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ARTICLE

The inheritance of resistance to bacterial leaf spot of lettuce caused by *Xanthomonas campestris* pv. *vitians* in three lettuce cultivars

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Lettuce yields can be reduced by the disease bacterial leaf spot (BLS) caused by the pathogen *Xanthomonas campestris* pv. *vitians* (*Xcv*) and host resistance is the most feasible method to reduce disease losses. The cultivars La Brillante, Pavane and Little Gem express an incompatible host–pathogen interaction as a hypersensitive response (HR) to California strains of *Xcv* resulting in resistance. Little was known about the inheritance of resistance; however, resistance to other lettuce pathogens is often determined by resistance gene candidates (RGCs) encoding nucleotide-binding leucine-rich repeat (NB-LRR) proteins. Therefore, we determined the inheritance of BLS resistance in the cultivars La Brillante, Little Gem and Pavane and mapped it relative to RGCs. The reaction to *Xcv* was analyzed in nine F_1, F_2 and recombinant inbred line populations of lettuce from HR×compatible or HR×HR crosses. The HR in La Brillante, Pavane and Little Gem is conditioned by single dominant genes, which are either allelic or closely linked genes. The resistance gene in La Brillante was designated *Xanthomonas resistance 1* (*Xar1*) and mapped to lettuce linkage group 2. *Xar1* is present in a genomic region that contains numerous NB-LRR encoding RGCs and functional pathogen resistance loci in the *RGC2* family. The *Xar1* gene confers a high level of BLS resistance in the greenhouse and field that can be introgressed into commercial lettuce cultivars to reduce BLS losses using molecular markers.

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INTRODUCTION

Lettuce is a popular leafy vegetable that is widely grown in environments with moderate temperatures around the world, although more than three quarters of commercial production occurs in China, the United States, India and Spain.[1] The US lettuce crop was worth more than $1.8 billion in 2012 and California produces ~75% of the US lettuce supply.[2] Eight general horticultural or market types of lettuce are known, although romaine and iceberg represent ~85% of US production.[2,3] Bacterial leaf spot (BLS) caused by *Xanthomonas campestris* pv. *vitians* (*Xcv*) is a potentially destructive disease throughout North America[4–7] and globally.[8–10] In California, BLS typically occurs during spring and fall harvested lettuce.[11,12] Occurrence of the disease is dependent on cool and wet conditions and the unpredictable nature of these weather conditions makes BLS outbreaks erratic.[12] Typical symptoms include angular black water soaked lesions on leaves that eventually dry out and become papery.[12] In severe outbreaks, lesions can coalesce to form large necrotic patches on leaves. Infected lettuce has lower quality and yield, and may experience greater postharvest losses.[4,13]

Current cultural management strategies include avoiding fields with a history of the disease, since the pathogen likely survives from crop to crop in soil and crop residue,[11] or growing crops during a time of year when environmental conditions are rarely conducive for BLS. Application of various bactericides and biopesticides can reduce disease when applied prior to infection;[4,12] however, in years where environmental conditions are not conducive to disease, their use only raises production costs. Use of resistant cultivars is the most sustainable, reliable approach to managing BLS. Screening lettuce cultivars for resistance revealed complete and incomplete resistance to BLS, although most cultivars used for large scale commercial production were susceptible.[4,6,14–16] Several iceberg and romaine breeding lines were bred that incorporated incomplete or partial resistance from the cultivars Iceberg, Salad Crisp and Batavia Reine des Glaces.[15,17] These cultivars and breeding lines develop lower severity infections compared to other cultivars, although they are not immune to the disease. Plant Introduction 358000-1 was reported to have no disease when inoculated with *Xcv* strain L7 in greenhouse experiments.[16] High level resistance is present in the cultivars Little Gem, La Brillante and Pavane when challenged with California strains of *Xcv* in field and greenhouse experiments[14,15] (Bull CT and Hayes RJ, 2014, unpubl. data). These cultivars express an incompatible host–pathogen interaction as a hypersensitive response (HR) to *Xcv*[18,19] (Bull CT and Hayes RJ, 2014, unpubl. data), a form of programmed cell death that is part of the plant immune response protecting against otherwise virulent strains of pathogens.[20,21] The HR in Little Gem was shown to limit growth of *Xcv* below what was observed in Batavia Reine des Glaces (a partially resistant cultivar that did not exhibit an HR) following infiltration of a California strain of *Xcv* into leaves.[18]

The genetics of pest and pathogen resistance has been extensively studied in many crop species.[22] Based on the inheritance of the resistance phenotype, resistance is often categorized as monogenic or polygenic. Monogenic resistance, also referred to as major gene resistance, often but not always, confers a high level of resistance. Polygenic resistance is determined by multiple minor genes or quantitative trait loci resulting in varying levels of incomplete to complete resistance. Numerous resistance loci have been cloned

