Identification of Major Quantitative Trait Loci Controlling Field Resistance to Downy Mildew in Cultivated Lettuce (*Lactuca sativa*)

Lorena Parra,^{1,2} Ivan Simko,³ and Richard W. Michelmore^{2,†}

¹ Plant Pathology Graduate Group, University of California Davis, One Shields Ave., Davis, CA 95616

² The Genome Center and Department of Plant Sciences, University of California Davis, One Shields Ave., Davis, CA 95616

³ United States Department of Agriculture–Agricultural Research Service, U.S. Agricultural Research Station, Crop Improvement and Protection Research Unit, 1636 E. Alisal Street, Salinas, CA 93905

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ABSTRACT

Lettuce downy mildew, caused by *Bremia lactucae* Regel, is the most economically important foliar disease of lettuce (*Lactuca sativa* L.). The deployment of resistant cultivars carrying dominant resistance genes (*Dm* genes) plays a crucial role in integrated downy mildew disease management; however, high variability in pathogen populations leads to the defeat of plant resistance conferred by *Dm* genes. Some lettuce cultivars exhibit field resistance that is only manifested in adult plants. Two populations of recombinant inbred lines (RILs), originating from crosses between the field resistant cultivars Grand Rapids and Iceberg and susceptible cultivars Salinas and PI491224, were evaluated for downy mildew resistance under field conditions. In all, 160 RILs from the Iceberg × PI491224 and 88 RILs from the Grand Rapids × Salinas populations were

genotyped using genotyping by sequencing, which generated 906 and 746 high-quality markers, respectively, that were used for quantitative trait locus (QTL) analysis. We found a QTL in chromosome 4 that is present in both Grand Rapids × Salinas and Iceberg × PI491224 populations that has a major effect on field resistance. We also found two additional significant QTLs in chromosomes 2 and 5 in the Iceberg × PI491224 RIL population. Marker-assisted gene pyramiding of multiple *Dm* genes in combination with QTLs for field resistance provide the opportunity to develop cultivars with more durable resistance to *B. lactucae*.

Keywords: *Bremia lactucae*, disease resistance, downy mildew, field resistance, genetics, *Lactuca sativa*, lettuce, quantitative trait loci

Lettuce (*Lactuca sativa* L.) is one of the most valuable crops in the United States, with an annual production of more than 8 billion pounds and a value of more than \$2.4 billion (USDA-NASS 2019). In the United States, more than 70% of the lettuce is grown in California (USDA-NASS 2019), with one of the most important regions of production in the Salinas Valley (CDFA 2016). The extensive lettuce cultivation combined with environmental conditions favorable to *Bremia lactucae*, the causal agent of downy mildew (DM), contribute to high disease pressure in the Salinas Valley (Wu et al. 2001).

Lettuce DM primarily affects the foliar tissue, reducing the quality and marketability of the crop; under severe infection, it can result in major yield losses. Lettuce plants can become infected by *B. lactucae* at any developmental stage but often show more disease severity in the field when they are close to harvest maturity (Wu

et al. 2001). DM is managed in commercial production by the combined use of fungicides and agronomic practices that minimize conditions favorable for the pathogen such as excess irrigation. The use of fungicides, however, is constrained by high costs and the development of fungicide-insensitive strains of the pathogen (Crute 1987; Schettini et al. 1991). The use of resistant lettuce varieties containing dominant resistance genes (*Dm* genes) is the most viable method for controlling this disease (Parra et al. 2016). However, the virulence specificity of *B. lactucae* can rapidly change because of its high variability, leading to the generation of new isolates that can defeat the resistance in cultivars containing *Dm* genes (Fletcher et al. 2019; Ilott et al. 1987).

