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ORIGINAL PAPER

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Genetic analysis and mapping of resistance to lettuce dieback: a soilborne disease caused by tombusviruses

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Abstract A diverse collection of modern, heirloom and specialty cultivars, plant introduction (PI) accessions, and breeding lines of lettuce were screened for susceptibility to lettuce dieback, which is a disease caused by soilborne viruses of the family *Tombusviridae*. Susceptibility was evaluated by visual symptom assessment in fields that had been previously shown to be infested with *Lettuce necrotic stunt virus*. Of the 241 genotypes tested in multiple field experiments, 76 remained symptom-free in infested fields and were therefore classified as resistant to dieback. Overall, resistant genotypes were as prevalent among modern cultivars as in heirloom cultivars or primitive germplasm. Within modern germplasm, however, all crisphead (iceberg) cultivars were resistant, while all romaine cultivars were susceptible. Using enzyme-linked immunosorbent assay, tombusviruses were detected in leaves of some plants of resistant genotypes that were grown in infested fields, suggesting that symptom-free plants are not immune to viral infection. The inheritance of resistance was studied for 'Salinas', a modern iceberg cultivar, and PI 491224, the progenitor of recently released romaine germplasm with resistance to lettuce dieback. Resistance was conferred by a dominant allele at a single locus in both genotypes. The tombusvirus resistance locus from 'Salinas', *Tvr1*, was mapped in an intraspecific *Lactuca sativa* population to a location that corresponds to linkage group 2 on the consensus map of *Lactuca*. The largest cluster of resistance genes in lettuce, the *Dm1*/*Dm3* cluster, is found on this linkage group; however, the precise position of *Tvr1* relative to this cluster has not yet been determined. To our knowledge, *Tvr1* is the first tombusvirus resistance gene identified for any plant host.

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Introduction

The disease lettuce dieback is caused by several members of the soilborne virus family *Tombusviridae*, including the type member, *Tomato bushy stunt virus* (TBSV), and *Lettuce necrotic stunt virus* (LNSV) (Obermeier et al. 2001). Symptoms of lettuce dieback include mottling and necrosis of older leaves, stunting, and plant death (Obermeier et al. 2001). The characteristic symptoms are diagnostic and usually appear after the plant has reached 6–8 weeks of age and render the plant unmarketable.

Although readily transmitted to experimental hosts by mechanical foliar inoculation, LNSV and TBSV systemically infect natural hosts through roots and do not require a vector for transmission (Martelli et al. 1988). Optimal cultural practices may lessen the impact of lettuce dieback by reducing incidence or delaying symptom development, but there are no known methods to prevent the disease in lettuce crops grown in infested fields (Davis et al. 2000; Wisler and Duffus 2000; Wintermantel and Anchieta 2003). Further, preliminary studies have provided no evidence that chemical treatment or rotation with nonhost crops can effectively reduce, remove, or destroy the virus in infested soil (W. Wintermantel, unpublished results). As a result, susceptible crops grown in fields that were previously infested consistently show symptoms, and genetic resistance remains the most viable option for disease control.

Most of the economically significant damage caused by tombusviruses occurs on tomato and lettuce (Davis et al. 2000; Gerik et al. 1990; Liu et al. 1999). Resistance has not been described for tomato, but some lettuce cultivars have been shown to remain completely free of symptoms when grown in infested soil (Grube and Ryder 2003). Because these cultivars show no effects of the disease, we have used the term ''resistance'' to describe the response of these cultivars; however, whether these cultivars are completely resistant or merely tolerant of infection has not been determined. The inheritance of this response has not been investigated.

Losses have been reported for romaine, red leaf, green leaf, and butterhead lettuce types, indicating that some commercial cultivars in each of these classes are susceptible to the disease. Thus far, however, symptoms of the disease have not been observed in modern crisphead (iceberg) cultivars. Prior to the initial reports of lettuce dieback in the late 1980s, the overwhelming majority of lettuce grown in the US was of the crisphead type. Since that time, the total acreage affected by lettuce dieback has increased in tandem with production of noncrisphead lettuces. The increased prevalence of the disease may be due to more widespread cultivation of susceptible germplasm, wider distribution of the pathogen, or both.