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and characterized from a broad range of plant species.[22,23] Most, but not all, major resistance genes encode nucleotide-binding site leucine-rich repeat (NB-LRR) proteins.[21,22] NB-LRR proteins detect pathogen effectors or the result of effector actions often leading to an HR.[20,24] Pathogens secrete numerous effector proteins into their hosts; plants that lack cognate resistance genes exhibit compatible host-pathogen interactions resulting in disease.[20,22]

More than 30 phenotypic resistance loci are known in lettuce and a few major genes have been cloned and sequenced.[25–28] Genomic sequencing of lettuce has identified hundreds of genes encoding NB-LRR proteins[26] (https://lgr.genomecenter.ucdavis.edu); these are referred to as resistant gene candidates (RGCs). Genetic analysis of disease resistance phenotypes has provided an efficient route for molecular marker development and for selecting RGCs for functional testing.[25,26,28,29] Most major genes and resistance quantitative trait loci colocalized to regions of the lettuce genome containing clusters of RGCs.[26] RGCs in lettuce can be categorized into 20 families based on sequence similarity.[26] *RGC2* is a large family that includes up to more than 40 members on chromosome 2 and confers resistance to several economically important oomycete, fungal and insect pests.[26,28,30]

Xanthomonads cause disease on a wide range of monocot and dicot crops.[31] While little was known about the inheritance of BLS resistance in lettuce,[15,16] resistance in pepper, tomato and rice against specific strains of their respective pathogenic *Xanthomonads* is conferred by dominant and recessive single genes.[32,33] Cloning and sequence analysis of several genes from these crops indicated that the genes conferring resistance against *Xanthomonads* are functionally diverse. In addition to genes encoding NB-LRR proteins, several dominant resistance genes were determined to encode receptor like kinase proteins involved in microbe-associated molecular pattern-triggered immunity.[32–34] Furthermore, the *Bs3* gene in pepper has sequence similarity to flavin monooxygenases and *Xa27* in rice encodes a novel protein.[35,36] Recessive genes for resistance against *Xanthomonads* in tomato, pepper and rice may condition HR-like lesions or a delayed or weak response that is nonetheless distinguishable from a compatible interaction.[32,33,37] Some recessive resistance genes are actually susceptibility genes targeted by transcription activator-like effectors and are involved in plant growth and development.[32,34]

The objectives of this study were to determine the inheritance of BLS resistance in the cultivars La Brillante, Little Gem and Pavane and to map BLS resistance in the lettuce genome relative to RGCs and other potentially resistance-related genes.

MATERIALS AND METHODS

Population development

Lettuce is a diploid ($2n=2x=18$) near obligately self-pollinating species and cultivars are highly uniform inbred lines.[3] All F_1 seed was generated using the hand pollination method of Ryder and Johnson[38] or by crossing to Ms7-Salinas, a line of the cultivar Salinas carrying the *Male sterile-7* (*Ms7*) gene.[39] Ms7-Salinas has been backcrossed to Salinas more than seven times to incorporate *Ms7*. Self-pollination was used to produce seed of F_2 and later generations and seed from each plant was kept separate, unless otherwise noted.

Multiple F_1 and F_2 populations were developed to evaluate segregation and dominance of the HR phenotype using crosses between cultivars expressing an HR (La Brillante, Pavane and Little Gem) to cultivars or breeding lines exhibiting compatible interactions (Salinas, Salinas 88, Ms-7 Salinas, Tiber, Waldmann's Green and Clemente). A population of 95 $F_{6:8}$ recombinant inbred lines (RILs) from the cross-Salinas 88×La Brillante[40] and 93 F_6 RILs from Clemente×Little Gem were developed using single seed descent.[41]

Populations from crosses involving two lines that express an HR were developed for allelism experiments to test for the possibility of different and unlinked dominant genes conferring the HR. These included F_2 La Brillante×Little Gem, F_2 La Brillante×Pavane, and progeny from crossing Ms7-Salinas as the female parent to a F_1 plant from La Brillante×Little Gem [Ms7-Salinas×(F_1-La Brillante×Little Gem)]. Lack of segregation indicates that genes are allelic or separate but linked genes.

