ in their reaction to the pathogen. In preliminary experiments, *L. serriola* accession UC96US23 was significantly more susceptible to the disease than crisphead-type cultivar Salinas. The mapping population was genotyped with amplified fragment length polymorphism markers (AFLPs), single nucleotide polymorphism markers (SNPs; Truco *et al.*, 2007) and simple-sequence repeat markers (SSRs; Simko, 2009; Rauscher & Simko, 2013). The AFLPs, SNPs and SSRs were combined into an integrated linkage map with similar distributions of markers across all linkage groups. Ninety F_8 recombinant inbred lines (RILs) from the mapping population plus the parents were assessed for their reaction to powdery mildew infection under greenhouse conditions.

In a greenhouse experiment with natural infection, 25 F_2 plants from a cross between butterhead cv. Soraya and crisphead cv. Salinas were categorized as resistant (limited pathogen sporulation) or susceptible (extensive pathogen sporulation). Three susceptible and seven resistant F_2 plants were subsequently used to produce $F_{2:3}$ seed lots. Using these 10 $F_{2:3}$ families, an additional 54 randomly selected $F_{3:4}$ families were produced.

Eighty lettuce accessions from the six horticultural types (butterhead, crisphead, leaf, oil, romaine and stem) and wild *L. serriola* species were screened for their reactions to powdery mildew in separate greenhouse experiments and field trials.

Assessment of resistance to powdery mildew in greenhouse experiments

Two experiments with the Salinas × UC96US23 mapping population were planted in December and May in randomized block designs, with three blocks per experiment. Each block contained six plants of each RIL and the two parents; totalling 1656 plants per experiment. Seeds of RILs and parents were seeded into 10 × 10 × 10 cm containers and grown in a greenhouse under ambient light conditions. Thirty day-old plants were exposed to naturally occurring powdery mildew. The sources of inoculum were infected plants of Salinas and UC96US23 that were planted in a separate greenhouse at an earlier date. These infected plants were evenly distributed throughout the experiment to ensure consistent distribution of inoculum over tested material.

Twelve days after exposure (DAE) to *G. cichoracearum* the RILs were evaluated for the percentage of leaf area covered by powdery mildew (Fig. 1). Percentage ratings were based on a visual guide that was developed using Assess v. 1.0 (American Phytopathological Society Press) image analysis software for measuring disease lesions. Evaluations of disease progress continued until the first RIL (or a parent) exceeded the average disease score of 80%. In experiment 1, evaluations were performed at 12, 20, 23, 26, 30, 35 and 42 DAE; in experiment 2, evaluations were performed at 12, 14, 21, 29, 35, 42, 49, 61 and 67 DAE. For each experiment the percentage data from individual evaluations were also combined into a single value that combined disease progress from the day of exposure (DAE of 0) until the most recent evaluation. This value, termed the area under the disease progress steps (AUDPS), was calculated according to Simko & Piepho (2012). The AUDPS approach improves the estimation of disease progress compared to the area under the disease progress curve (AUDPC) by giving a weight closer to optimal to the first and last observations.

Figure 1 Percentage ratings of leaf area covered by powdery mildew. Calculations were performed with Assess v. 1.0 software. Photographs of infected leaves were then converted into a visual guide that was used for evaluation of disease progress. Leaves are approximately 10 cm long.

Parents of the mapping population differ in their growth rate and the position of leaves on plants. UC96US23 grows more slowly with leaves mostly in a prostrate position, whereas Salinas grows rapidly with leaves in a more upright position. To evaluate the effect of plant growth rate and habit on resistance to powdery mildew, both the plant phenological stage and the leaf position were assessed on the mapping population. Plant phenological stage was evaluated, after exposure to powdery mildew, three times in weekly intervals using the scale of Jenni & Bourgeois (2008). Leaf position was evaluated twice on the scale from 1 (horizontal leaf position) to 3 (vertical leaf position).

Separate experiments with lettuce accessions were designed to evaluate resistance to the disease. These plants were evaluated only once when *c.* 80–100% of the plant leaf area of the most susceptible accession was covered with powdery mildew. The reaction of accessions to powdery mildew was recorded either as percentage rating of the infected leaf area, or on a 0–5 categorical scale (Table 1).

Assessment of resistance to powdery mildew in field trials

In 2004 (year 1) and 2005 (year 2) field experiments were conducted in Yuma, Arizona. Crop cultivation was done according to standard commercial practices for the area. Plant dates were 9 November 2004 and 17 November 2005 with evaluation dates on 14 March 2005 and 9 March 2006, respectively. The crops were naturally infected with powdery mildew, which is common in lettuce not treated with fungicides during this growing part of the season in Yuma. Resistance was evaluated at harvest maturity using a 0–4 rating scale (Table 1) modified from Turini (2003). In year 1, ten $F_{2:3}$ families derived from a cross between butterhead cv. Soraya and crisphead cv. Salinas were tested in a completely randomized design together with parental lines and 61 additional accessions. Five of the families, the parental lines and 27 accessions were evaluated in two replications, while the remaining five families and 34 accessions were not replicated due to scarcity of seeds. In year 2, 54 randomly selected $F_{3:4}$ families that were derived from the ten $F_{2:3}$ families, the parental lines and 51 accessions were tested in a randomized complete block

Table 1 Visual rating scale for evaluating reaction of lettuce to powdery mildew infection in greenhouse and field experiments