Some lettuce varieties exhibit resistance in the field that cannot be attributed to *Dm* genes. Field resistance has been observed in lettuce cultivars such as Iceberg and Grand Rapids, which can tolerate infections of *B. lactucae* in the field without major yield losses (Crute and Norwood 1981; Milbrath 1923; Verhoeff 1960). Cultivars with field resistance to the disease can be infected with multiple races of the pathogen but the DM lesions typically remain small and the sporulation of the pathogen is limited. This quantitative resistance phenotype is manifested in adult plants and has been shown to be inherited polygenically (Grube and Ochoa 2005; Norwood et al. 1983; Simko et al. 2013). Such resistance has not been frequently used in lettuce breeding programs due to its complex inheritance and the difficulty of phenotyping under field conditions. Three quantitative trait loci (QTLs) for field resistance were identified in a recombinant inbred line (RIL) population derived from a cross between two field resistant cultivars, Grand Rapids and Iceberg; transgressive segregation was observed in this population, with some RILs exhibiting greater resistance than either parent (Simko et al. 2013).

We analyzed two RIL populations derived from crosses of each resistant line to a susceptible line to search for new QTLs for field resistance and to validate the previously identified QTLs. RILs derived from Grand Rapids × Salinas and Iceberg × PI491224 were

†Corresponding author: R. W. Michelmore; rwmichelmore@ucdavis.edu

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Author contributions: L.P. designed the experiments; carried out the DNA extractions, library construction, analysis of sequencing data, RIL phenotyping, collection and evaluation of *B. lactucae* isolates, QTL mapping, statistical analysis; and drafted the manuscript. I.S. developed the RILs used in this study and supervised the field experiments. R.M. conceived the study and made significant contributions to the final manuscript. All authors read and approved the final manuscript.

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evaluated for DM severity under field conditions. Two QTLs were confirmed and one new QTL was identified. All three were positioned on the lettuce reference genome; none collocated with canonical resistance genes. The knowledge of QTLs for field resistance will allow for the introgression of these resistances into multiple horticultural lettuce types using marker-assisted selection. Alternatively, genomic selection may be applied to predict DM resistance in lettuce genotypes originating from these resistant cultivars (Hadasch et al. 2016). The combination of quantitative and qualitative DM resistance could be used to achieve more durable resistance against *B. lactucae*.

MATERIALS AND METHODS

Plant material. In total, 88 and 160 F₇ RILs derived from Grand Rapids × Salinas and Iceberg × PI491224, respectively, were developed using single-descent seed from individual F₂ plants. RILs were multiplied under greenhouse conditions in earlier generations while a larger quantity of seed of the F₇ generation was produced in a field. Both Grand Rapids (leaf type) × Salinas (crisphead type) and Iceberg (Batavia type) × PI491224 (romaine type) RIL populations segregated for plant shape, color, head formation, and plant size.

DM evaluations in the field. Both populations were planted during spring 2015 (year 1) and fall 2016 (year 2) in Salinas, CA and evaluated for response to natural infection by *B. lactucae*. RIL populations, dates, the location of the experiments, and the average disease infection are summarized in Table 1. Each RIL and parent was planted in rows containing approximately 20 plants/plot following a randomized complete block design with three replicates. A DM-susceptible lettuce cultivar (Little Gem or PI491224) was used to spread the inoculum in the field and to provide disease severity data that were used to adjust the DM score of each RIL plot, thus improving the sensitivity to detect phenotypic differences among RILs. The susceptible cultivar was planted in the same double-row bed next to each RIL plot. Phenotyping DM severity was conducted twice using scores from 0 to 5, where 0 = no DM lesions on the plant; 1 = few small lesions on some plants in the plot; 2 = few lesions on most of the plants in the plot or several lesions on some of the plants in the plot; 3 = several lesions on most of the plants in the plot; 4 = multiple lesions on most of the plants in the plot; and 5 = multiple lesions on most of the plants, with the presence of dead tissue. RILs, parents, and the susceptible control were scored as the average phenotype of all of the plants from each plot. The first phenotyping was conducted when the lettuce had reached harvest maturity (average of the parents) followed by the second phenotyping 1 week later. The DM observed severity scores of the RILs for each plot were adjusted using the DM severity score of the susceptible control line growing beside it (covariable). The regression-adjusted response variable Z was defined as $Z_i = Y_i - b(X_i - \bar{X})$, where Y_i is the observed DM severity of a RIL in the plot i , X_i is observed DM severity of the spreader row next to the plot i , $\bar{X}$ is the average of the DM severity in all spreader plots, and b is the

slope of the residual regression between X and Y . The statistical analyses and calculation of the adjusted DM severity scores (Z) were carried out using SAS (SAS Institute Inc.).