Field experiments

Ninety RILs from Salinas 88×La Brillante and the cultivars Salinas 88 and La Brillante were planted on 2 September, 2011 and grown to market maturity in a Salinas, CA field experiment. RILs and parents were seeded and grown in 6.1-m-long plots of a single seed line on a 1-m wide double seed line bed. Recombinant inbred lines were assigned to plots using a randomized complete block design with three replications. An additional replicate plot of Salinas 88 and La Brillante were included in each block resulting in six replicates of each parent. All trials were maintained using standard cultural practices appropriate for the region.[3] Natural infection of *Xcv* causing BLS occurred in this experiment and plots were evaluated on 21 November and 28 November for BLS severity using a 0–10 scale that corresponds to the percentage of leaves with BLS symptoms divided by 10. Five randomly selected plants per plot were evaluated and then averaged into a plot mean for use in all subsequent data analyses.

Greenhouse experiments

Greenhouse experiments used either a mixture of three *Xcv* strains (BS340, BS339 and BS347) or the strain BS347 alone.[14] Inoculum for use in infiltration and spray-on inoculations was prepared and adjusted to approximately 1×10^8 colony-forming-unit mL^{-1} using the methods of Hayes *et al*.[15]

Greenhouse experiments were mostly conducted using four week old seedlings grown in plug trays with 31 rows of 11 cells that are 19×19×60 mm. *Xcv* infiltration experiments were conducted in a growth chamber maintained at 20 ℃ and 16-hday length, while *Xcv* spray inoculations were conducted in a greenhouse with ambient lighting and temperatures maintained between 18 ℃ and 24 ℃. The Clemente×Little Gem RILs were the only lines not evaluated in plug trays. These RILs were grown for 4 weeks in plug trays, transplanted into 6-inch pots and then grown for another 8 weeks in the greenhouse prior to being infiltrated with *Xcv*.

Expression of the HR was determined in all RIL, F_2 and F_1 populations by infiltrating *Xcv* into leaves using a needless 1-mL syringe according to the methods of Bull *et al*.[19] The HR was observed 18–48 h after infiltration as light-brown papery lesions at the infiltration site (Supplementary Figure S1). Compatible interactions appeared healthy up to 24–48 h, but subsequently developed black watery lesions that resembled BLS disease. All F_1 progeny were tested in a single experiment while each RIL population and each F_2 population were tested in separate experiments. Experiments with Salinas 88×La Brillante RILs evaluated six plants per RIL. Nine RILs were re-tested using 29 plants per RIL to determine if these RILs were segregating for the HR. $F_{6:8}$ RILs that were mixtures of plants with an HR and plants having a compatible interaction were considered to be derived from a heterozygous F_6 plant. In all these experiments, parental cultivars and in some cases, the BLS susceptible cultivar Vista Verde, were included as controls. The Clemente×Little Gem RILs were evaluated using a single F_6 plant per RIL and the experiment did not include the parental cultivars.

Disease severity (DS) was assessed on Salinas 88×La Brillante RILs in two greenhouse experiments by spraying *Xcv* inoculum onto seedlings until run off using the approach described by Bull *et al*.[14] Lines (RIL or parent) were arranged as a randomized complete block design with three blocks with nine seedlings per treatment replicate. Disease severity was assessed using a 0–5 rating scale described by Hayes *et al*.[15] where '0=no disease; 1⩽10 lesions of <3 mm in size; 2=individual disease lesions >3 mm or more than 10 lesions; 3=large coalesced lesions covering less than 20% of leaf area; 4=lesions covering 20–50% of leaf area; 5=lesions covering >50% of leaf area'. The average DS of each plot was calculated and used for data analysis.

Analysis of segregation for the HR and genetic mapping

The frequency of RILs or plants expressing the HR and those expressing a compatible interaction was tabulated and the ratio was calculated. The observed ratio was compared to the expected segregation ratio for a single gene in F_2 and RIL populations using Chi-square. The expected number of segregating $F_{6:8}$ Salinas 88×La Brillante RILs was calculated assuming that at a single locus 1/32 F_6 plants will be heterozygous. The HR to *Xcv* in the Salinas 88×La Brillante population was positioned on a genetic linkage map alongside amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism (SNP) markers in the Salinas 88×La Brillante population. Generation of AFLP marker data for this population is described in Hayes *et al*.[40] while generation of SNP data is described in Hayes *et al*.[29] A full map of this population using this AFLP and SNP data was previously

published and has fourteen linkage groups ranging in length from 13.1 to 194.4 cM and totaling 904 cM.[40] The Salinas 88×La Brillante linkage group (LG) 2 genetic map was aligned to a map of LG 2 from Salinas×UC96US23 comprised of three AFLP markers, three anonymous SNP markers and 107 markers derived from EST sequences previously reported in McHale *et al*.[26] The three AFLP markers mapped in Salinas×UC96US23 also segregated in Salinas 88×La Brillante and were used for map alignment. In addition, segregation data for nine SNP markers in Salinas×UC96US23 assayed using an Affymetrix oligonucleotide array were obtained from Truco *et al*.[42] These SNPs were chosen because they segregated in Salinas 88×La Brillante and Salinas×UC96US23. Ninety Salinas×UC96US23 RILs had segregation data for all markers and were used in linkage analysis. Linkage mapping for both populations was conducted in the software MapDisto v 1.7.6.5[43] using the Kosambi mapping function and the unidirectional growth algorithm. The Salinas 88×La Brillante and Salinas×UC96US23 maps were aligned using the software MapChart v 2.2.[44]