Rating	Visual observation in greenhouse
0	No powdery mildew colonies, whole leaf area is green
1	1–5 colonies of light grey colour
2	6–20 colonies of mostly grey colour
3	>20 colonies of mostly white colour with grey background
4	Most of the leaf area is covered with white colonies, few leaf areas are green
5	Almost whole leaf is covered with white colonies

Rating	Visual observation in field[a]
0	No powdery mildew present on plant
1	Powdery mildew present on lower wrapper leaves or lower leaves only
2	Powdery mildew present on lower wrapper leaves or lower leaves and on upper wrapper leaves or middle leaves
3	Powdery mildew present on lower wrapper leaves, or lower leaves and on upper wrapper leaves, or middle leaves and on cap leaf or upper leaves
4	Extensive powdery mildew present on the entire plant

[a]Rating scale for visual observation in field was modified from Turini (2003).

design with three replications. The average score was calculated from $F_{3:4}$ families that were derived from the same $F_{2:3}$ family to compare results from 2 years.

Linkage mapping

Disease scores were averaged across all blocks within experiments ($3 \times 6 = 18$ plants per RIL or parent) prior to QTL analyses. For phenological data, modal values for each RIL (or parents) were used in QTL mapping and statistical analyses. Two hundred and ninety-three markers were selected from a pool of available mapped molecular markers in the RIL population. AFLP and SNP data were downloaded on 1 April 2010 from the Compositae Genome Project database (http://compgenomics.ucdavis.edu); SSR markers had been previously developed within the authors' laboratory (Simko, 2009; Rauscher & Simko, 2013). The selected AFLP, SNP and SSR markers were distributed across all nine linkage groups with an average distance of 7·1 cM between two adjacent markers.

QTLs were mapped using the composite interval mapping (CIM) feature of the QGene v. 4.3.9 software (Joehanes & Nelson, 2008). The forward-cofactor selection option was applied for automatic selection of cofactors. Significance thresholds for the LOD (logarithm of the odds) scores were determined for each evaluation through resampling with 1000 iterations. Calculated significance thresholds for individual evaluations were in the range from LOD 5·4 to LOD 5·7 at genomewide $\alpha_{0.05}$ and from LOD 6·9 to LOD 7·2 at genomewide $\alpha_{0.01}$. To limit detection of false positive QTLs a conservative approach was applied that requires that a QTL had to be detected in at least two different evaluation dates of the same experiment to be declared significant.

Computation of integrated ratings for the germplasm evaluation

Evaluation of disease resistance on 80 accessions was performed in 23 greenhouse experiments and field trials. Reactions to the pathogen in the greenhouse were recorded either as percentage of plant leaf area covered by powdery mildew or using a 0–5 categorical scale (Table 1), whereas under field conditions, reaction to powdery mildew infection was recorded using a 0–4 scale (Table 1). Because only a subset of all accessions was evaluated in each experiment, the rank-aggregation approach (Simko & Piepho, 2011; Simko *et al.*, 2012) was used to combine results from different rating scales into a single value. Rank-aggregating calculations were carried out using the Bradley–Terry model for paired comparisons. The model for a pair of accessions is:

$$P(\text{accession } i \text{ outperforms accession } j) = \pi_i/(\pi_i + \pi_j),$$

where π_i and π_j are non-negative parameters that represent the performance of accessions i and j from all paired comparisons of the two accessions (Simko & Pechenick, 2010). The rank-aggregating method produces a rating of X for each accession on a latent scale. Values from the latent scale were normalized as: $z = (X - \bar{X})/s$, where X is the rating value to be normalized, $\bar{X}$ is the arithmetic mean of all rating values, and s is the standard deviation of all rating values. Higher values of z-score indicate higher susceptibility to the disease.

Statistical analysis

The normality of data distribution in greenhouse experiments was evaluated with the Shapiro–Wilk goodness-of-fit test. Differences between disease ratings of the two parents of the mapping population were compared by the Student's t-test. Pearson correlation coefficient was applied to measure the relationship between field trials in different years. Disease severity means from field experiments with Soraya × Salinas progeny that were resistant and susceptible in greenhouse tests were compared using t-tests. Dunnett's multiple comparison test was used to compare the disease score of the control cv. Salinas with those observed for $F_{2:3}$ and $F_{3:4}$ families and Soraya. Differences among horticultural types of lettuce were evaluated by analysis of variance (ANOVA) and comparison for all pairs of types was performed using the Tukey–Kramer HSD test. All statistical analyses were performed with computer software JMP v. 6.0.3 (SAS Institute).

Results

Powdery mildew lesions were visible on RILs in greenhouse experiment 1 at the first evaluation (12 DAE) when the average disease score was 7·6% (Fig. 2). The disease gradually progressed and reached the average value of 48·7% 42 DAE. Experiment 2 showed a different pattern of disease progress. There was very little disease visible by 42 DAE, when the average disease score was only 4·7%. Therefore plants were exposed to *G. cichoracearum* again. The new sources of inoculum were plants of Salinas and UC96US23 infected with powdery mildew. After this second exposure, the disease progressed faster and reached the average value of 35·8% at 67 DAE.

Distributions of disease scores at the end of both experiments (Fig. 3) were not significantly different from normal distribution as indicated by the Shapiro–Wilk W

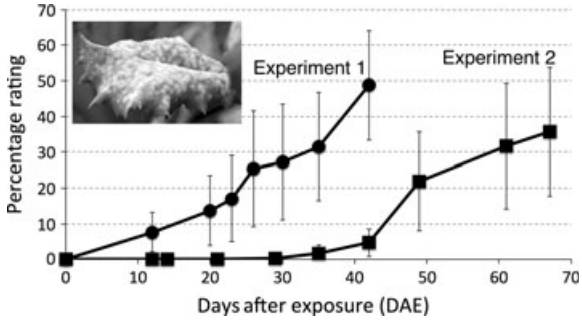

Figure 2 Disease progress in greenhouse experiments 1 and 2. The average percentage ratings were calculated from evaluations of the Salinas × UC96US23 mapping population. Vertical lines represent standard deviations of the mean values.