The simultaneous increases in demand for romaine lettuce and in crop losses resulting from lettuce dieback have created an urgent need for romaine cultivars with resistance to this disease. Our primary objectives were to evaluate the susceptibility of cultivated and exotic lettuce germplasm to this new disease, to determine the inheritance of the resistance response observed for crisphead cultivars and romaine germplasm, and to map the locus or loci that confer resistance in the crisphead 'Salinas' ('Saladin'). Finally, as a first step toward characterizing the mechanism of resistance, we sought to determine whether it was possible to detect tombusviruses in tissues of cultivars that remained free of symptoms when grown in the presence of the pathogen.

Materials and methods

Genetic materials

The germplasm evaluated included 112 modern and 69 heirloom cultivars, 60 landraces or accessions of *Lactuca sativa*, and nine wild relatives of lettuce including *L. saligna*, *L. serriola*, and *L. virosa*. The term ''heirloom'' describes those varieties that are not grown extensively in major production areas, but that were previously cultivated or that are now grown on a small scale. All types of lettuce were tested, including romaine, leaf, butterhead, Latin, crisphead, and specialty cultivars, such as those grown solely for mesclun/salad mixes (baby romaine) or for consumption of mature stems (stem lettuces). The term ''crisphead'' includes both modern heading cultivars (iceberg lettuces) as well as modern and heirloom Batavia cultivars, which form looser heads (Ryder 1999). The cultivar named 'Iceberg', which was used as a susceptible control for our studies, is not an iceberg lettuce, but actually an heirloom Batavia type lettuce.

Most germplasm was obtained either from commercial seed sources or from the USDA–ARS lettuce germplasm collection in Salinas, Calif. Seeds of *L. saligna* UC96US23, *L. virosa* IVT280, the *L. sativa* breeding line SVR6603A (now being marketed as 'Triple Threat'), and *L. sativa* 'Saladin' were provided by R. Michelmore (University of California, Davis, Calif., USA), B. Maisonneuve (INRA, France), W. Waycott (Seminis Vegetable Seeds, Arroyo Grande, Calif., USA), and D. Astley (HRI, UK), respectively.

For inheritance studies, two resistant genotypes were used: the romaine landrace PI 491224 and the modern iceberg 'Salinas'/'Saladin' (synonyms used in the U.S. and Europe, respectively). Six susceptible genotypes were used: 'Iceberg', the red leaf 'Lolla Rossa', and the modern romaine 'Green Towers', 'Lobjoits', 'Parris Island Cos' (PIC), and 'Valmaine'. Controlled reciprocal crosses were made as described by Ryder and Johnson (1974). Morphological markers were used to confirm F_1 identity, and F_2 and F_3 populations were produced by self-pollinating individual F_1 and F_2 plants. Recombinant-inbred lines (RILs) were produced from the cross 'Saladin' × 'Iceberg' for genetic mapping. RILs were generated from unselected F_2 plants by self-pollinating and advancing by single-seed descent until the $F_{2:6}$ generation.

Evaluation of susceptibility to lettuce dieback

Susceptibility was evaluated by seeding lettuce directly into in fields from which LNSV had previously been isolated from plants exhibiting characteristic dieback symptoms. Experiments were conducted at several field sites throughout the Salinas Valley of California over a period of 5 years. Each experiment comprised two complete blocks, with 20–60 plants per genotype per block. Plants were seeded between March and August in two rows on 1.0-m wide beds and were thinned to obtain spacing of 30 cm between plants. Standard commercial practices were used for irrigation, fertilization, and pest control.

