Resistance to Downy Mildew in Lettuce ‘La Brillante’ is Conferred by *Dm50* Gene and Multiple QTL

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ABSTRACT

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Many cultivars of lettuce (*Lactuca sativa* L.) are susceptible to downy mildew, a nearly globally ubiquitous disease caused by *Bremia lactucae*. We previously determined that Batavia type cultivar ‘La Brillante’ has a high level of field resistance to the disease in California. Testing of a mapping population developed from a cross between ‘Salinas 88’ and La Brillante in multiple field and laboratory experiments revealed that at least five loci conferred resistance in La Brillante. The presence of a new dominant resistance gene (designated *Dm50*) that confers complete resistance to specific isolates was detected in laboratory tests of seedlings

inoculated with multiple diverse isolates. *Dm50* is located in the major resistance cluster on linkage group 2 that contains at least eight major, dominant *Dm* genes conferring resistance to downy mildew. However, this *Dm* gene is ineffective against the isolates of *B. lactucae* prevalent in the field in California and the Netherlands. A quantitative trait locus (QTL) located at the *Dm50* chromosomal region (*qDM2.2*) was detected, though, when the amount of disease was evaluated a month before plants reached harvest maturity. Four additional QTL for resistance to *B. lactucae* were identified on linkage groups 4 (*qDM4.1* and *qDM4.2*), 7 (*qDM7.1*), and 9 (*qDM9.2*). The largest effect was associated with *qDM7.1* (up to 32.9% of the total phenotypic variance) that determined resistance in multiple field experiments. Markers identified in the present study will facilitate introduction of these resistance loci into commercial cultivars of lettuce.

Cultivated lettuce (*Lactuca sativa* L.) is susceptible to downy mildew disease caused by the biotrophic oomycete *Bremia lactucae* Regel. This pathogen can infect lettuce plants at any developmental stage, causing yellow to pale-green lesions that eventually become necrotic. Severe downy mildew infection results in loss of yield. Moreover, downy mildew lesions on leaves provide a hospitable environment for survival and proliferation of human enteric pathogens such as *Escherichia coli* O157:H7 and *Salmonella enterica* (Simko et al. 2015). Chemical control of downy mildew with metalaxyl-based fungicides is possible but ineffective against metalaxyl-insensitive isolates (Schettini et al. 1991). The improvement of resistance to downy mildew is a major objective of lettuce breeding programs. The breeding effort frequently focuses on utilization of single dominant genes (*Dm* genes) that confer high levels of resistance to the disease in both young seedlings and mature plants. Over 30 *Dm* genes have been identified in lettuce (Lebeda et al. 2002; Michelmore et al. 2009; Michelmore and Wong 2008) and 20 of them have been mapped predominantly to four major resistance clusters (Christopoulou et al. 2015; Maisonneuve et al. 1994; McHale 2008; McHale et al. 2009; Michelmore 2010; Paran et al. 1991; Paran and Michelmore 1993). The resistance based on single dominant genes, however, has not proved to be durable

because new isolates of the pathogen have evolved that render this race-specific resistance ineffective (Lebeda and Zinkernagel 2003; Michelmore et al. 1984). Lettuce resistance to downy mildew may also be determined polygenically (Crute and Norwood 1981; Eenink 1981; Eenink et al. 1982; Grube and Ochoa 2005; Norwood et al. 1983; Simko et al. 2013). Polygenic resistance tends to be quantitative, with phenotypic reactions ranging from partial to near-complete resistance. Although polygenic resistance may prove to be more durable than *Dm*-mediated resistance (Michelmore et al. 2013; Parlevliet 2002; St. Clair 2010; Stuthman et al. 2007), polygenic resistance has not been successfully used thus far in lettuce breeding programs due to its complex inheritance, sensitivity to environmental conditions, and difficulty in phenotyping.

The Salinas Valley of California is the most important lettuce producing area in the United States (Simko et al. 2014b). Extensive production of lettuce crops together with favorable environmental conditions for downy mildew development contribute to high disease pressure in this area (Wu et al. 2001). We previously assessed reactions to downy mildew in 582 lettuce cultivars, plant introductions, unadapted germplasm, wild *Lactuca* spp. related to cultivated lettuce, and breeding lines grown in the Salinas region (Simko et al. 2012). Accessions with consistently high levels of resistance to the disease have been incorporated into our breeding programs (Simko et al. 2014a) and for studies of field resistance to downy mildew (Simko et al. 2013). One of the cultivars with consistently high levels of field resistance to *B. lactucae* was ‘La Brillante’ (Simko et al. 2012). This old, Batavia type cultivar, has been known since 1924 in France (<http://compositdb.ucdavis.edu/database/lettcv2/display>) but is not used for commercial production in the United States. To identify genes for resistance

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to *B. lactucae* and other diseases in La Brillante, a population of recombinant inbred lines (RIL) was developed from a cross between La Brillante and ‘Salinas 88’, which is a modern iceberg cultivar susceptible to many California isolates of *B. lactucae*. This mapping population has previously been used to map genes for resistance to Verticillium wilt (Hayes et al. 2011) and bacterial leaf spot (Hayes et al. 2014b), and also genes involved in postharvest decay of fresh-cut lettuce (Hayes et al. 2014a).

The objectives of this study were to dissect the genetics of resistance to *B. lactucae* observed in La Brillante, map positions of resistance loci on the molecular linkage map relative to candidate genes for resistance, and evaluate stability of resistance in field and laboratory experiments.

MATERIALS AND METHODS

Plant material and genotyping with molecular markers.