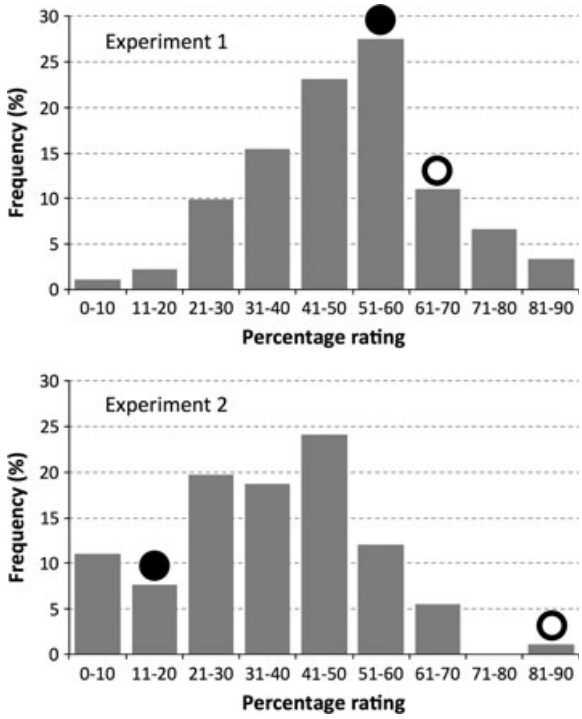

Figure 3 Distribution of disease percentage ratings in the Salinas × UC96US23 mapping population at the end of greenhouse experiments 1 (42 days after exposure, top panel) and 2 (67 days after exposure, bottom panel). Powdery mildew was evaluated on 90 RILs of the mapping population. Closed circles show values for cv. Salinas, while open circles show values for accession UC96US23.

test ($P = 0{\cdot}925$ for experiment 1; $P = 0{\cdot}575$ for experiment 2). In experiment 1 the difference between disease scores of the two parents was not significant (percentage rating for Salinas = 53%, and for UC96US23 = 68%, $P = 0{\cdot}069$). In experiment 2, UC96US23 (percentage rating = 85%) was significantly more susceptible ($P < 0{\cdot}0001$) to powdery mildew than Salinas (percentage rating = 17%).

Table 2 Logarithm of the odds (LOD) scores for significant quantitative trait loci (QTLs) for resistance to powdery mildew detected at different days after exposure (DAE) based on percentage ratings and area under the disease progress steps (AUDPS) values

			Percentage ratings		AUDPS	
QTL[a]	Experiment	DAE[b]	LOD	R^2%[c]	LOD	R^2%
pm-1.1	1	23	5·5	23	6·4	29
	1	26	8·2	35	6·3	28
	1	30	6·4	28	6·2	28
	1	35	5·6	26	7·4	32
	1	42	n.s.[d]	n.s.	6·0	27
pm-2.1	2	61	6·3	28	n.s.	n.s.
	2	67	11·0	42	9·1	38
pm-2.2	1	26	6·6	30	n.s.	n.s.
	1	30	5·9	27	6·5	29
	1	35	10·0	41	6·7	30
	1	42	6·5	29	7·2	31
	2	42	9·5	39	5·9	27
	2	49	n.s.	n.s.	8·6	36
	2	61	n.s.	n.s.	7·0	31
pm-7.1	2	61	n.s.	n.s.	8·4	36
	2	67	n.s.	n.s.	6·3	28

[a]Location of four QTLs on the molecular linkage map is shown in Figure 4.

[b]In experiment 1, evaluations were performed at 12, 20, 23, 26, 30, 35, and 42 DAE. In experiment 2, evaluations were performed at 12, 14, 21, 29, 35, 42, 49, 61, and 67 DAE.

[c]R^2%: percentage of phenotypic variation explained by the QTL. Additive effects of alleles are shown in Figure 5.

[d]n.s.: LOD score was not significant at genomewide $\alpha_{0{\cdot}05}$.

Location of QTLs, origin of the resistance alleles and their stability

Using the CIM method combined with a conservative approach that required that a QTL had to be detected at least twice in one of the experiments to be declared significant, a total of four QTLs were detected (Table 2). One QTL was located on LG 1, two QTLs on LG 2, and one QTL on LG 7 (Fig. 4). The QTL on LG 1 (*pm-1.1*) was detected in experiment 1 only, two QTLs on LG 2 (*pm-2.1*) and LG 7 (*pm-7.1*) were detected in experiment 2 only, and one QTL on LG 2 (*pm-2.2*) was detected in both experiments. Three out of the four QTLs were detected by both disease assessment methods (percentage rating and AUDPS). The QTL on LG 7 (*pm-7.1*) was significant only when AUDPS scores were used for calculations (Fig. 5). The largest LOD score and the highest percentage of phenotypic variation (R^2) explained by the QTL was detected for *pm-1.1* in experiment 1 at 26 DAE by percentage rating (LOD = 8·2, R^2 = 35%), *pm-2.1* in experiment 2 at 67 DAE by percentage rating (LOD = 11·0, R^2 = 42%), *pm-2.2* in experiment 1 at 35 DAE by percentage rating (LOD = 10·0, R^2 = 41%) and