'Iceberg' and 'Salinas' were used as susceptible and resistant controls, respectively. In each experiment, the presence of LNSV in susceptible controls was confirmed by mechanical inoculation onto indicator plant species, by reverse transcriptase-polymerase chain reaction (RT-PCR), or both, using the methods described by Obermeier et al. (2001). Susceptibility of each genotype was determined by visually assessing plants for the presence of characteristic symptoms of lettuce dieback. Diagnostic symptoms include chlorotic flecking, necrotic patches on maturing leaves, and stunting. Disease incidence (DI), the percentage of plants that showed

symptoms, was recorded at harvest maturity. Genotypes with one or more diseased plants were classified as susceptible, and genotypes that exhibited no symptoms in at least two independent experiments were considered resistant.

Detection of the pathogen by double-antibody sandwich enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was used to determine whether LNSV was present in field-grown plants without symptoms (Clark and Adams 1977). Mature plants of four susceptible and five resistant lettuce genotypes were assayed. A single partially expanded leaf was tested from each mature plant. For susceptible plants, leaves that showed strong symptoms without extensive necrosis were selected. Entire leaves were placed on ice and immediately transferred to −20°C for storage. ELISA was performed on 0.1 g tissue removed from the same region of each leaf, avoiding the midrib.

All steps of the ELISA were performed in polystyrene plates, using a total volume of 50 µl per well. Plates were washed four times with PBST (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.4 mM KH_2PO_4, 0.05% Tween 20, pH 7.2) between each step of the procedure. LNSV coat protein (CP) antisera (Obermeier et al. 2001) was diluted 1:1000 in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6), added to plates and incubated for 24 h at 4°C. Leaf tissue was ground and diluted 1:5 (w:v) in sample extraction buffer [PBST containing 100 mM Na_2SO_3, 2% polyvinylpyrrolidone (PVP), 0.2% grade II powdered egg albumin, 2% Tween 20, pH 7.4], added to plates and was incubated for 24 h at 4°C. LNSV CP antiserum conjugated to alkaline phosphatase (Obermeier et al. 2001) was diluted 1:2,500 in ECI buffer (PBST containing 0.2% bovine serum albumin and 2% PVP, pH 7.4), added to plates and incubated 2–4 h at 4°C. Finally, *p*-nitrophenyl phosphatase (1 mg/ml in diethanolamine, pH 9.8) was added and plates were incubated at room temperature 4–24 h until color had developed fully in positive controls. Symptomatic leaves from *Nicotiana benthamiana* plants infected with LNSV-L2 were included as a positive control. A sample was declared positive if its absorbance (405 nm) was higher than the mean absorbance plus three standard deviations of samples from noninfested fields. Because LNSV titers are variable and often low in infected lettuce (Obermeier et al. 2001), ELISA tests were used only to determine presence or absence of virus in a sample, and not to provide quantitative data.

Molecular genotyping and mapping

Genetic linkage maps were constructed using two populations from the same 'Saladin' × 'Iceberg' cross: 254 $F_{2:5}$ RILs and an additional 125 F_2 individuals (P. Hand and D. Pink, unpublished results). Amplified fragment length polymorphism (AFLP) markers were mapped in both F_2 and F_5 populations, and random amplified polymorphic DNA (RAPD) markers were mapped only in the F_2 population. In brief, DNA was extracted using either the Qiagen DNeasy 96 Plant kit (Qiagen, Crawley, UK) or using the procedure described by Doyle and Doyle (1990). RAPD analysis was performed using random ten-mer oligonucleotide primers (Operon Technologies, Alameda, Calif., USA) and standard conditions (Welsh and McClelland 1990). AFLP analysis was performed according to the method of Vos et al. (1995). A total of 28 *Eco*RI (E)/*Mse*I (M) or *Pst*1 (P)/*Mse*1 primer pairs were scored in both mapping populations. The selective nucleotides for the primers used were E35-ACA, E36-ACC, E40-AGC, E41-AGG, E45-ATG, M47-CAA, M48-CAC, M49-CAG, M59-CTA, M60-CTC, M61-CTG, and P38-ACT.