Determination of the variation of virulence phenotype of *B. lactucae* collected from experimental fields. Twenty *B. lactucae* isolates were collected from the experimental fields in order to determine their virulence phenotypes and mating types. Small vein-delimited lesions were cut from infected leaves, washed with distilled water, and placed inside Magenta boxes with seedlings of Green Towers (GT), a susceptible cultivar without any known *Dm* genes, until sporulation of the lesions occurred. Isolates were then propagated asexually on GT seedlings as described previously (Brown et al. 2004). The virulence phenotype of each isolate was determined by the reactions on a differential set of 24 lettuce cultivars carrying known *Dm* genes, as described previously (Brown et al. 2004). For the determination of the mating type, *B. lactucae* isolates C82P24 (mating type B₂) and SF5 (mating type B₁) were individually coinoculated with the field isolates, followed by the scoring for sexual and asexual structures under a microscope.

Genotyping by sequencing and QTL identification. The DNA of each RIL and parental cultivar was extracted from seed and genotyped using genotyping by sequencing, as described by Macias-González et al. (2019). DNA was extracted from approximately 50 seeds of each line, digested with *Ava*II, ligated to sequencing adapters, and pooled along with the parents for both populations. The pooled multiplexed libraries were size-selected using BluePippin (Sage Science) and sequenced using an Illumina Hi-seq 2500 platform (Illumina Inc.). TASSEL 3 was used for downstream analysis to obtain individual genotypes anchored to the lettuce reference genome, using a minimum coverage of six reads for single-nucleotide polymorphism (SNP) detection (Glaubitz et al. 2014; Reyes-Chin-Wo et al. 2017). Microsoft Excel was used for visualization and selection of markers. SNP markers with <10% missing data across the different RILs and <0.2 allelic distortion were selected for further analysis; therefore, 87 Grand Rapids × Salinas individuals and 137 Iceberg × PI491224 individuals were included in downstream analyses. The SNP genotypes were converted to alleles A, H, or B, where A was homozygous for the resistant parent, B was homozygous for the susceptible parent, and H was heterozygous. The data were haplotyped by scaffolding on the lettuce reference genome using custom Python scripts (SumByGroup.py and GroupHaplotyper.py, <http://github.com/alex-kozik/atgc-xyz>). The linkage maps for both populations were constructed using MSTMap (Wu et al. 2008); linkage groups were assigned to the nine chromosomes based on the Salinas reference genome v8 (Reyes-Chin-Wo et al. 2017). QTL analysis was conducted with R/qtl using a standard interval mapping model (Broman et al. 2003). The significance threshold for the logarithm of odds (LOD) scores for QTLs was calculated by 1,000 permutations at the genome-wide significance threshold of $\alpha < 0.05$ and $\alpha < 0.01$. The QTL intervals were calculated using a 1-LOD drop interval. QTL effects were estimated as the proportion of phenotypic variance explained by the QTL (R^2).

TABLE 1. Details of field experiments of two recombinant inbred line (RIL) populations used to study the genetics of field resistance of lettuce (*Lactuca sativa*) to downy mildew (DM) (*Bremia lactucae*)

RIL population	N ^a	Location ^b	Season, year	DM spreader	Average disease severity ^c	
					At HM ^d	1 week after HM ^d
Grand Rapids × Salinas	88	Alisal	Spring, year 1	Little gem	2.6	3.5
Iceberg × PI491224	160	Spence	Spring, year 1	PI491224	2.9	4.2
Grand Rapids × Salinas	88	Spence	Fall, year 2	Little gem	2.4	3.5
Iceberg × PI491224	160	Spence	Fall, year 2	Little gem	2.1	3.3

^a Number of RILs.

^b Locations in Salinas, CA.

^c Average disease severity was assessed as the DM severity score in the DM-susceptible control line.

^d HM = harvest maturity.

RESULTS

Disease distribution in the field and adjusted DM scores.