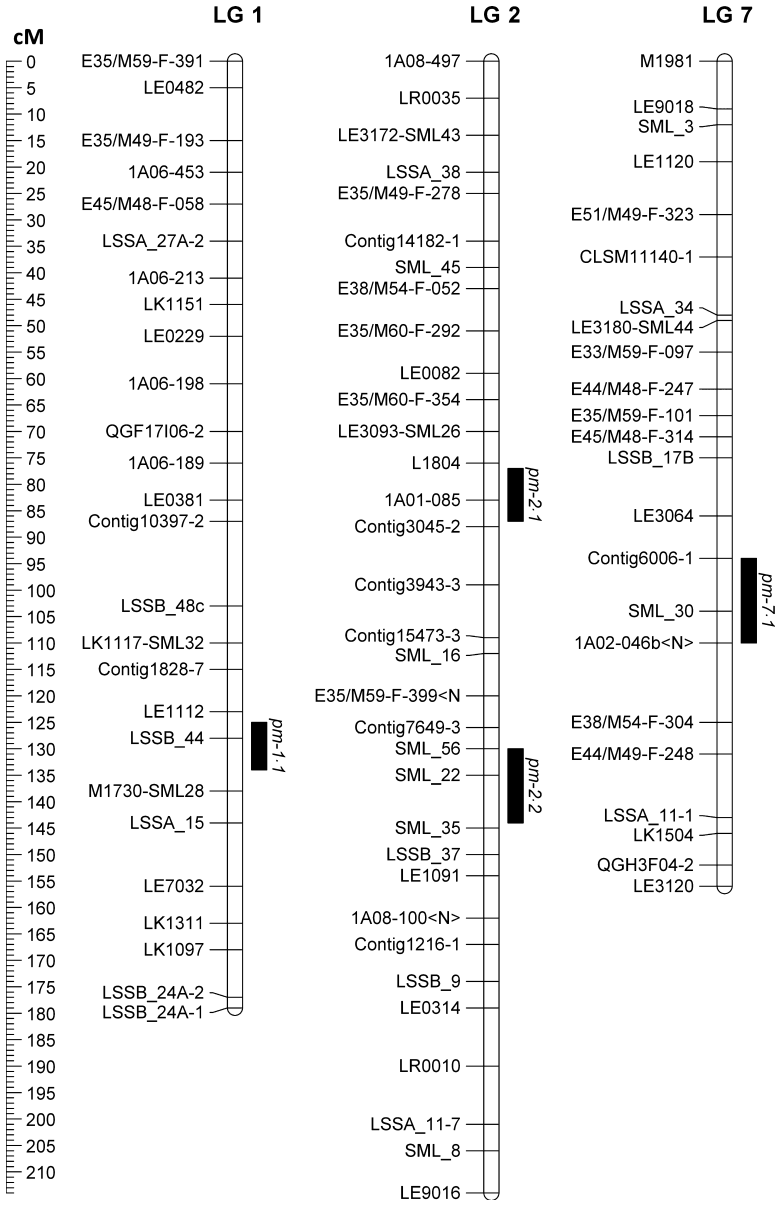

Figure 4 Location of four QTLs for resistance to powdery mildew on the molecular linkage map of lettuce. Bars indicate regions significantly associated with resistance to the disease. Resistance alleles at *pm-1.1* and *pm-2.1* originated from cv. Salinas, while those at *pm-2.2* and *pm-7.1* originated from accession UC96US23.

pm-7.1 in experiment 2 at 61 DAE by AUDPS (LOD = 8·4, R^2 = 36%).

Alleles for higher disease resistance at two of the QTLs (*pm-1.1* and *pm-2.1*) originated from cv. Salinas, while at the other two QTLs (*pm-2.2* and *pm-7.1*) resistance alleles originated from the more susceptible UC96US23. The *pm-2.2* is the only QTL that was detected in both experiments and by both disease assessment methods. The effect of the other three resistance QTLs appears to be limited to the specific experiment. This experiment × QTL interaction may be caused by different growing conditions. Experiment 1 was planted in December, while experiment 2 in May. Although both experiments were carried out under the greenhouse conditions, temperatures were generally higher and natural light length was longer in experiment 2.

No significant QTL for plant phenological stage or leaf position was detected in the present study. The largest LOD score for the leaf position was detected on LG 5 (LOD = 5·3). No powdery mildew QTL was located at this linkage group. The largest LOD score for plant phenological stage was detected on LG 1 (LOD = 5·1), approximately 9 cM from the *pm-1.1*.

Differences in detecting QTLs using percentage rating vs AUDPS values

QTLs that were detected by both disease assessment methods always had higher LOD scores when using percentage rating. For example, the LOD scores in experiment 1 were 8·2 for percentage rating and 7·4 for AUDPS for *pm-1.1*, and 10·0 and 7·2 for *pm-2.2*. In