Linkage analysis was performed independently for each population. Polymorphic markers were assigned to linkage groups based on pairwise recombination frequencies and LOD (logarithm of odds) values, using JoinMap, version 3.0, software (van Ooijen and Voorips 2001). LOD values of 4.0 and 5.0 were used for the F_2 and F_5 populations, respectively. Within linkage groups, markers were localized using the Kosambi mapping function, with a pairwise recombination upper limit of 0.45 and a LOD threshold of 0.01. The F_2 and F_5 maps were then combined to produce an integrated map using JoinMap, based on shared AFLP markers. To align this map with published lettuce maps, markers were considered common if a fragment of similar estimated size (within 15 bp for RAPD and 5 bp for AFLP) were amplified using the same primer or primer combination.

To map the resistance locus, $F_{2:6}$ progeny from 48 of the 'Saladin' × 'Iceberg' $F_{2:5}$ plants were evaluated for lettuce dieback symptoms in an infested field in a randomized complete block design with two complete replicates. The resistance phenotype was mapped as a single qualitative marker using JoinMap.

Results

Reaction of lettuce germplasm in dieback-infested fields

In all field experiments, characteristic symptoms were observed for 'Iceberg' and other cultivars that had previously shown symptoms of lettuce dieback in commercial production fields. The DI for susceptible controls ranged from 50% to 100% among field experiments. Within an experiment, the timing of symptom development varied for plants of the same genotype. Symptoms were first observed when plants were 6–8 weeks old, and the DI increased over time (Fig. 1). Although the final DI varied between cultivars

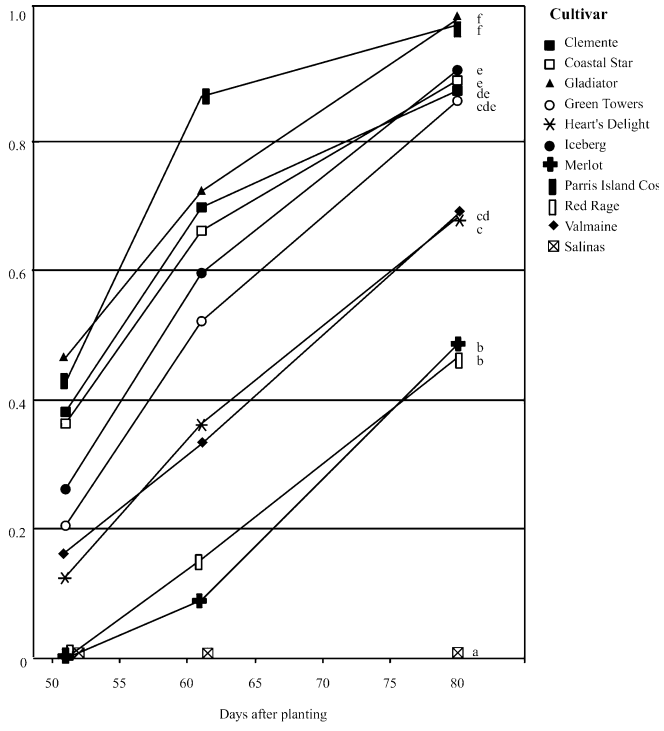

Fig. 1 Mean incidence of lettuce dieback for several cultivars grown in a field infested with *Tomato bushy stunt* and *Lettuce necrotic stunt* tombusviruses. With the exception of the resistant control, 'Salinas', all cultivars were susceptible. Data are from one representative experiment. Data points followed by the same letter were not significantly different at the final evaluation date (Tukey's test, $P = 0.05$)

and between tests, all genotypes showed the same response in different experiments and different fields, providing no evidence for field × cultivar interactions. Cultivars that had one or more diseased plants in any experiment were classified as susceptible. No plants of the resistant control 'Salinas' showed symptoms in any experiment. Genotypes that were completely free of symptoms after confirmation in at least two independent replicated experiments were considered resistant. Resistant cultivars and noncultivated germplasm are listed according to horticultural type (Table 1). The same information for susceptible cultivars and germplasm is provided as electronic supplementary material (S1).