Analysis of disease severity data

Disease severity data from the field experiment were analyzed to compare the resistance of RILs carrying a gene conferring the HR (described in the results section) to RILs having a compatible interaction to *Xcv*. Recombinant inbred lines were coded according to their genotype for the gene controlling the HR (homozygous for the HR, segregating or homozygous for the compatible interaction), hereafter referred to as the HR genotype. Data were right skewed and exhibited unequal variances between treatments. Therefore, data were analyzed using analysis of variance type statistics of ranked DS data using the PROC Mixed procedure in SAS.[45,46] Data for parents (Salinas 88 and La Brillante) and RILs were analyzed separately. The LD_CI macro was used to generate relative treatment effects (RTEs) for each treatment and 95% confidence intervals for detection of statistical differences between treatments. The median and third quartile (Q3) were calculated for each treatment.

Disease severity data for RILs (excluding parents) observed in greenhouse experiments were analyzed using Proc Mixed of SAS (version 9.3; SAS Institute, Cary, NC, USA) following the methods of Littell *et al*.[47] to compare HR genotypes for BLS resistance. The probability that variance estimates for HR genotypes were different from zero was determined using a Wald test. Least square means were requested for each HR genotype and were compared using the least significant difference procedure. Mean DS of Salinas 88 and La Brillante were calculated for each experiment and compared using a *t*-test assuming unequal variances. To further explore genetic variation for DS within each HR genotype, data from each HR genotype in each experiment was analyzed separately in Proc Mixed. Least square means for RILs were requested and compared using 99% confidence intervals.

RESULTS

Inheritance of HR to *Xcv*

Parental cultivars, RILs, and F_2 and F_1 plants exhibited qualitative differences for their response following infiltration with *Xcv* and were categorized as HR or compatible. Segregation among RILs within the Clemente×Little Gem and Salinas 88×La Brillante populations did not deviate significantly from ratios expected for a single gene controlling the HR (Table 1); similarly, F_2 progeny from Clemente×Pavane, La Brillante×Tiber and Little Gem×Waldmann's Green exhibited segregation ratios that did not deviate significantly from a 3 : 1 ratio expected for a single dominant gene (Table 2). The F_1 plants from crossing Ms7-Salinas to Little Gem, Pavane and La Brillante uniformly expressed the HR, further indicating that HR was dominant (Table 2). In all experiments, an HR was observed in the cultivars Pavane, La Brillante and Little Gem while Clemente, Tiber, Waldmann's Green, Salinas, Salinas 88, Ms-7 Salinas and Vista Verde exhibited compatible interactions (Tables 1 and 2).

Evaluation of the HR in F_2 progeny from intercrossing parents that exhibit HR detected no plants with a compatible interaction (Table 3). Additionally, a population of 194 plants derived from crossing Ms7-Salinas to an F_1 plant from La Brillante×Little Gem also uniformly exhibited the HR. As with previous experiments, Little Gem, Pavane and La Brillante uniformly exhibited an HR, while plants of Ms7-Salinas and Vista Verde had compatible interactions. These results suggest that the HR in La Brillante, Little Gem and Pavane is conditioned by the same gene or separate but linked genes.

The single dominant gene conferring the HR in La Brillante was mapped to LG2 near the BOMS SNP marker in Salinas 88×La Brillante and was designated *Xanthomonas resistance 1* (*Xar1*; Figure 1). Alignment of the Salinas 88×La Brillante map to the map from Salinas×UC96US23 positioned *Xar1* within an 8.1 cM region between AFLP marker E35/M49-189 and the BOMS SNP marker. This region of the Salinas×UC96US23 map contains a cluster of at least seven RGCs that encode NB-LRR proteins, five of these belong to the *RGC2* family.[26]