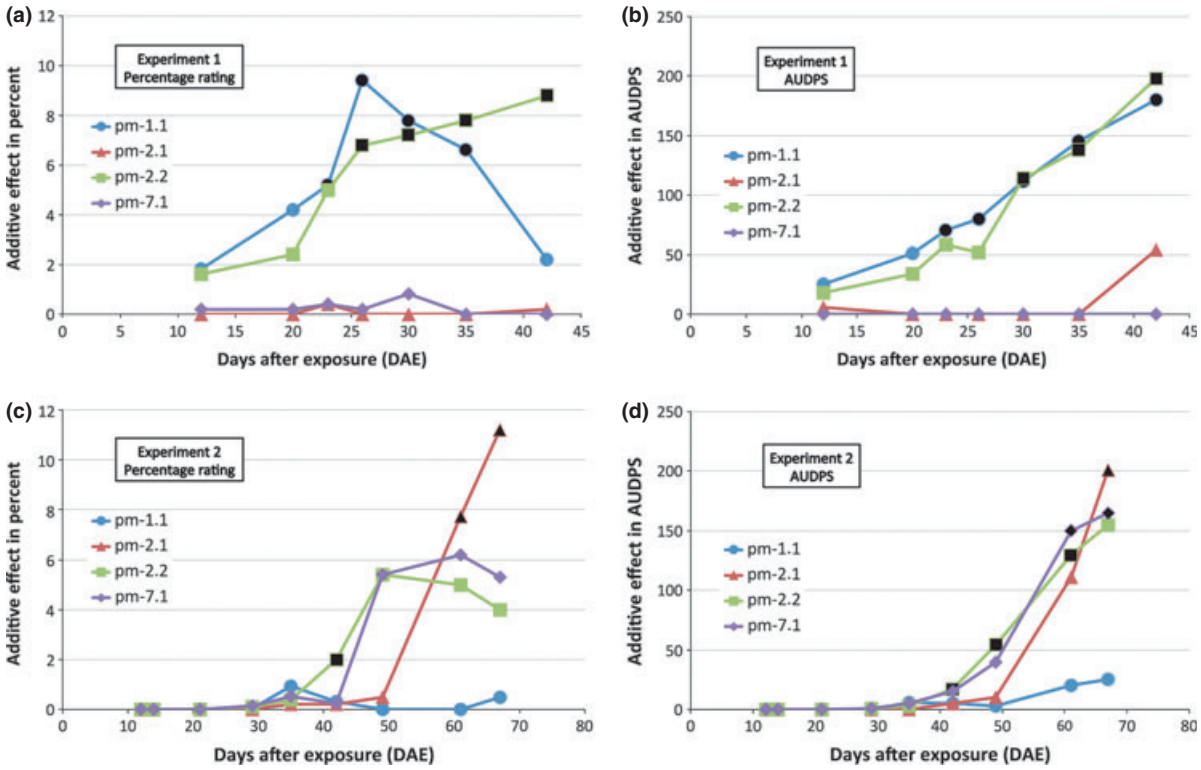

Figure 5 Additive effects of alleles at four QTLs. (a, b) experiment 1; (c, d) experiment 2. (a, c) Results from percentage rating; (b,d) results from AUDPS. Black markers indicate analyses where QTLs effects were significant at $P \leq 0{\cdot}05$. Resistance alleles at QTLs *pm-1.1* and *pm-2.1* originate from cv. Salinas, while resistance alleles at QTLs *pm-2.2* and *pm-7.1* originate from accession UC96US23.

experiment 2 the LOD values for *pm-2.1* were 11·0 and 9·1, respectively, and for *pm-2.2*, 9·5 and 8·6, respectively. Although LOD scores for QTLs that were detected by both methods were always higher when calculated from percentage ratings, AUDPS allowed detection of a unique QTL. This QTL was located on LG 7 (*pm-7.1*) and was detected in experiment 2 through use of AUDPS scores only (Fig. 5).

The maximum LOD scores calculated from percentage ratings peaked earlier than LOD scores calculated from AUDPS values. In experiment 1 the highest LOD score was detected for *pm-1.1* at 26 DAE for percentage rating and 35 DAE for AUDPS; for *pm-2.2*, the peak LOD was observed at 35 DAE for percentage rating and 42 DAE for AUDPS. Similarly, in experiment 2, the highest LOD score for *pm-2.2* was reached at 42 DAE for percentage rating and 49 DAE for AUDPS. Both disease assessment methods reached the maximum LOD score at 67 DAE for *pm-2.1*. However this was the last evaluation day of experiment 2, thus it is possible that a higher LOD value calculated from AUDPS data could be reached later.

Inheritance of powdery mildew resistance in field trials

Field trials were performed in 2 years with families derived from a cross between Soraya and Salinas. The progeny derived from greenhouse-grown F_2 plants designated resistant and susceptible were not significantly different for mean powdery mildew severity in 2004 (resistant = 2·5 and susceptible = 2·4) and 2005 (both groups = 2·3) field experiments. Greenhouse selection was ineffective, which may have resulted from the small number of progeny tested, different growing conditions, or from pathogen isolate × genotype interactions. In year 1, the disease scores of all ten $F_{2:3}$ families ranged from 0·85 to 2·70; while the scores for the parental lines were 1·75 (Soraya) and 3·90 (Salinas). All families, with a single exception, and Soraya had significantly ($P < 0{\cdot}01$) less disease than Salinas. In year 2, the average disease scores for $F_{3:4}$ families ranged from 0·83 to 2·53. Soraya reached the score of 1·07 and Salinas 3·00. As in year 1, Soraya and nine $F_{3:4}$ families had significantly ($P < 0{\cdot}01$) less disease than Salinas. Disease ratings in the two years were highly and significantly correlated ($r = 0{\cdot}936$, $P < 0{\cdot}0001$), which indicates a genetic basis for resistance that was effective in both years (Fig. 6).