A mapping population of 95 F₇ RIL was derived from a cross between cultivars Salinas 88 and La Brillante (S88 × LB) using single-seed descent (Hayes et al. 2011, 2014a). Experiments were planted using F₈ seed lots produced from approximately 20 field-grown F₇ plants of each RIL.

The DNA of the RIL was extracted using 300 mg of tissue using GenElute Plant Genomic DNA Miniprep kit (Sigma-Aldrich, St. Louis) according to the manufacturer’s instructions. The indexed library for sequencing the RIL was generated using a double-digestion protocol developed for legume species (R. V. Pennmetts and D. Cook, *unpublished data*) with minor modifications. Restriction enzymes (*Hind*III and *Nla*III) and ligase were purchased from New England BioLabs (Ipswich, MA); adapters were synthesized by LifeTechnologies (Frederick, MD). Paired-end reads (100 bp sequenced from each end) were generated using a single lane on an Illumina HiSeq2500 (Illumina, San Diego, CA). The reads from each individual RIL were mapped to the reference genome sequence of Salinas. RIL were then genotyped using high-quality single-nucleotide polymorphisms (SNP) to haplotype assembly scaffolds. Additional SNP and amplified fragment length polymorphism (AFLP) markers were used for genotyping of RIL as was previously described for this population (Hayes et al. 2014b). Markers were clustered based on the reference map (Truco et al. 2013) and linkage maps developed for each chromosome using MSTMap (Wu et al. 2008). A set of evenly distributed framework markers for quantitative trait loci (QTL) analysis was developed by imputing haplotypes for 1-centimorgan (cM) windows along each linkage group and used to construct a second map. The resultant map was checked for colinearity with the reference map (Supplementary Fig. S1).

Evaluation of resistance to downy mildew. Reaction of the S88 × LB RIL and two parents to *B. lactucae* was assessed in five field experiments and four laboratory experiments (Table 1). The name of experiments refers to the planting year (first two digits), the planting month (a single digit after a period), and the location of experiments (two capital letters). Small letters distinguish between multiple experiments performed concurrently (e.g., 12.8LAa and 12.8LAb). Individual evaluations of experiments are shown in the consecutive order after a dash; for example, 11.8NL-2 means the second evaluation of the experiment planted in August 2011 in The Netherlands.

Plants in field experiments became infected naturally with populations of *B. lactucae* present in the test areas. Isolates were collected and phenotyped from the California field experiment in 2010 (10.7FA) and 2011 (11.8FA and 11.9SP). The virulence phenotypes of the pathogen isolates were determined through laboratory testing on a set of differential cultivars expressing all known *Dm* genes (Christopoulou et al. 2015; Ilott et al. 1987; Michelmore and Wong 2008). Four experiments (10.7FA, 11.3FA, 11.8FA, and 11.9SP) were conducted at two locations in Salinas in 2010 and 2011 and one experiment was conducted in The

Netherlands in 2011 (11.8NL). Experiments in Salinas were performed using a randomized complete block design with three replications for experiments 11.3FA, 11.8FA, and 11.9SP and two replications for experiment 10.7FA. Plants were seeded in two rows 35 cm apart on 102-cm-wide beds (center-to-center). Plots of each genotype were 5 to 7 m long with approximately 30 cm between plants within a seedline after thinning, resulting in over 30 plants per replicate. Crop cultivation was done using standard cultural practices for each area, except that no fungicide treatment was applied to allow for disease development. Experiments were sprinkler irrigated for approximately 10 min every other evening to maintain high relative humidity favorable to downy mildew. The 11.8NL experiment was not replicated and consisted of 20 plants per RIL or parent organized into four rows, with 5 plants per row.

Experiments were regularly inspected and the first rating was performed approximately a week after disease symptoms were observed on the susceptible parent Salinas 88. Subsequent disease evaluations were performed at weekly intervals. The last evaluation was always performed at the stage of harvest maturity. If disease developed late in the growing season (11.3FA), only a single evaluation at the harvest maturity stage was performed. Disease was visually assessed and an overall rating was given to the whole plot with 30+ plants in Salinas and 20 plants in The Netherlands. In Salinas, a 0-to-5 rating scale was used that combined both disease incidence and severity (Simko et al. 2013). The 11.8NL experiment was scored on a 0-to-9 rating scale that was subsequently converted into the 0-to-5 scale used in Salinas. Data analyses were performed on disease scores averaged across all blocks within the experiment. When two or more disease evaluations were performed for the experiment, multiple ratings were combined into a single value. The combined value, termed the area under the disease progress steps (AUDPS), reflects disease progress from the first until the last evaluation (Simko and Piepho 2012). A standardized version of AUDPS (sAUDPS) that expresses an average disease score (Simko and Piepho 2012) was used in QTL analyses in addition to the actual scores from individual evaluations. Results obtained from analyses of sAUDPS data are indicated by a capital letter A after the name of the experiment (e.g., 10.7FA-A).