The field experiments were carried out during two seasons in sequential years and relied on natural infections of *B. lactucae*. For this reason, we observed variation in the average disease severity (denoted by the DM severity in the DM-susceptible control line) between seasons and fields (Table 1). We also observed an uneven distribution of DM within the field for experiments with both the Grand Rapids × Salinas (Supplementary Data S1) and the Iceberg × PI491224 populations (Supplementary Data S2). The effect of the uneven distribution of DM was more pronounced in the Grand Rapids × Salinas experimental field, where 17.5% of the variation in

the DM severity of the RIL population can be attributed to the distribution of DM in the field during year 1 (Supplementary Data S1A and S6). The Iceberg × PI491224 population was less affected by disease distribution, with an average of 6% of the variation of the disease severity attributed to the distribution of DM in the field (Supplementary Data S6).

There was no significant correlation between the RIL genotypes and the susceptible control line phenotypes, suggesting that using the susceptible control line as a covariable for the DM RIL phenotype could remove the variation associated with the distribution of disease in the field from the error variance of the RILs. The adjustment of the disease severity scores of the RILs resulted in a higher correlation between replicates (Supplementary

Fig. 1. Distribution of downy mildew DM severity scores for Grand Rapids × Salinas (GR×SA) and Iceberg × PI491224 (ICE×PI) recombinant inbred line populations. Parental DM severity scores are indicated with arrows.

		Lettuce genotype																									Mating type	Host line	RIL DM-score	Allele			
		Green towers	Ledricky	UC DM2	Dandle	R4157D	Valmaine	Sabine	LSE 57/15	UC DM10	Num DM15	CGDM16	LSE /18	NunDM17	Colorado	Ninja	Discovery	Argelles	Anplus	RYZ-2164	RYZ-910457	Bedford	Balesta	Bellisimo	Femke	Iceberg				Grand Rapids	qDMR2.1	qDMR4.4	qDMR5.1
Dm/R gene		0	1	2	3	4	5/8	6	7	10	15	16	16	17	18	36	37	38	40	?	?	?	?	?	?	?	?						
B. lactucae isolate	C15L1756	+	+	+	+	-	+	+	+	+	+	-	+	-	+	+	+	-	-	-	-	+	-	-	-	+	+	B2	RIL-IP215	0.5	R	R	R
	C15L1776	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	+	+	B2	RIL-IP105	0.7	R	R	R
	C15L1743	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	+	B2	Iceberg	0.8	R	R	R
	C15L1744	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	-	+	+	B2	RIL-IP188	1.9	S	S	R
	C15L1746	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	B2	RIL-IP222	2.2	S	R	R
	C15L1742	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	B2	PI491224	3.3	S	S	S
	C15L1754	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	+	+	-	-	-	+	B2	RIL-IP259	3.3	H	H	S
	C15L1745	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	-	+	B2	RIL-IP228	3.7	S	S	S
	C15L1773	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-	-	+	B2	RIL-IP140	3.7	R	S	S
	C15L1747	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	-	+	B2	RIL-IP164	3.9	S	H	S
	C15L1774	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	B2	RIL-GS106	0.3		R	
	C15L1755	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	B2	RIL-GS105	0.5		R	
	C15L1750	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	B2	RIL-GS154	2.9		S	
	C15L1775	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	B2	RIL-GS125	3.1		S	
	C15L1751	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	B2	RIL-GS130	3.6		S	
	C15L1749	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	B2	Salinas	3.5		S	
	C15L1752	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	B2	Salinas	3.5		S	
C15L1757	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	B2	Salinas	3.5		S		
C15L1777	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	B2	Salinas	3.5		S		

Fig. 2. Diversity of virulence phenotypes of *Bremia lactucae* collected from experimental fields. Symbols + and - indicate isolates virulent or avirulent, respectively, on lettuce seedlings (recognized by a resistance [R] gene). Host line: lettuce recombinant inbred line (RIL) or line where the isolate was collected; RIL DM-score: downy mildew (DM) phenotype of the host line; Allele: allele of the host line at the quantitative trait locus regions (R = allele from the resistant parent, S = allele from the susceptible parent, and H = heterozygous allele).