Resistance to powdery mildew in lettuce accessions

Resistance of 80 lettuce accessions to powdery mildew was assessed in 23 greenhouse experiments and field trials. Data from individual experiments and trials were combined into an integrated (and normalized) rating,

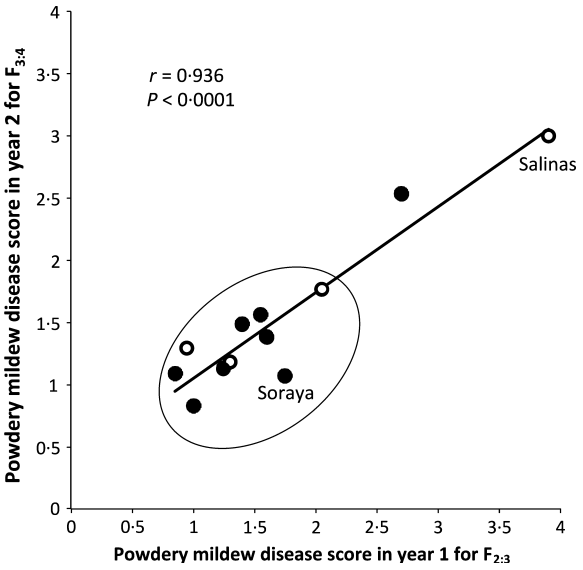

Figure 6 Relationship between powdery mildew disease scores in 2 years of field trials. The *x*-axis shows disease scores for $F_{2:3}$ families in year 1, while the *y*-axis shows disease scores for the $F_{2:3}$-derived $F_{3:4}$ families in year 2. All families originated from a cross between butterhead cv. Soraya and crisphead cv. Salinas. Circled values are significantly different ($P < 0{\cdot}01$) in both years from those recorded for Salinas. Closed and open circles indicate families and cultivars that were originally classified in greenhouse tests as resistant (closed circle) or susceptible (open circle).

with increasing negative values indicating a higher disease resistance (Table 3). The highest resistance to powdery mildew was observed in the leaf cultivar Two Star ($z = -2{\cdot}19$, eight tests), butterhead cultivars Clarion ($z = -1{\cdot}69$, 10 tests), Cindy ($z = -1{\cdot}47$, seven tests) and Bremex ($z = -1{\cdot}43$, 13 tests), and *L. serriola* accession PI 255665 ($z = -1{\cdot}56$, four tests) among those evaluated in three or more tests. The overall highest susceptibility was observed in the *L. serriola* accession UC96US23 ($z = 3{\cdot}08$, six tests) and crisphead cultivars Autumn Gold ($z = 1{\cdot}73$, 18 tests), Grizzly ($z = 1{\cdot}59$, four tests), Silverado ($z = 1{\cdot}43$, four tests) and Wolverine ($z = 1{\cdot}41$, three tests). Salinas, a parent of the mapping population, also showed a relatively high susceptibility with $z = 1{\cdot}11$ from 21 tests.

Overall, resistance to powdery mildew was significantly higher ($P < 0{\cdot}0001$) in leaf (z-score $= -0{\cdot}88 \pm 0{\cdot}18$, mean value $\pm$ standard error) and butterhead (z-score $= -0{\cdot}69 \pm 0{\cdot}13$) types than in crisphead (z-score $= 0{\cdot}77 \pm 0{\cdot}11$) lettuces. Romaine cultivars were also less resistant (z-score $= 0{\cdot}18 \pm 0{\cdot}16$) than leaf and butterhead lettuce at $P < 0{\cdot}05$.

Discussion

Greenhouse experiments 1 and 2 showed a different pattern of disease progress (Fig. 2). It was previously observed that resistance to powdery mildew in lettuce is influenced by many factors, including temperature, moisture, light intensity and mineral nutrition (Schnathorst, 1965). The possibility that different, naturally occurring races of the pathogen were present in the greenhouse when experiments were planted 6 months apart cannot be excluded. Unfortunately, the races of the pathogen could not be determined because a set of differentials to classify pathogen races did not exist at the time of the experiments; such a set was developed only recently (Lebeda & Mieslerová, 2011).

Four QTLs for resistance to powdery mildew in greenhouse-grown lettuce were detected on linkage groups 1, 2 and 7 (Fig. 4; Table 2). These linkage groups harbour resistance genes to a number of lettuce diseases in addition to QTLs for resistance to powdery mildew. At least five race-specific *R*-genes (*Dm5/8*, *Dm10*, *Dm17*, *Dm43*, *Dm45*) for resistance to downy mildew (caused by *Bremia lactucae*) are located on LG 1 (McHale, 2008; McHale *et al.*, 2009; Michelmore, 2010) together with genes for resistance to *Lettuce mosaic virus* (*Mo-2*; McHale *et al.*, 2009), *Turnip mosaic virus* (*Tu*; Robbins *et al.*, 1994), root downy mildew (*plr*; Kesseli *et al.*, 1993) and fusarium wilt (*FUS1*; Michelmore, 2010). LG 2 harbours a large cluster of *R* genes for resistance to downy mildew (*Dm1*, *Dm2*, *Dm3*, *Dm6*, *Dm14*, *Dm15*, *Dm16*, *Dm18*; McHale, 2008; McHale *et al.*, 2009; Michelmore, 2010) and resistance genes against lettuce dieback (*Tvr1*; Simko *et al.*, 2009), fusarium wilt (*FUS2*, *RRD2*; Michelmore, 2010; Aruga *et al.*, 2012) and anthracnose (*ANT2*; McHale *et al.*, 2009). On LG 7 the only known major resistance gene is the *FUS3* gene conferring resistance to fusarium wilt (Michelmore, 2010). Locations of the powdery mildew resistance QTLs mapped in this study appear to be different from all other previously published major resistance genes with the possible exception of *pm-1.1* that is located at approximately 10 cM from *Dm43* (McHale, 2008; McHale *et al.*, 2009). Similarly, the majority of QTLs for resistance to downy mildew in lettuce do not coincide with known *R* gene clusters (Zhang *et al.*, 2009). Comparisons of map positions indicate that QTLs for resistance to downy mildew (Jeuken *et al.*, 2008) and to powdery mildew reside within the same general region of LG 2 and possibly also LG 7. A more detailed comparison of the linkage maps was not possible because of the limited number of shared molecular markers. Four QTLs described in the present study are so far the only reported resistance QTLs to powdery mildew in lettuce. Mapping populations derived from crosses with accessions having high levels of resistance to powdery mildew, such as those identified in these tests, are expected to yield additional QTLs.