The responses of different types of cultivated and noncultivated lettuce germplasm to lettuce dieback are summarized in Table 2. For all of the *L. sativa* genotypes tested, 68/235, or 29%, were resistant, and the overall proportion of resistant cultivars was similar for modern cultivars (33%), heirloom cultivars (23%), and noncultivated germplasm (32%). Of the wild *Lactuca* species that were tested, one of the four *L. serriola* tested was susceptible, but all of the *L. saligna* and *L. virosa* were resistant.

Within cultivated germplasm, at least one resistant cultivar was identified for each type of lettuce. Modern crisphead germplasm was unique in that all cultivars tested were resistant. In contrast, nearly all modern red leaf and romaine cultivars were susceptible. Out of 23 tested, only one modern red leaf cultivar, 'Ruby Ruffles', was resistant. The two resistant romaine cultivars, 'Defender' and 'Skyway', were both specialty cultivars that are not suited for the production of full-size heads or hearts.

Resistance was also rare for heirloom and noncultivated red leaf and romaine genotypes. Two resistant heirloom cultivars were identified, but neither meets current commercial production needs. The romaine 'Blonde Lente à Monter' had a light green color that is considered undesirable, and the red leaf 'Cracoviensis' had an unusual primitive growth habit. Within noncultivated germplasm, three romaine accessions, PI 491209, PI 491214, and PI 491224, and the breeding line 'SVR6603A' were resistant. The three PI accessions exhibited varying degrees of early bolting and unacceptable leaf color and texture. PI 491224 exhibited the best horticultural characteristics and was therefore used to develop the resistant romaine breeding lines 01-778M, 01-781M, and 01-789M (Grube and Ryder 2003).

Pathogen detection

To determine whether the absence of lettuce dieback symptoms was associated with an absence of detectable virus, resistant and susceptible genotypes were tested for the presence of tombusviruses, using ELISA. Leaf samples were collected from randomly selected symptomatic plants of susceptible genotypes ('Valmaine', 'Lobjoits', and 'Iceberg') and asymptomatic plants of resistant genotypes (01-778-1, 'Salinas 88', 'Glacier', 'Imperial' and 'Grand Rapids') grown in an infested field. When two subsets of these plants were tested using ELISA in two independent experiments, LNSV was detected in 13/13 and 12/14 of the symptomatic plants tested (Table 3). Although the incidence of LNSV detection by ELISA was much lower (2/37 and 10/37) for healthy plants of resistant genotypes than for diseased plants of susceptible genotypes, LNSV was detected in at least one healthy plant of each resistant genotype (Table 3).

Genetic analysis

The inheritance of resistance responses of both 'Salinas'/'Saladin' and PI 491224 were studied using segregating populations in four separate field experiments (Tables 4, 5, 6). As was expected, the cultivars 'Saladin' and 'Salinas' were morphologically indistinguishable. All resistant parents (PI 491224, 'Saladin', and 'Salinas') remained free of disease symptoms in all tests. For susceptible genotypes, the percentage of diseased plants was consistently high but was not always 100%. Plants of susceptible genotypes that had not developed

Table 1 Lettuce cultivars and noncultivated germplasm that never exhibited symptoms of lettuce dieback when grown in infested fields[a]