BLS disease severity in Salinas 88×La Brillante RILs

Disease severity of Salinas 88×La Brillante RILs in the field experiment was dependent on the genotype of the RILs at the *Xar1* locus (Table 4). At the first evaluation time point, no differences for the median DS were observed among the RIL *Xar1* genotypes. However, the Q3 DS of the *Xar1Xar1* RILs was lower than the segregating RILs and the *xar1xar1* RILs. Recombinant inbred lines with the *Xar1Xar1* genotype had lower median and Q3 DS than the segregating RILs and the *xar1xar1* RILs at the second evaluation time point. The RTE for *Xar1Xar1* RILs was significantly lower than those with an *xar1xar1* genotype, while the segregating RILs were not significantly different from either group. Bacterial leaf spot disease was observed on Salinas 88, while disease was generally absent on La Brillante. The median and Q3 DS for Salinas 88 on 21 November and 28 November were greater than La Brillante. The November 21 DS RTE for La Brillante (0.27) and Salinas 88 (0.77) had standard errors of zero and no confidence intervals for comparison of RTE could be calculated. The November 28 RTE for these two cultivars was significantly different. Recombinant inbred lines with the *Xar1Xar1* genotype generally had similar DS as La Brillante. In contrast, no RILs were observed with DS as high as Salinas 88.

Table 1. Distribution of the HR or compatible interaction in lettuce RIL populations derived from Clemente×Little Gem and Salinas 88×La Brillante and the parents Salinas 88 and La Brillante after infiltration of leaves with a mixture of *Xanthomonas campestris* pv. *vitians* strains BS339, BS340 and BS347 in growth chamber or greenhouse experiments

RIL population	RILs or plant tested (no.)[1]	RILs or plants with HR (no.)	RILs segregating (no.)	RILs or plants with compatible interactions (no.)	*P* of χ^2
Experiment 1					
F_6-Clemente×Little Gem	93	49	na	44	0.60[2]
Experiment 2					
F_8-Salinas 88×La Brillante	90	41	5	44	0.48[3]
Salinas 88	12	0		12	
La Brillante	12	12		0	

[1] One plant per RIL was tested for Clemente×Little Gem and six plants per RIL were tested for Salinas 88×La Brillante. Twelve plants of Salinas 88 and La Brillante were tested.
[2] Probability of acceptable fit to a 1 HR:1 compatible with 1 df.
[3] Probability of acceptable fit to a 31 HR:2 segregating:31 compatible with 2 df.

Table 2. The distribution of plants expressing an HR or compatible interaction in F_2 and F_1 progeny and their parents after infiltration of leaves with *Xanthomonas campestris* pv. *vitians* strain BS347 in growth chamber experiments

Population and generation	Plants tested (no.)	Plants with HR (no.)	Plants with compatible interactions (no.)	$P(\chi^2, 1\ df)$[1]
Experiment 1				
F_2-Clemente×Pavane	316	231	85	0.61
Pavane	12	12	0	
Clemente	11	0	11	
Vista Verde	12	0	12	
Experiment 2				
F_2-La Brillante×Tiber	181	137	44	0.83
La Brillante	5	5	0	
Tiber	7	0	7	
Vista Verde	4	0	4	
Experiment 3				
F_2-Little Gem×Waldmann's Green	168	127	41	0.86
Little Gem	5	5	0	
Waldmann's Green	6	0	6	
Vista Verde	9	0	9	
Experiment 4				
F_1-Ms7-Salinas×Little Gem	8	8	0	
F_1-Ms7-Salinas×La Brillante	6	6	0	
F_1-Ms7-Salinas×Pavane	7	7	0	
Little Gem	8	8	0	
Pavane	7	7	0	
La Brillante	8	8	0	
Salinas	8	0	8	
Ms7-Salinas	8	0	8	
Salinas 88	8	0	8	

[1] Probability of acceptable fit to 3 HR:1 compatible segregation in the F_2 progeny.

Table 3. The distribution of plants expressing an HR or compatible interaction after infiltration of leaves with *Xanthomonas campestris* pv. *vitians* strain BS347 in three lettuce populations from HR×HR crosses, their parents and the susceptible cultivar Vista Verde tested in growth chamber experiments

Population	Plants tested (no.)	Plants with HR (no.)	Plants with compatible interactions (no.)
Experiment 1			
F_2-La Brillante×Little Gem	214	214	0
Little Gem	16	16	0
La Brillante	16	16	0
Vista Verde	15	0	15
Experiment 2			
F_2-La Brillante×Pavane	214	214	0
Pavane	18	18	0
La Brillante	17	17	0
Vista Verde	18	0	18
Experiment 3			
Ms7-Salinas×(F_1-La Brillante×Little Gem)	194	194	0
La Brillante	17	17	0
Little Gem	17	17	0
Ms7-Salinas	17	0	17
Vista Verde	18	0	18