Alleles for higher disease resistance to powdery mildew originated from both parents (Table 2). Appearance of resistance alleles in highly susceptible UC96US23 indicates transgressive segregation that is frequently observed in plants (de Vicente & Tanksley, 1993). One of the 90 tested RILs (RIL-068) showed significantly higher resistance to powdery mildew than Salinas ($P < 0{\cdot}01$) in both experiments (percentage rating of 24 vs 54% in experi-

Table 3 Integrated ratings (*z*-score) of relative resistance to powdery mildew exhibited by 80 lettuce cultivars and accessions

Cultivar or accession[a]	Lettuce type[b]	No. of tests	*z*-score[c]	Cultivar or accession	Lettuce type	No. of tests	*z*-score
Two Star	LF	8	−2·19	Clemente	RO	11	0·04
PI 234204	LS	1	−1·94	Allegiance	BU	5	0·06
Clarion	BU	10	−1·69	Hilde	BU	5	0·06
Sabine	BU	1	−1·60	Kofa	CR	7	0·16
Salad Bowl	LF	1	−1·60	Pacific	CR	4	0·17
PI 255665	LS	4	−1·56	Desert Spring	CR	7	0·23
Cindy	BU	7	−1·47	Del Rio	CR	4	0·25
Bremex	BU	13	−1·43	Fresh Heart	RO	4	0·32
Optima	BU	2	−1·33	Bubba	CR	6	0·33
Waldmann's Green	LF	8	−1·29	Dark Green Boston	BU	6	0·35
Red Tide	LF	4	−1·12	Tiber	CR	4	0·38
Balady Aswan – Red	ST	2	−1·10	Diamond	CR	4	0·43
Soraya	BU	12	−1·03	Vanguard 75	CR	4	0·43
Big Boston	BU	7	−1·01	Green Forest	RO	4	0·45
Red Fox	LF	4	−1·00	Cibola	CR	4	0·49
Tehama	LF	5	−0·87	Barcelona	CR	2	0·51
Prizehead	LF	1	−0·85	King Henry	RO	4	0·62
White Boston	BU	1	−0·85	Coolguard	CR	3	0·64
Shining Star	LF	4	−0·84	Calicel	CR	4	0·72
Arctic King	BU	3	−0·76	El Dorado	CR	1	0·82
Anthem	BU	7	−0·71	Gladiator	RO	1	0·82
Green Towers	RO	7	−0·70	Klamath	RO	1	0·82
Imperial 850	CR	6	−0·66	Sharpshooter	CR	1	0·82
Margarita	BU	9	−0·56	Jackal	CR	4	0·82
Esmeralda	BU	2	−0·52	Athena	RO	2	0·87
Darkland	RO	8	−0·47	PI 251246	OL	1	0·89
Corelli	BU	7	−0·39	Winterhaven	CR	14	1·00
Supercoach	CR	4	−0·33	Del Oro	CR	1	1·01
Red Rage	LF	2	−0·31	Coyote	CR	4	1·04
Fila	BU	5	−0·30	Salinas	CR	21	1·11
Parris Island Cos	RO	5	−0·30	Big Sur	CR	4	1·15
Amanda Plus	BU	1	−0·29	Head Master	CR	1	1·37
Balady Aswan – Green	ST	1	−0·29	Icon	CR	1	1·37
Big Red	LF	1	−0·29	Red Coach	CR	1	1·37
Dynamite	BU	1	−0·29	Yuma	CR	1	1·37
Red Oakleaf	LF	1	−0·29	Wolverine	CR	3	1·41
Valmaine	RO	4	−0·17	Silverado	CR	4	1·43
Conquistador	RO	3	−0·11	Grizzly	CR	4	1·59
Grappa	BU	2	−0·01	Autumn Gold	CR	18	1·73
Vulcan	LF	2	−0·01	UC96US23	LS	6	3·08

[a]Seeds originate from the US Agricultural Research Station in Salinas, California.
[b]Lettuce horticultural types or species: BU, butterhead; CR, crisphead; LF, leaf; OL, oil; RO, romaine; ST, stem; LS, *Lactuca serriola*.
[c]*z*-score: normalized score of aggregated ranking. Increasing negative values indicate a higher relative resistance to powdery mildew.

ment 1, and 0 vs 17% in experiment 2, respectively). This RIL possesses the most favourable combination of the four resistance alleles originating from both parents. These results show that even a highly susceptible accession such as UC96US23 may be a source of resistance alleles to powdery mildew.