Data S3). Both the Grand Rapids × Salinas and the Iceberg × PI491224 populations showed a normal distribution of adjusted DM severity scores (Fig. 1). In both years, the susceptible parents Salinas and PI491224 showed a high adjusted DM severity score, ranging from 3.3 to 3.7 and from 3.6 to 3.7, respectively. A low

adjusted DM severity score was observed for the resistant parental cultivars, ranging from 0.8 to 1.0 for Grand Rapids and from 0.04 to 0.9 for Iceberg (Fig. 1). In both years, the disease severity values in the susceptible control Little Gem were not high at the first assessment of DM severity, likely due to low disease pressure in the

Fig. 3. Quantitative trait loci (QTLs) for field resistance to *Bremia lactucae*. QTLs in green cells were identified in this study. Chr = chromosome. Downy mildew resistance QTLs that have been identified in previous studies (Parra et al. 2016) are represented in speckled cells. Solid gray areas represent the chromosomal location of the major resistance clusters (Reyes-Chin-Wo et al. 2017).

Fig. 4. Quantitative trait locus mainscan plot for Grand Rapids × Salinas (GR×SA, left) and Iceberg × PI491224 (ICE×PI, right) linkage maps using combined phenotypes from spring 2015 and fall 2016 field experiments. Significance thresholds for the logarithm of odds (LOD) scores are represented with horizontal dotted lines. The dotted lines indicate $\alpha \leq 0.05$ or $\alpha \leq 0.01$.

field. Therefore, only the DM severity values from the second assessments were used for the QTL analysis.

Diversity of *B. lactucae* in experimental fields. The isolates collected from the experimental fields had highly variable avirulence phenotypes relative to their ability to sporulate on seedlings expressing *Dm4*, *Dm6*, *Dm16*, *Dm36*, *Dm37*, and *Dm40* in the lab (Fig. 2). In contrast, the reactions on lines expressing *Dm1*, *Dm2*, *Dm5*, *Dm7*, *Dm10*, *Dm15*, *Dm16*, *Dm17*, and *Dm18* were consistent among all of the isolates collected from the experimental fields (Fig. 2). Most of the isolates had novel virulence phenotypes and did not belong to any previously designated race (Fig. 2). All of the isolates collected from the field were of the B₂ mating type (Fig. 2). Seedlings of Iceberg and Grand Rapids were fully susceptible to all of the isolates collected from the experimental fields. There was no correlation between virulence phenotype and the QTL genotype as determined below of the source RIL.

Map construction and QTL identification. We initially detected 1,633 and 947 markers for the Iceberg × PI491224 and

Grand Rapids × Salinas populations, respectively, which were distributed throughout the nine linkage groups. Markers positioned in the same genetic bin were discarded, reducing the number of markers to 906 and 746 for the Iceberg × PI491224 and Grand Rapids × Salinas linkage maps, respectively. A few gaps were detected in both linkage maps (Supplementary Data S4), where no markers were found due to either a lack of polymorphisms between the parents or insufficient read coverage to make an SNP call. The largest gaps were located in chromosome 7 for the Grand Rapids × Salinas linkage map (31 centimorgans [cM] in length) and in chromosome 8 for the Iceberg × PI491224 linkage map (29 cM length). Overall, the average distance between markers was 2 cM for the Iceberg × PI491224 linkage map and 3 cM for the Grand Rapids × Salinas linkage map. The minimum distance between markers for both linkage maps was 1 cM.

QTL analysis revealed three QTLs for field resistance located in chromosomes 2, 4, and 5 of the lettuce genome (Figs. 3 and 4). Three QTLs were detected in the Iceberg × PI491224 population

Fig. 5. Additive effect of quantitative trait loci detected in Iceberg × PI491224 RIL population. The average phenotype was calculated using all of the individuals with the corresponding allelic combinations. DM = downy mildew, AA = homozygous for the resistant allele, and BB = homozygous for the susceptible allele.