To test for resistance in laboratory conditions (11.4LA, 11.5LA, 12.8LAa, and 12.8Lab) (Table 1), seedlings were sprayed with a conidial suspension of *B. lactucae* (1 × 10⁴ conidia/ml) 7 days after sowing. Inoculated seedlings were maintained in clear plastic boxes at 15°C with a 14-h photoperiod. Sporulation (emergence of *B. lactucae* conidiospores) was assessed on 15 to 30 seedlings per genotype (Simko et al. 2013). In experiment 11.4LA, assessments were performed at 7, 13, and 18 days after inoculation (DAI); in experiment 11.5LA, assessments were performed at 16 DAI; and in experiments 12.8LAa and 12.8Lab, sporulation assessments were performed at 7 and 12 DAI. The number of seedlings with *B. lactucae* sporulation was counted in 11.4LA and 11.5LA experiments but estimated in 12.8LAa and 12.8Lab experiments. The percentage of seedlings with sporulation out of the total number of seedlings was used in data analyses. Because bimodal distribution of resistance data from three laboratory experiments (11.4LA, 11.5LA, and 12.8Lab) indicated a possible effect of a single, major resistance gene, percentage values were dichotomized into two distinct groups using Ward’s hierarchical clustering (WHC) analysis implemented in computer software JMP (v. 11.1.1; SAS Institute, Cary, NC). The observed number of RIL in the two groups was then compared with the expected number of resistant and susceptible RIL in the F₇ generation (approximately 1:1 ratio) based on the assumption of a single-gene and Mendelian inheritance. Goodness of fit between the observed (dichotomized) and the expected data were calculated by χ^2 analysis in JMP.

In total, 23 evaluations for disease resistance were performed on the S88 × LB RIL population in five field and four laboratory experiments. Reaction of La Brillante to 17 additional isolates of

B. lactucae was tested in the laboratory as described for the 11.5LA experiment. These supplementary tests were performed to assess the efficacy of a *Dm* gene present in La Brillante. Isolates of *B. lactucae* tested were Bl:22, Bl:24, Bl:25, Bl:26, Bl:27, Bl:28, Bl:29, Bl:30, Bl:31, C83M47, C12O1393, C12O1385, C13C1414, C14C1428, C14C1470, C14C1492, and C14C1500 (Table 2).

Data analyses. QTL mapping was performed in QGene v. 4.3.9 software (Joehanes and Nelson 2008) using the composite interval mapping (CIM) feature, with the automatic forward selection of cofactors and scan interval set at 1 cM. Normality of data distribution was tested with the D'Agostino-Pearson test implemented in QGene. Experiments with nonnormal distribution of data

TABLE 1. Description of field and laboratory experiments evaluating Salinas 88 × La Brillante population reaction to downy mildew^a

Experiment	Location	Seeding month and year	Number of disease evaluations	Number of tested RIL	Downy mildew isolate (<i>Avr</i> genes)	Rating scale	Scores at last evaluation			
							RIL mean	RIL range	Salinas 88	La Brillante ^b
10.7FA	Salinas, CA	July 2010	4	86	Natural population	0–5	2.9	1.3–4.3	4.1	1.8*
11.3FA	Salinas, CA	March 2011	1	90	Natural population	0–5	1.8	0–2.8	2.3	0.7*
11.8FA	Salinas, CA	August 2011	3	90	Natural population	0–5	2.4	1.7–4	4.2	1.7*
11.8NL	Netherlands	August 2011	3	79	Natural population	0–5	3.5	1.7–4.4	4.4	2.8 ^c
11.9SP	Salinas, CA	September 2011	4	88	Natural population	0–5	3.2	2.3–4.2	4.3	2.1*
11.4LA	Laboratory	April 2011	3	80	C01O879 (<i>Avr17, 37, 38</i>)	0–100	64.9	0–100	100	16.7*
11.5LA	Laboratory	May 2011	1	89	C01O879 (<i>Avr17, 37, 38</i>)	0–100	35.7	0–100	71.4	0*
12.8LAa	Laboratory	August 2012	2	90	C11O1352 (<i>Avr17, 36</i>)	0–100	63.7	33.3–100	76.6	50
12.8LAb	Laboratory	August 2012	2	90	C11O1345 (<i>Avr6, 17, 37, 38</i>)	0–100	68.9	33.3–100	100	50*

^a RIL = recombinant inbred line and *Avr* = avirulence gene.

^b Asterisk indicates significant difference ($P \leq 0.05$) between the mean disease scores of Salinas 88 and La Brillante within the experiment.

^c Statistical analysis of a difference between the mean disease scores of Salinas 88 and La Brillante was not performed because of unreplicated design of this experiment.

TABLE 2. Interaction of La Brillante and the set of lettuce differentials with 20 isolates of *Bremia lactucae*^a