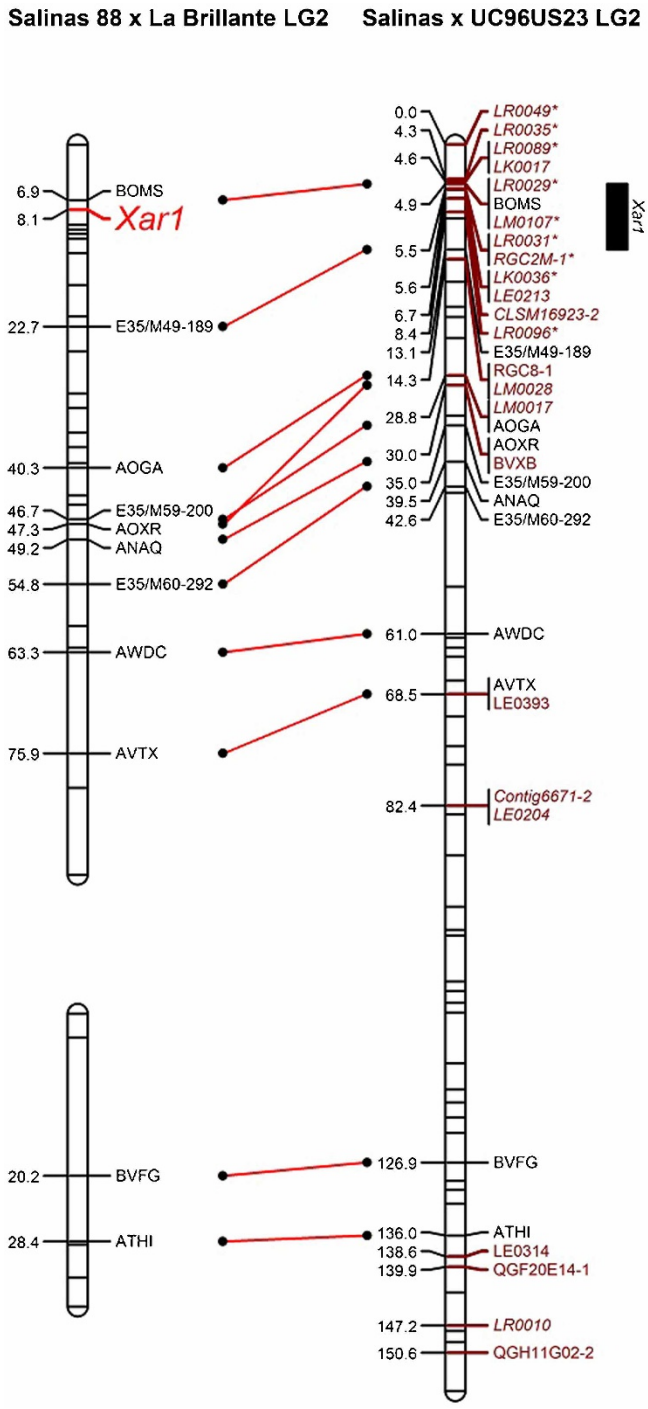

Figure 1. Position of the *Xanthomonas resistance 1* (*Xar1*) gene, 10 SNP markers and 36 AFLP markers on lettuce LG2 in the recombinant inbred line population Salinas 88×La Brillante. The Salinas 88×La Brillante genetic map was aligned to a map of LG2 from a cross between *L. sativa* cultivar Salinas and the *L. serriola* accession UC96US23 (Salinas×UC96US23) comprised of 122 markers previously reported in McHale *et al.*[26] and Truco *et al.*.[42] Codes for markers segregating in both Salinas×UC96US23 and Salinas 88×La Brillante are shown on both maps. In the Salinas×UC96US23, codes are also shown for markers that are resistance-related genes. Markers codes shown in brown text are resistance-related genes, those in italics are NB-LRR encoding genes and those with an asterisk (*) are in the *RGC2* family (McHale *et al.* 2009).[26] The black bar adjacent to the Salinas×UC96US23 map indicates the location of *Xar1.*

Table 4. Bacterial leaf spot disease severity for RILs from Salinas 88×La Brillante that are homozygous or segregating at the *Xar1* locus in a replicated Salinas, CA field experiment with natural infection