TABLE 2. Summary of quantitative trait loci (QTLs) detected from *Lactuca sativa* ‘Grand Rapids’ × ‘Salinas’ and ‘Iceberg’ × ‘PI491224’ recombinant inbred line populations^a

QTL	Chr	Source, cultivar	Exp	LOD	R ² (%)	Linkage map ^b	Reference genome v8 ^b
QDMR4.4	Chr 4	<i>L. sativa</i> , Grand Rapids	Year 1	5.9	27	...	...
QDMR4.4	Chr 4	<i>L. sativa</i> , Grand Rapids	Year 2	8.9	38	...	...
QDMR2.1	Chr 2	<i>L. sativa</i> , Iceberg	Year 1	ns	...	...	...
QDMR4.4	Chr 4	<i>L. sativa</i> , Iceberg	Year 1	ns	...	...	...
QDMR5.1	Chr 5	<i>L. sativa</i> , Iceberg	Year 1	7.6	23	...	...
QDMR2.1	Chr 2	<i>L. sativa</i> , Iceberg	Year 2	5.8	18	...	...
QDMR4.4	Chr 4	<i>L. sativa</i> , Iceberg	Year 2	6.8	20	...	...
QDMR5.1	Chr 5	<i>L. sativa</i> , Iceberg	Year 2	4.5	14	...	...
QDMR4.4	Chr 4	<i>L. sativa</i> , Grand Rapids	Comb	8.3	36	86–92 cM	107–116 Mb
QDMR2.1	Chr 2	<i>L. sativa</i> , Iceberg	Comb	4.3	13	180–183 cM	197–205 Mb
QDMR4.4	Chr 4	<i>L. sativa</i> , Iceberg	Comb	4.8	15	54–78 cM	91–110 Mb
QDMR5.1	Chr 5	<i>L. sativa</i> , Iceberg	Comb	5.9	18	132–140 cM	155–162 Mb

^a Abbreviations: Chr = chromosome, Exp = experiment, LOD = logarithm of the odds score for QTLs calculated by simple interval mapping, R² = percentage of the total phenotypic variation of the trait explained by the QTL, ns = not significant, Comb = combined, and cM = centimorgans.

^b Position of the QTL using a 1-LOD interval.

that had an additive effect (Fig. 5); the QTL on chromosome 5 explained 18% of the phenotypic variation, while the QTL on chromosome 2 explained 13% of the phenotypic variation. These two QTLs collocated with the QTLs previously identified in a population derived from Grand Rapids × Iceberg (Simko et al. 2013) (Fig. 3). A third QTL was detected on chromosome 4 that explained 15% of the phenotypic variation in the Iceberg × PI491224 population (Figs. 3 and 4). Only one QTL was detected in the Grand Rapids × Salinas population; this was located at the same position on chromosome 4 and explained 36% of the phenotypic variation. Only the QTLs in chromosome 4 from Grand Rapids and chromosome 5 from Iceberg were consistently identified in both years. The new QTL on chromosome 4 was designated *qDMR4.4* according to the conventional QTL denomination (“q” followed by the phenotype and chromosome location). The QTLs detected in this study are summarized in Table 2. Interestingly, this QTL collocated with a QTL for light green color in lettuce (Simko et al. 2016) and in both the Grand Rapids × Salinas and Iceberg × PI491224 populations, the resistant genotypes were clearly lighter green than the susceptible genotypes (Supplementary Data S5).

DISCUSSION

To isolate the true effect of the RIL genotype from the effect of DM distribution in the field, we used an experimental design that incorporated the use of a DM-susceptible control cultivar as a covariable of the RIL disease severity. This greatly increased the correlation between replicates in the RCBD experiments and the precision of the statistical model, and allowed us to estimate the disease distribution in the field. The QTL in chromosome 2 did not reach the significance threshold without including the covariable. Moreover, by using a covariable in our analysis, we were able to isolate the true effect of genotype on DM severity in the field from the effect of DM distribution in the field, which greatly increased the sensitivity of the study. We were able to consistently detect QTLs with only two field experiments; in contrast, previous studies needed multiple field evaluation to detect significant QTLs (Simko et al. 2013, 2015).