Accession	<i>Dm</i> genes	Sextet number ^c	Sextet value ^c	LG ^d	The Netherlands										Davis, CA ^b									
					Bl: 22	Bl: 24	Bl: 25	Bl: 26	Bl: 27	Bl: 28	Bl: 29	Bl: 30	Bl: 31	879	1352	1345	M47	1393	1385	1414	1428	1470	1492	1500
La Brillante	n.a.	n.a.	n.a.	2	–	–	–	(–)	+	–	–	–	+	–	(–)	(–)	–	+	–	–	–	+	–	–
Cobham																								
Green ^e	0	n.a.	n.a.	n.a.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lednický	1	1	1	2	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
UCDM2	2	2	2	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dandie	3	3	4	2	–	–	–	+	+	–	–	–	+	+	+	+	+	+	–	(–)	+	+	(–)	–
R4T57D	4	4	8	4	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	(–)
Valmaine	5/8	5	16	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Sabine	6	6	32	2	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–	+	–	–
LSE57/15	7	1	1	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
UCDM10	10	2	2	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Capitan	11	3	4	4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Hilde II	12	4	8	n.a.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pennlake	13	5	16	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
UCDM14	14	6	32	2	+	–	–	–	+	(–)	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NunDm15	15	1	1	2	+	–	–	–	+	–	+	+	–	+	+	+	–	+	–	+	–	+	+	+
CGDm16	16	2	2	2	–	+	+	+	–	+	+	–	–	+	+	+	+	+	–	+	(–)	(–)	+	+
NunDm17	17	3	4	1	–	–	–	–	+	–	+	+	+	–	–	–	–	–	–	–	+	–	–	–
Colorado	18	4	8	2	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	(–)	(–)
Ninja	36	5	16	n.a.	–	–	–	+	–	–	–	–	–	+	–	+	–	+	–	–	–	–	–	–
Discovery	37	6	32	n.a.	–	–	+	+	–	–	+	–	–	–	+	–	–	+	(–)	–	(–)	–	(–)	(–)
Argelès	38	1	1	n.a.	(–)	+	(–)	+	+	+	+	–	–	+	–	+	–	–	–	–	+	–	–	–
RYZ2164	“Silvinas”	2	2	n.a.	–	–	–	–	+	–	+	+	+	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	–	–	–	–	–
RYZ910457	“Murai”	3	4	n.a.	–	–	–	–	–	+	–	–	+	n.a.	n.a.	n.a.	n.a.	n.a.	(–)	(–)	–	(–)	(–)	(–)
Bedford	monogenic	4	8	n.a.	(–)	–	–	–	–	–	–	–	–	n.a.	n.a.	n.a.	n.a.	n.a.	+	+	+	–	+	+
Balesta	monogenic	5	16	n.a.	+	–	–	–	+	–	–	–	–	n.a.	n.a.	n.a.	n.a.	n.a.	–	–	–	–	–	–
Bellissimo	monogenic	6	32	n.a.	–	–	–	–	–	(–)	–	–	–	n.a.	n.a.	n.a.	n.a.	n.a.	–	–	(–)	–	–	+
Amplus ^f	2, 4, 40	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	+	n.a.	+	–	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

^a Tests were performed as described for 11.5LA experiment in Materials and Methods. In all, 9 isolates starting with the ‘Bl’ letters were tested at Enza Zaden in The Netherlands and the remaining 11 isolates were tested at University of California, Davis; – indicates incompatible interaction (accession resistant), (–) indicates sparse sporulation (accession incompletely resistant), + indicates compatible interaction (accession susceptible), and n.a. = data not available or not applicable.

^b Abbreviation for University of California-Davis isolates: 879 = C01O879, 1352 = C11O1352, 1345 = C11O1345, M47 = C83M47, 1393 = C12O1393, 1385 = C12O1385, 1414 = C13C1414, 1428 = C14C1428, 1470 = C14C1470, 1492 = C14C1492, and 1500 = C14C1500.

^c Sextets recommended by The International Bremia Evaluation Board for descriptions of the *Bremia lactucae* virulence pattern (<http://www.worldseed.org/ist/ibeb.html>).

^d Location of *Dm* gene on linkage group (McHale et al. 2009).

^e Green Towers was used instead of Cobham Green in tests of isolates C12O1385, C13C1414, C14C1428, C14C1470, C14C1492, and C14C1500.

^f Not included in the set of differentials used by The International Bremia Evaluation Board, but it was previously included in the set of differentials used by University of California, Davis.

were tested twice. Analyses were first performed on the actual data with QGene. Presence of significant QTL was then confirmed by a nonparametric Kruskal-Wallis test implemented in MapQTL (v.6; Kyazma, Wageningen, The Netherlands). Significance thresholds for the logarithm of the odds (LOD) scores were determined through permutations with 10,000 iterations (Churchill and Doerge 1994). The threshold for suggestive QTL was set at the genome-wide α value of 0.63 (Lander and Kruglyak 1995), while the threshold for significant QTL was set at the genome-wide α value of 0.05. The percentages of phenotypic variation ($R^2\%$) explained by QTL, 1-LOD, and 2-LOD support intervals of the QTL locations were calculated by QGene. In addition to analyses performed in QGene, mixed-model CIM (MCIM) was applied to test for QTL and QTL-environment interactions. MCIM was performed using the default setting of QTLNetwork v. 2.137 (Yang et al. 2008). The nomenclature convention for significant QTL is qDM#.#; where the first numeral identifies linkage group and the second numeral distinguishes among multiple QTL detected on the same linkage group in this or the previous analyses (Simko et al. 2013).

The lettuce genome sequence (<https://lgr.genomecenter.ucdavis.edu>) and ultrahigh-density molecular linkage map (Truco et al. 2013) were examined to detect candidate resistance genes (McHale 2008; McHale et al. 2009) located in the chromosomal regions of significant QTL. Candidate resistance genes were identified as those having BLAST (Altschul et al. 1990) similarity ($\leq 1 \times e^{-20}$ threshold) to genes involved in resistance in lettuce or other species (McHale 2008; McHale et al. 2009).

The Student's t test was applied to test significance of difference between disease scores measured on the two parental cultivars. Similarity between RIL disease scores assessed at different experiments and evaluations was analyzed using Pearson and Spearman correlation coefficients; however, because of the high similarity of the results, only values obtained by Pearson correlation coefficient are presented. Clustering of 23 disease evaluations (from nine experiments) into groups with similar performance was based on the first two principal components of the principal component analysis. These principal components were used as an input for WHC analysis (Husson et al. 2010). The optimum number of clusters in WHC was identified through cubic clustering criterion calculated for 1 to 10 clusters. Statistical analyses were performed with computer software JMP.