			November 21 disease severity (0–10)[2]						November 28 disease severity (0–10)					
					Relative treatment effect[3]						Relative treatment effect			
							95% CI						95% CI	
Parent cultivar or RIL *Xar1* genotype	*Xcv* infiltration response[1]	RILs (no.)	Median	Q3[4]	Estimate	s.e.	Lower limit	Upper limit	Median	Q3	Estimate	s.e.	Lower limit	Upper limit
Parent cultivar														
La Brillante[5]	HR		0.0	0.0	0.27	0.00	—	—	0.3	0.4	0.29	0.02	0.27	0.44
Salinas 88	Compatible		2.8	5.4	0.77	0.00	—	—	7.4	8.0	0.75	0.03	0.57	0.77
RIL *Xar1* genotype														
Xar1Xar1	HR	41	0.0	0.0	0.42	0.01	0.40	0.45	0.0	0.5	0.39	0.01	0.36	0.42
xar1xar1	Compatible	44	0.0	0.6	0.57	0.01	0.54	0.60	1.0	3.0	0.61	0.02	0.58	0.64
Segregating	HR/compatible	5	0.0	0.6	0.60	0.07	0.45	0.72	0.3	4.0	0.57	0.08	0.40	0.71

[1] HR and compatible interactions determined by infiltrating leaves of 4-week-old plants with *Xcv* in previous growth chamber experiments.
[2] Disease severity rated as 0=no disease to 10=severe disease.
[3] The relative treatment effect and 95% confidence intervals (CI) were calculated from analysis of rank values of the disease severity data.
[4] Q3=quartile three, s.e.=standard error and CI=confidence interval.
[5] Data from RILs and data from parents (La Brillante and Salinas 88) were analyzed separately. Separate statistical comparisons are made between parents or within RIL *Xar1* genotype.

Disease severity of Salinas 88×La Brillante RILs in the greenhouse experiments were also dependent on the *Xar1* genotype (Table 5). In greenhouse experiment 1, *Xar1Xar1* RILs had a DS mean of 1.5, which was significantly lower than the mean of 2.9 observed in *xar1xar1* RILs. Similar differences were observed in greenhouse experiment 2. In both greenhouse experiments La Brillante had significantly ($P<0.01$) lower DS than Salinas 88. The DS variances between RILs within *Xar1Xar1* and *xar1xar1* groups were significantly different from zero, indicating the possibility of additional resistance genes segregating within each group. However, no significant differences between RIL DS means within the compatible group or within the HR group were observed in both greenhouse experiments (Supplementary Table S1).

DISCUSSION

We have identified a new gene in the cultivar La Brillante conferring resistance to specific strains of the bacteria *Xanthomonas campestris* pv. *vitians* through an HR (Tables 1 and 2 and Figure 1). We named this gene *Xanthomonas resistance 1*, as the first major gene characterized in lettuce conferring resistance to this pathogen. The cultivars Little Gem and Pavane likely also carry *Xar1*, though it is possible these cultivars carry different genes that are closely linked to *Xar1* (Table 3). *Xar1* maps to a region of the lettuce genome harboring a cluster of NB-LRR encoding *RGC2* genes as well as phenotypic loci conferring resistance against downy mildew (*Dm* genes) and root aphid.[28] Consequently, *Xar1* may be involved in pathogen recognition. Mapping *Xar1* within this *RGC* cluster provides candidate genes for functional testing and for development of molecular markers to use in breeding using marker assisted selection.[26,28]

The dominant *Xar1* allele confers a high level of resistance to BLS in greenhouse and field experiments (Tables 4 and 5). In greenhouse experiments, most RILs with an *Xar1Xar1* genotype were rated with DS greater than zero, although in rare cases DS ratings as high as three were observed. These values resulted from the occurrence of small tan spots that occasionally coalesced (Supplementary Figure S2). It was difficult to determine if these

Table 5. Bacterial leaf spot disease severity of recombinant inbred lines (RIL) from Salinas 88×La Brillante that are homozygous or segregating at the *Xar1* locus in two replicated greenhouse experiments inoculated with a three strain mixture of *Xcv*

			Disease severity (0–5)[1]			
			Greenhouse experiment 1		Greenhouse experiment 2	
Parent cultivar or RIL *Xar1* genotype	*Xcv* infiltration response[2]	RILs (no.)	Mean	Variance among RILs	Mean	Variance among RILs
Parents cultivar						
La Brillante[3]	HR		0.9[4]		2.2[4]	
Salinas 88	Compatible		3.3		4.5	
RIL *Xar1* genotype						
Xar1Xar1	HR	44	1.5a[5]	0.23*	2.5a[e]	0.18*
xar1xar1	Compatible	5	2.9b	0.04*	4.5b	0.10*
Segregating	HR/compatible	41	2.4ab	0.15	3.0a	0.12

[1] Disease severity rated as 0=no disease to 5=severe disease.
[2] HR and compatible interactions determined by infiltrating leaves of 4-week-old plants with *Xcv* in previous growth chamber experiments.
[3] Data from RILs and data from parents (La Brillante and Salinas 88) were analyzed separately.
[4] Means of Salinas 88 and La Brillante significantly different at $P<0.01$ using a *t*-test assuming unequal variances.
[5] RIL *Xar1* genotype means with different letters are significantly different at $P<0.05$.
* Variance estimate significantly different from zero at $P<0.05$.