The isolates of *B. lactucae* collected from the experimental fields were fully virulent on the seedlings of Grand Rapids and Iceberg, indicating that the resistance phenotype observed in the field is not controlled by race-specific *Dm* genes (monogenic resistance) with large phenotypic effect. The isolates collected from the experimental fields varied in virulence phenotype (Fig. 2); the lack of a correlation between the virulence phenotype and the QTL genotype of the source RIL is consistent with the field resistance not being race specific.

We identified three QTLs conferring field resistance to *B. lactucae*. The two QTLs on chromosomes 2 and 5 were only detected in the Iceberg × PI491224 population. These QTLs overlapped with *qDMR2.1* and *qDMR5.1* that had been previously detected by QTL analysis of a Grand Rapids × Iceberg population (Simko et al. 2013), with the resistance alleles coming from Iceberg. Our data confirm the existence of these QTLs and their efficacy on DM field resistance, making them high-confidence targets for marker-assisted selection.

The third QTL, *qDMR4.4* located in chromosome 4, was detected in both populations of this study. It was not detected in the previous study because both parents carried a resistance allele at this QTL and, thus, this QTL did not segregate. The resistance allele at *qDMR4.4* of Grand Rapids had a major effect on DM field resistance, explaining 36% of the phenotypic variation observed. We cannot exclude the possibility that additional, small-effect resistance alleles could be present at other QTLs in Grand Rapids, which we were unable to detect due to the small size of the Grand Rapids × Salinas RIL population. Interestingly, *qDMR4.4* overlapped two QTLs previously reported in populations segregating for resistance from cultivar La Brillante and in *L. saligna* CGN05271, which also contribute to DM resistance in adult lettuce plants (Fig.

3); however, the resistance from *L. saligna* was evaluated on leaf discs rather than under natural conditions in the field and the QTL in La Brillante only had a minor effect on DM resistance (Jeuken et al. 2008; Simko et al. 2015; Zhang et al. 2009).

There were three RILs from the Grand Rapids × Salinas population that had consistently higher levels of resistance than Grand Rapids (Fig. 1), which may be indicative of transgressive segregation and the presence of resistance alleles at minor QTLs in Salinas. Transgressive segregation was also observed in one RIL from the Grand Rapids × Iceberg population (Simko et al. 2013). These transgressive phenotypes need to be confirmed by additional field testing.

The 1-LOD QTL intervals for the field resistance QTLs found in this study ranged from 3 to 10 cM, which represent a physical size range of 6.7 to 19 Mb. The lettuce reference genome contains 136 to 395 gene models in these intervals, making identification of candidate genes unfeasible without further high-resolution genetic dissection. None of the genes located in the QTL intervals in the reference genome encode nucleotide-binding leucine-rich repeat receptors (NLRs) or receptor-like kinases (RLKs), genes that commonly determine disease resistance. This suggests that the field resistant phenotype is not determined by genes encoding NLRs or RLKs unless such genes are absent in the reference genome within the QTL region. There are several RILs from both the Grand Rapids × Salinas and Iceberg × PI491224 populations that are heterozygous in at least one of the QTLs associated with field resistance. Progeny from these individuals can be used for future fine mapping of the QTL intervals, allowing for refinement of the regions and, potentially, the identification of the causal genes. This will shed light on the mechanisms involved in the quantitative resistance phenotype, which has thus far been poorly understood (Poland et al. 2009).

New races of *B. lactucae* constantly overcome race-specific resistance genes; therefore, continuous integration of new *Dm* genes needs to be applied to the lettuce breeding platforms. Quantitative field resistance has not been exploited in lettuce breeding programs for DM, mainly due to the difficulties of transferring multiple QTL loci into different cultivars and the incomplete resistance phenotype. To date, at least 16 QTLs for DM resistance have been identified (including the 3 detected in this study) that have different additive effects and significance (den Boer et al. 2013; Jeuken et al. 2008; Parra et al. 2016; Simko et al. 2015; Zhang et al. 2009); *qDMR4.4* detected in this study has a major effect on the DM resistance phenotype. Future fine mapping of the QTLs with major effects on DM field resistance will refine the QTL intervals, facilitating the identification of reliable molecular markers for marker-assisted breeding. Combining QTLs for field resistance with *Dm* genes might increase the durability of *Dm* genes and provide more reliable resistance to DM.

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