RESULTS

Field and laboratory resistance screening. All field experiments became naturally infected with indigenous populations of *B. lactucae*. In all, 91% of linear correlations between pairs of field evaluations were significant (and positive) at $P \leq 0.05$. The correlations ranged from $r = 0.003$ ($P = 0.979$) between 11.3FA-1 and 11.9SP-2 to $r = 0.821$ ($P < 0.0001$) between 11.8FA-2 and 11.8FA-3. The highest correlation between evaluations from different field experiments was $r = 0.685$ ($P < 0.0001$) calculated between 11.8FA-3 and 11.9SP-4. Isolates were collected from the 10.7FA (three isolates), 11.8FA (one isolate), and 11.9SP (one isolate) trials in Salinas in 2010 and 2011. Virulence phenotyping on the differential set of resistant cultivars revealed that two isolates from 10.7FA (C10D1278 and C10D1279) had avirulence (*Avr*) genes *Avr17*, *Avr36*, *Avr37*, and *Avr38*, while the third isolate from 10.7FA (C10D1293) and the isolate from 11.8FA (C11O1339) had the same set of *Avr* genes plus *Avr4*. The isolate from 11.9SP (C11O1355) had *Avr4*, *Avr16*, *Avr17*, and *Avr38* *Avr* genes. These virulence phenotypes are similar to the predominant isolates prevalent in the Salinas Valley in recent years (Simko et al. 2013) (http://bremia.ucdavis.edu/bremia_database.php). All of these isolates were virulent on La Brillante in laboratory seedling tests. The mean disease scores for RIL at each field experiment's last evaluation ranged from 1.8 in 11.3FA to 3.5 in 11.8NL (Table 1). Similarly, the lowest and the highest disease scores for Salinas 88

and La Brillante were observed in 11.3FA (2.3 and 0.7, respectively) and 11.8NL (4.4 and 2.8, respectively) experiments. Disease scores for Salinas 88 were significantly ($P \leq 0.05$) higher than those for La Brillante in all California field experiments. Differences between parents were not statistically analyzed in the 11.8NL experiment because this experiment was unreplicated. However, both the disease progress and the sAUDPS values were consistent with a higher susceptibility in Salinas 88 (ratings of 1.7, 3.3, and 4.4 at three evaluations, and sAUDPS of 3.2) than in La Brillante (0.6, 1.7, 2.8, and 1.7).

Four laboratory experiments (11.4LA, 11.5LA, 12.8LAa, and 12.8LAb) were performed to evaluate resistance of RIL and parents at the seedling stage. The 11.4LA and 11.5LA experiments used *B. lactucae* isolate C01O879 that is virulent on all known *Dm* genes, except *Dm17*, *Dm37*, and *Dm38*; the 12.8LAa experiment used C11O1352 that is not virulent on *Dm17* and *Dm36*; and the 12.8LAb experiment used isolate C11O1345 that is not virulent on *Dm6*, *Dm17*, *Dm37*, and *Dm38* (Tables 1 and 2). La Brillante showed complete (0% at 11.5LA) or near-complete (16.7% at 11.4LA) resistance to isolate C01O879 in two laboratory experiments. When seedlings were inoculated with isolates C11O1352 and C11O1345, La Brillante was partially resistant (score of 50%). Salinas 88 was highly susceptible (scores from 71.4 to 100%) to all tested isolates of *B. lactucae*. Differences between disease scores of Salinas 88 and La Brillante were significant ($P \leq 0.05$) in all but one laboratory experiment (12.8LAa). Mean disease scores for RIL in experiments 11.4LA, 12.8LAa, and 12.8LAb were similar and ranged from 63.7 to 68.9%. The mean RIL score in experiment 11.5LA was 35.7%. In all four laboratory experiments, the most susceptible RIL had disease scores of 100%. RIL with the highest resistance to the disease had scores comparable with those observed in La Brillante. Complete resistance was observed for the most resistant RIL when tested with isolate C01O879 (score of 0% at 11.4LA and 11.5LA) and partial resistance of the most resistant RIL was observed when tested with isolates C11O1352 (score of 33.3% in 12.8LAa) and C11O1345 (score of 33.3% in 12.8LAb). All disease evaluations from the three laboratory experiments where La Brillante showed a significantly higher level of resistance to *B. lactucae* than Salinas 88 (11.4LA, 11.5LA, and 12.8LAb) grouped into a single cluster (Fig. 1).

Detection of new *Dm* gene. QTL analysis of data obtained from laboratory experiments identified a single resistance locus on linkage group 2 (Table 3). Alleles conferring elevated resistance originated from La Brillante and explained approximately 39 to 71% of the total phenotypic variation of the trait in experiments 11.4LA and 11.5LA, which were performed with isolate C01O879 that possesses *Avr* genes *Avr17*, *Avr37*, and *Avr38*. In the 12.8LAb experiment, where isolate C11O1345 (*Avr6*, *-17*, *-37*, and *-38*) was used, the locus on LG2 explained approximately 25 to 39% of the total phenotypic variation of the trait. The observed number of RIL in two dichotomized groups (resistant versus susceptible) did not significantly deviate from the 1:1 ratio in experiments 11.4LA ($P = 0.842$), 11.5LA ($P = 0.290$), and 12.8LAb ($P = 0.142$). The high level of $R^2\%$ explained by the locus on LG2 together with the 1:1 segregation of resistant and susceptible RIL found in the mapping population suggested that this resistance locus could be a major *Dm* gene. This *Dm* gene provides partial resistance to isolate C11O1352 with *Avr17* and *Avr36* (12.8LAa) and to isolate C11O1345 with *Avr6*, *Avr17*, *Avr37*, and *Avr38* (12.8LAb) in laboratory tests with high levels of inoculum. To further determine the specificity of the *Dm* gene, La Brillante was inoculated with an additional 17 isolates of *B. lactucae* with known *Avr* genes (Table 2). La Brillante was resistant to 12 isolates (Bl:22, Bl:24, Bl:25, Bl:28, Bl:29, Bl:30, C83M47, C12O1385, C13C1414, C14C1428, C14C1492, and C14C1500), partially resistant to one isolate (Bl:26), and susceptible to four isolates (Bl:27, Bl:31, C12O1393, and C14C1470). The reactions to the tested isolates provided evidence that the *Dm* gene in La Brillante is new. We designated this *Dm* gene *Dm50*. Further

studies are needed to determine whether the resistance conferred by this *Dm* locus is due to a single or multiple genes.