symptoms were BLS disease or a consequence of the HR in an environment highly conducive to *Xcv* and inoculated with high populations of the pathogen. Macroscopic symptoms have been reported with other major resistance genes, such as the *Dm16* gene, which confers an HR to specific strains of *Bremia lactucae* Regel. causing lettuce downy mildew.[28] In field experiments, disease was essentially absent on La Brillante and *Xar1Xar1* RILs. RILs with an *xar1xar1* genotype were diseased, but not at the levels observed on Salinas 88. Random selection during inbreeding is expected to result in at least a few RILs having similar BLS susceptibility as Salinas 88 and it is not known why Salinas 88 was so highly diseased compared to the RILs in this experiment.

La Brillante, Little Gem and Pavane are not popular types of lettuce for the US market and are not widely grown in California. The *Xar1* gene will need to be bred into romaine and iceberg cultivars in order for the lettuce industry to achieve reduced disease losses from BLS. The numerous resistance genes on LG2 may present challenges for breeders attempting to combine *Xar1* with other disease resistance genes. Twelve loci conferring resistance to six lettuce pathogens and pest are located on LG2 in the general region of *Xar1* (Figure 1 and Ref. 26). Other resistance loci are also known to be on LG2, but the distance between these genes and *Xar1* has not been accurately determined.[27,48,49] Molecular markers amenable to high through put screening will be useful for selecting the recombinant genotypes with *cis* linkage between *Xar1* and other valuable pathogen resistance loci. Downy mildew is of particular concern, as several *Dm* loci are present on LG2. BLS and downy mildew are promoted by similar environmental conditions and BLS often occurs in combination with downy mildew. Therefore, combining resistance to both pathogens in *cis* would be desirable as the resistance to the two pathogens could then be manipulated in breeding programs as a single Mendelian unit. As an alternative to using marker-assisted selection to identify recombinants combining closely linked resistance genes on LG2, breeders could choose parents possessing *Dm* genes not located on LG 2; several are known on LGs 1, 3, 4 and 9.[25,26] Additionally, La Brillante possesses resistance to downy mildew in addition to BLS, which makes it a more preferable breeding parent than Little Gem and Pavane.[50] Additionally, an iceberg breeding line was selected and publically released from Salinas 88×La Brillante for combining resistance to BLS, downy mildew and race 1 isolates of *V. dahliae*.[51] The breeding line is not suitable for commercial production, but is expected to be used as a parent to develop new breeding populations.

The *Xar1* gene has demonstrated resistance to three California strains of *Xcv* (BS340, BS339 and BS347), though the breadth of *Xcv* diversity to which *Xar1* confers resistance is not fully known. Isolates of *Xcv* collected from around the globe were reported to be homogenous.[52,53] However, strains previously identified as *Xcv* were shown to belong to two different species, *X. hortorum* and *X. axonopodis*.[54] Furthermore, Sahin *et al*. [55] reported two groups of *Xcv* based on distinct disease phenotypes; these groupings were supported by a range of phenotypic and molecular assessments. Recent data indicates that the strains used in these experiments belong to *X. hortorum* and are in group B of the Sahin *et al*.[55] classification (Bull CT, 2014, unpubl. data). In other crop species, major genes conferring resistance to *Xanthomonads* operate in a race specific manor.[32,33] Little Gem was classified as resistant in this and other reports,[14,15] but was considered to be only moderately resistant in research conducted in Florida, the USA.[56] Differences in the level of resistance reported in Little Gem may result from the use of different *Xcv* strains, potentially including strains that have a compatible interaction with Little Gem. It seems likely that *Xar1* and additional genes for *Xcv* resistance will be needed to manage BLS, a strategy used in other crops to control diseases caused by *Xanthomonads*.[22,32–34] In addition to La Brillante, Pavane and Little Gem, several other lettuce cultivars and accessions are resistant to *Xcv*.[14–16,51] While some of these additional cultivars have been used to breed *Xcv*-resistant lettuce,[15,17] determining the inheritance of their resistance may encourage their more widespread use in lettuce breeding.

CONCLUSION

Xar1 is a single dominant gene on chromosome 2 found in the cultivar La Brillante that confers high-level resistance to specific strains of *Xcv* causing BLS. Resistance genes in the cultivars Pavane and Little Gem are either allelic or are closely linked to *Xar1*. *Xar1* can be breed into improved cultivars that can reduce losses from BLS.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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