Identified QTLs reduced powdery mildew during a limited period of time. For example, the *pm-1.1* QTL in experiment 1 (percentage rating) had the highest LOD and percentage of variation explained (R^2) at 26 DAE and then gradually decreased (Table 2). The additive effect of alleles at *pm-1.1* showed the same pattern as LOD and R^2, with the largest effect observed at 26 DAE (Fig. 5, top left panel). Similar, but less pronounced, trends were observed for QTLs *pm-2.2* and *pm-7.1* in experiment 2, whereas the additive effect of alleles increased in time for QTLs *pm-2.2* in experiment 1 and *pm-2.1* in experiment 2 (Fig. 5, left panels). Thus, polygenic resistance to powdery mildew in the Salinas × UC96US23 mapping population appears to be conferred by a cumulative effect of QTLs operating through successive, possibly overlapping, limited time periods. Additional experiments are needed to determine if these QTLs are functioning only at specific environmental conditions or plant developmental phases. Previously, developmental phase-specific QTLs were reported for lettuce resistance to downy mildew. Evaluation of the *L. sativa* × *L. saligna* mapping population revealed that some QTLs reduce infection in the

young plant stage only, or the adult plant stage only, or both stages (Zhang *et al.*, 2009).

Three significant QTLs were detected when using percentage data while four QTLs (one of them unique) were detected using AUDPS scores (Table 2). Comparison of the two disease assessment methods indicated that neither method consistently outperformed the other when used for QTL mapping. The AUDPS score combines disease resistance data from the first to the most recent evaluation. This method may allow detection of QTLs that show consistently large, but not significant effects in multiple evaluations with percentage rating (e.g. *pm*-7.1 in experiment 2). In contrast, percentage rating allows detection of QTLs that show a substantial effect in a single or very few evaluations. Such QTLs may not reach the significance threshold when calculated from AUDPS scores (e.g. *pm*-2.2 if experiment 1 were to be finished at 26 DAE). Thus, percentage ratings and AUDPS scores complement each other when used for QTL analysis.

The integrated rating approach enabled the combining of data from 23 experiments into a single data set (Table 3). This approach can be used to compare relative resistances of accessions that were never tested in the same experiment (Simko & Piepho, 2011). However, it is essential to proceed with caution when combining data from phenotypic assessments of disease resistance. Substantial accession × experiment interaction (such as race-specific resistance) will, for example, make the integrated rating a function of the experiment in which the accession was included (Simko *et al.*, 2012).

Although there is only a limited overlap in cultivars that were tested in the present and older studies, the integrated rating results are in good agreement with previous assessments that were performed over a period of 50 + years (1958–2012) in various environments using different disease assessment assays and scoring systems, and, probably, with different races or mixed races of the pathogen. Accessions previously described as having moderate or high resistance to powdery mildew were always among the most resistant ones in the present tests (those with negative *z*-scores). High or moderate resistance was previously reported in cvs Salad Bowl (current *z*-score = −1·60 from one test), Big Boston ($z = -1{\cdot}01$, seven tests) and Arctic King ($z = -0{\cdot}76$, three tests) (Schnathorst & Bardin, 1958); Two Star ($z = -2{\cdot}19$, eight tests) (Matheron & Porchas, 2003); Bremex ($z = -1{\cdot}43$, 13 tests) and Amanda Plus ($z = -0{\cdot}29$, one test) (Lebeda, 1985b); Clarion ($z = -1{\cdot}69$, 10 tests), Cindy ($z = -1{\cdot}47$, seven tests), Soraya ($z = -1{\cdot}03$, 12 tests) and Corelli ($z = -0{\cdot}39$, seven tests) (Knight *et al.*, 1986); Sabine ($z = -1{\cdot}60$, one test) (Knight *et al.*, 1986; Lebeda & Mieslerová, 2011; Lebeda *et al.*, 2012); and *L. serriola* accession PI 255665 ($z = -1{\cdot}56$, four tests) (Lebeda, 1985a). One notable exception to this general trend was Imperial 850 that was completely resistant in the earlier trials (Whitaker & Pryor, 1941), but whose *R*-gene-based resistance was defeated by the current population of the pathogen present in Salinas, California. However, Imperial 850 still possesses a certain level of resistance ($z = -0{\cdot}66$, six tests), probably either due to a residual effect of the defeated *R* gene or presence of another resistance gene(s). Resistance was previously reported in cvs Bath Cos (Schnathorst & Bardin, 1958), Big Green Cos (Matheron & Porchas, 2003), Susan (Knight *et al.*, 1986), Suttons A-1 and Maruraj (Husain & Akram, 1996), and Colorado (Lebeda & Mieslerová, 2011; Lebeda *et al.*, 2012), that were not tested in this study.

Generally low resistance of crisphead cultivars to powdery mildew was observed (Table 3). Therefore, if crisphead-type lettuces are cultivated in an area where powdery mildew can be an economic concern (e.g. Yuma, Arizona), development of cultivars with improved resistance is vital. However, modern crisphead-type lettuces have very limited genetic diversity (Simko, 2009), indicating that novel resistance genes (or alleles) may need to be introgressed into this type from other lettuce types or wild species. Results of the field trials with $F_{2:3}$ and $F_{3:4}$ families derived from a cross between moderately resistant butterhead cultivar Soraya and susceptible crisphead cultivar Salinas (Fig. 6) demonstrated effective transfer of resistance to powdery mildew in this material. Results of the present study will be used in the authors' lettuce breeding and genetics programmes.

Acknowledgements

The authors would like to thank R. Michelmore, M. Truco, O. Ochoa and L. McHale for providing marker information and seeds of the mapping population. This work was partly supported by the California Leafy Greens Research Program and the Arizona Iceberg Lettuce Research Council. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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