Detection of QTL. Five significant QTL were identified at the genome-wide α of 0.05 when analyzing disease scores from field experiments with CIM (Table 3). These QTL were located on LG2 (*qDM2.2*), LG4 (*qDM4.1* and *qDM4.2*), LG7 (*qDM7.1*), and LG9 (*qDM9.2*) (Fig. 2). The most frequently detected QTL was *qDM7.1*

found in seven analyses (four in 10.7FA, two in 11.8FA, and one in 11.9SP). The *qDM4.2* QTL was detected in two analyses (10.7FA and 11.9SP), while each of three remaining QTL was detected only once (*qDM2.2* in 10.7FA, *qDM4.1* in 11.9SP, and *qDM9.2* in 11.8FA). No significant QTL was identified in 11.3FA and 11.8NL experiments. The $R^2\%$ of QTL ranged from 23.0% for *qDM9.2* at evaluation 11.8FA-2 to 32.9% for *qDM7.1* calculated from the

Fig. 1. Positions of 23 evaluations according to their first two principal components coordinates. Clustering of experiments was determined by Ward's hierarchical clustering analysis using the first two principal components of the principal component analysis for input (see detailed explanation in Material and Methods). Three clusters are boxed and labeled with capital letters. Gray boxes next to clusters show percentage of evaluations in which the indicated resistance loci were detected at significant level (genome-wide $\alpha \leq 0.05$) or suggestive level (genome-wide $\alpha \leq 0.63$) in parenthesis.

TABLE 3. Loci for resistance to downy mildew in the Salinas 88 $\times$ La Brillante population

Resistance locus	Linkage group	Nearest marker	Evaluation ^a	QTL location (cM) ^b	QTL support interval (cM) ^c	LOD ^d	$R^2\%$ ^e	A^f
<i>Dm50</i> & <i>qDM2.2</i>	2	Lsat_1_v3_g_0_460	10.7FA-1	12.2	9.9–13.9	7.4	32.8	0.28
		BOMS	11.4LA-1	0.0	0.0–0.4	22.6	68.9	40.9*
		E54/M48-290.76	11.4LA-2	6.6	6.6–6.6	11.7	45.4	28.4*
		BOMS	11.4LA-3	0.1	0.0–1.8	17.6	59.7	28.4*
		BOMS	11.4LA-A	0.0	0.0–1.6	23.8	70.8	35.2*
		Lsat_1_v3_g_0_3497	11.5LA-1	12.1	9.9–13.6	9.6	39.0	24.8*
		BOMS	12.8LAb-1	0.0	0.0–3.1	9.7	38.7	10.9*
		E54/M48-290.76	12.8LAb-2	3.7	0.0–9.9	5.7	25.2	12.6*
		BOMS	12.8LAb-A	0.0	0.0–4.9	9.7	38.7	11.2*
<i>qDM4.1</i>	4	Lsat_1_v3_g_0_8627	11.9SP-A	53.2	47.7–54.4	5.6	25.4	0.11
<i>qDM4.2</i>	4	Lsat_1_v3_g_4_474	10.7FA-4	116.9	112.7–118.7	5.0	23.6	0.39
		Lsat_1_v3_g_4_474	11.9SP-4	116.8	112.8–119.2	7.3	31.9	0.22
<i>qDM7.1</i>	7	Lsat_1_v3_g_7_201	10.7FA-1	74.6	65.4–76.9	5.0	23.6	0.25
		Lsat_1_v3_g_7_2940	10.7FA-2	33.0	31.7–35.1	6.6	29.8	0.62
		Lsat_1_v3_g_7_2940	10.7FA-3	33.3	31.7–35.1	6.7	30.2	0.37
		Lsat_1_v3_g_7_2940	10.7FA-A	33.1	31.7–35.0	7.5	32.9	0.34
		Lsat_1_v3_g_0_7264	11.8FA-1	50.7	49.1–51.5	5.6	25.0	0.21
		Lsat_1_v3_g_0_7264	11.8FA-A	50.7	49.2–51.1	6.3	27.6	0.21
		BICL	11.9SP-1	31.7	27.4–33.0	7.0	30.7	0.23
<i>qDM9.2</i>	9	Lsat_1_v3_g_0_7903	11.8FA-2	23.5	22.5–24.5	5.1	23.0	0.22

^a Evaluations within experiments are indicated by consecutive numbers after the experiment name and a dash. Letter “A” indicates results obtained from area under the disease progress steps scores.

^b Quantitative trait loci (QTL) in centimorgans (cM).

^c Range of 2-LOD support interval for QTL detected by composite interval mapping.

^d LOD = logarithm of the odds score.

^e $R^2\%$ = Percentage of the total phenotypic variation of the trait explained by the QTL.

^f Additive effect of alleles as estimated by QGene. Positive values indicate that alleles for higher resistance originate from ‘La Brillante’. Asterisk indicates values that were estimated from a 0 to 100 percentage scale, while values without asterisk were estimated from a 0 to 5 rating scale (detailed information is in Table 1).

Fig. 2. Locations of five loci for resistance to downy mildew on the Salinas 88 × La Brillante genetic linkage map. Black bars indicate 1-logarithm of the odds (LOD) support interval around significant loci on LG2 (*Dm50/qDM2.2*), LG4 (*qDM4.1* and *qDM4.2*), LG7 (*qDM7.1*), and LG9 (*qDM9.2*). Whiskers on bars correspond to 2-LOD support intervals. Black diamonds indicate approximate positions of the major resistance gene clusters. The cluster on LG2 harbors *Dm1*, *Dm2*, *Dm3*, *Dm6*, *Dm14*, *Dm15*, *Dm16*, and *Dm18* conferring resistance to downy mildew (McHale et al. 2009); *Xar1* gene for resistance to bacterial leaf spot (Hayes et al. 2014b); and *Ra* gene for resistance to lettuce root aphid (Wroblewski et al. 2007). The cluster on LG4 harbors *Dm4*, *Dm7*, *Dm11*, *Dm44*, *Dm48*, and *Dm49* (McHale et al. 2009; Michelmore 2010) for lettuce resistance to downy mildew. The cluster on LG9 harbors *Dm39* for resistance to downy mildew (McHale et al. 2009) and *Vr1* for resistance to Verticillium wilt (Hayes et al. 2011). White bars show positions of QTL for resistance to downy mildew detected in a ‘Grand Rapids’ × ‘Iceberg’ (GR × Ice) mapping population (Simko et al. 2013); *qDM2.1* on LG2 and *qDM9.1* on LG9. Scale is in Kosambi centimorgans (cM).

average disease score of experiment 10.7FA. Analysis of data from field experiments with MCIM confirmed significant QTL, including the two separate QTL on LG4 (*qDM4.1* and *qDM4.2*). All QTL showed significant additive and additive–environment interaction effects.

Further statistical analyses were performed with QGene to identify suggestive linkage (at genome-wide α of 0.63) between markers and traits in the chromosomal regions of *Dm50/qDM2.2*, *qDM4.1*, *qDM4.2*, *qDM7.1*, and *qDM9.2*. This a posteriori analysis tests whether tendencies in all evaluations are the same as in the evaluations that yielded significant QTL. The suggestive linkages were detected in experiments 10.7FA, 11.8FA, 11.8NL, 11.9SP, and 12.8LAa for *qDM4.1*; 11.9SP and 12.8LAa for *qDM4.2*; 10.7FA, 11.8FA, 11.8NL, 11.9SP, and 12.8LAB for *qDM7.1*; and 11.8FA and 11.9SP for *qDM9.2* (Supplementary Fig. S2). Consistent with previous observations, elevated resistance to the disease at all suggestive linkages was associated with La Brillante alleles. Evaluations 11.3FA-1, 11.8NL-1, and 12.8LAa-2, plus the sAUDPS value from the 11.8NL experiment (11.8NL-A), did not yield any significant or suggestive QTL.

DISCUSSION

Significant QTL for resistance to downy mildew were found in 8 of 15 field evaluations (53%). When a ‘Grand Rapids’ × ‘Iceberg’ (GR × Ice) mapping population was tested at different times in the same locations, QTL were detected in 55% of field evaluations (Simko et al. 2013). In both studies, the lowest disease pressure was observed when experiments were sown in spring; spring and summer environmental conditions in Salinas are generally less conducive for development of downy mildew. More downy mildew is usually observed in the fall growing season, when prolonged morning leaf wetness and cool morning and midday temperatures favor infection and disease development (Wu et al. 2001).

Twenty-three disease evaluations on RIL were grouped into three clusters based on correlations between scores (Fig. 1). The most obvious difference among the clusters was detection of significant effects in the *Dm50* chromosomal region in all evaluations of cluster B but not in any of the evaluations of cluster C and in only a single evaluation of cluster A (*qDM2.2* in 10.7FA-1). This indicates that clustering of evaluations corresponds, to a certain degree, with QTL activity. In cluster A, the most frequently detected QTL was *qDM7.1*, which was not identified in evaluations of clusters B and C at the genome-wide $\alpha \leq 0.05$ and was detected in only a single evaluation of cluster B at a suggestive level. Cluster C yielded only two suggestive QTL (both on LG4) and no significant QTL. These results are consistent with significant additive–environment interactions detected for QTL by MCIM analysis.

QTL for field resistance to downy mildew were detected at LG2 (*qDM2.2*), LG4 (*qDM4.1* and *qDM4.2*), LG7 (*qDM7.1*), and LG9 (*qDM9.2*). Previous studies performed on the GR × Ice population identified three QTL (Simko et al. 2013) but these resistance QTL were located on either different linkage groups (*qDM5.1* at LG5) or a different chromosomal region of the same linkage group (*qDM2.1* at LG2 and *qDM9.1* at LG9) (Fig. 2). Several QTL for resistance to *B. lactucae* originating from *L. saligna* were mapped to multiple linkage groups, including LG2, LG4, LG7, and LG9 (den Boer et al. 2013; Jeuken et al. 2008; Zhang et al. 2009). However, it is not possible to accurately determine whether these QTL for resistance to *B. lactucae* from *L. saligna* are located in the same chromosomal regions as *qDM2.2*, *qDM4.1*, *qDM4.2*, *qDM7.1*, and *qDM9.2* because the two studies used different sets of molecular markers. However, it appears that *qDM9.2* is located at the same chromosomal region as *Dm39* for resistance to downy mildew (McHale et al. 2009) and *Vr1*, a single dominant gene for resistance to Verticillium wilt (caused by *Verticillium dahliae*) race 1 (Hayes et al. 2011). The La Brillante alleles at *Vr1* and *qDM9.2* are associated with resistance to both Verticillium wilt and downy

mildew. Linkage group 4 harbors at least six *Dm* genes (*Dm4*, *Dm7*, *Dm11*, *Dm44*, *Dm48*, and *Dm49*) (McHale et al. 2009; Michelmore 2010) for lettuce resistance to downy mildew. Comparison of molecular linkage maps indicates that *qDM4.1* is located in the proximity of the six *Dm* genes while the second QTL detected at LG4 (*qDM4.2*) is well separated from this cluster (Fig. 2). QTL on linkage groups 4, 7, and 9 do not appear to be coincident with known candidate resistance genes (M. Christopoulou and R. W. Michelmore, unpublished data).

Three of four laboratory experiments identified the effect of the *Dm50* gene on LG2. This gene conferred complete resistance to isolate C010879 and incomplete resistance to isolate C1101345. It is possible that the effector gene recognized by *Dm50* might be differentially expressed in tested isolates or there might be a variable recognition due to variation in the effector protein. Alternatively, there is a possibility that the *Dm50* locus has a different mode of action when interacting with an *Avr* gene, or that the locus consists of two or more closely linked resistance genes, each interacting with a different *Avr* gene. No significant effect of the *Dm50* gene was detected when C1101352 was used for inoculation, though seedlings of La Brillante were partially resistant. Incomplete race-specific resistance to *B. lactucae* is known for several *Dm* genes, including *Dm6* and *Dm17* (Crute and Norwood 1978). The reactions of La Brillante to a range of isolates of *B. lactucae* expressing known *Avr* genes determined that the *Dm* gene in this cultivar has a different specificity from the known *Dm* genes expressed by the set of lettuce differentials (Table 2) (<http://www.worldseed.org/isf/ibeb.html>). Linkage analysis confirmed that *Dm50* is distinct from *Dm5/8*, *Dm10*, *Dm17*, *Dm43*, and *Dm45* located on LG1; *Dm13* located on LG3; *Dm4*, *Dm7*, *Dm11*, *Dm44*, *Dm48*, and *Dm49* located on LG4; and *Dm39* located on LG9 (Maisonneuve et al. 1994; Mchale 2008; Mchale et al. 2009; Michelmore 2010; Paran et al. 1991; Paran and Michelmore 1993).

The *Dm50* gene is located in the major resistance cluster on LG2 that contains at least eight major, dominant genes (*Dm1*, *Dm2*, *Dm3*, *Dm6*, *Dm14*, *Dm15*, *Dm16*, and *Dm18*) conferring resistance to downy mildew (McHale et al. 2009). *Xar1* gene for resistance to bacterial leaf spot (Hayes et al. 2014b), and *Ra* gene for resistance to lettuce root aphid (Wroblewski et al. 2007). It is likely that *Dm50* is another resistance gene located in this cluster. Analysis of the lettuce genome sequence identified a candidate resistance gene (a member of the RGC2 gene family) in the chromosomal area of *Dm50* locus (M. Christopoulou and R. W. Michelmore, unpublished data). Significant effect on LG2 was detected under field conditions in only a single evaluation (*qDM2.2* at 10.7FA-1). It was not possible to determine through linkage mapping whether *Dm50* and *qDM2.2* are the same or closely linked loci. Our data show that *Dm50* is ineffective against isolates of *B. lactucae* prevalent in both California and The Netherlands and that presence of the gene does not result in high levels of resistance observed in mature plants of La Brillante in the field. Our analyses also did not detect any significant effect of *Dm* genes in Salinas 88 on resistance in the field to downy mildew. Salinas 88 was developed by backcrossing to ‘Salinas’ and has the same set of race-specific resistance genes to downy mildew as Salinas (Ryder 1991). The studies reported here provided additional evidence that none of the *Dm* genes (*Dm5/8*, *Dm7*, and *Dm13*) present in Salinas or Salinas 88 are effective against *B. lactucae* isolates prevalent in California and The Netherlands (Grube and Ochoa 2005).

Only two (*qDM4.2* and *qDM7.1*) of the five significant QTL were detected in two or more experiments. This is not unusual, because evaluation of polygenic resistance to *B. lactucae* in field conditions is complicated by the quantitative nature of the plant phenotypic reactions (Lebeda and Jendrulek 1988). Plant reactions to the disease may range from almost complete resistance to high susceptibility. Genotypically identical plants growing next to each other may show a certain level of variability in their reaction due to uneven disease pressure, microclimate, and other unspecified

factors. Similarly, in laboratory experiments, some differences may be observed even within homozygous cultivars grown in essentially identical conditions. When mapping populations are tested in multiple experiments, additional factors may substantially affect congruence among detected QTL, including environmental conditions and differences in the pathogen population (Simko 2002). Furthermore, lower disease pressure in 11.3FA may have caused less accurate scoring (Simko et al. 2013), while delayed disease onset could lead to differences in QTL due to plant stage-dependent resistance (den Boer et al. 2013; Zhang et al. 2009).

The resistance observed in La Brillante in field conditions is conferred by the effect of at least five QTL located at four linkage groups. La Brillante also harbors at least one major resistance factor (designated *Dm50*) located in the resistance cluster on LG2. The *Dm50* gene conferred high levels of resistance against specific isolates of *B. lactucae* when tested on seedlings in laboratory conditions; however, its effect was not evident under field conditions (with a possible single exception) when field plots were infected with indigenous populations of the pathogen.

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