Germplasm	Type[b]	Cultivar[c]		
Cultivated	BAT	AvonCrisp (H)	Express (H)	La Brillante (H)
		Batavia Beaujolais (H)	Great Lakes 54 (H)	River Green
		Drumhead White Cabbage (H)	Imperial (H)	
	BUTT	Bibb	Dynamite	Pirat
		Big Boston (H)	Encanto	Pontiac
		Buttercrunch (H)	Esmerelda	Salamander (H)
		Cinnamon Red	Margarita	Tania
		Dark Green Boston	Ostinata	Tennis Ball (H)
	ICE	Calmar	Heritage	Salinas 88
		Climax	Pacific	Sharp Shooter
		Empire	Sea Green	Vanguard
		Glacier	Salinas	Winterhaven
	LAT	Little Gem		
	GLF	Fanfare	Royal Green	Tehama
		Grand Rapids (H)	Salad Bowl (H)	Two Star
		Green Valley	Shining Star	Waldmann's Green
		Pybas Green	Slobolt (H)	
	RLF	Cracoviensis (H)	Ruby Ruffles	
	ROM	Blonde Lente à Monter (H)	Defender (BR)	Skyway (BR)
	STM	Celtuce		
Noncultivated	PRIM	PI 171666	PI 250020	PI 273589
		PI 177418	PI 251246	PI 289064
		PI 178924b	PI 251247	
	ROM	PI 491209	PI 491224	
		PI 491214	SVR6603A	
	STM	Balady Banha	Balady Barrage	Balady Behara
	WILD	PI 490999 (sal)	PI 491178 (ser)	PI 273597 (vir)
		PI 509525 (sal)	UC96US23 (ser)	IVT 280 (vir)
		PI 491204 (sal)	PI 271940 (ser)	

[a]Genotypes that had no diseased plants in at least two independent experiments in which a significant proportion of susceptible controls were diseased

[b]Lettuce type classes are *BAT* Batavia, *BUT* butter, *ICE* iceberg, *LAT* Latin, *GLF* green leaf, *RLF* red leaf, *ROM* romaine, *STM* stem, *PRIM* primitive and *WILD Lactuca* spp. other than *L. sativa*

[c]Heirloom or cultivars with specialty uses are designated as follows: *H* heirloom, *BR* baby romaine. Wild species are *Lactuca saligna*, sal, *L. serriola*, ser, and *L. virosa*, vir

Table 2 Summary of the reactions of cultivated and noncultivated lettuce germplasm grown in fields infested with *Lettuce necrotic stunt virus*

Description/type[a]	Cultivated						Noncultivated		
	Modern			Heirloom					
	S	R	%R	S	R	%R	S	R	%R
Batavia	2	1	33	9	7	44	–	–	–
Butter	5	11	69	6	4	40	1	0	0
Iceberg	0	12	100	–	–	–	–	–	–
Latin	3	1	25	1	0	0	–	–	–
Leaf, green	10	8	44	5	3	38	–	–	–
Leaf, red	22	1	4	12	1	8	5	0	0
Romaine	32	2	6	20	1	5	24	4	14
Stem	1	1	50	–	–	–	2	3	60
Primitive *L. sativa*	–	–	–	–	–	–	7	8	53
Wild relatives	–	–	–	–	–	–	1	8	89
Total	75	37	33	53	16	23	40	23	32

[a]For each major type of lettuce, the number of susceptible (*S*) and resistant (*R*) genotypes are presented. *%R* Percentage of cultivars that were classified as resistant to lettuce dieback. Cultivated germplasm included both modern and heirloom varieties, and noncultivated germplasm included landraces, plant introduction (PI) accessions, and experimental lines. Genotypes classified as R had no diseased plants in at least two independent experiments in which a significant proportion of susceptible controls were diseased

symptoms at the conclusion of the experiment were considered escapes. The frequency of escape was generally low, ranging from 0 to 0.09 for the susceptible control 'Iceberg', but occasionally reached moderate proportions, e.g., 0.29 (29/99) for 'Valmaine' in August 2002 (Table 5).

Table 3 Detection of tombusviruses by enzyme-linked immunosorbent assay in leaf tissues of mature lettuce plants that were seeded in an infested field site in August 2002 in Salinas, Calif.

Genotype	Field symptoms	Test 1		Test 2	
		No. positive [a]	Total	No. positive	Total
Valmaine	Yes	11	11	9	10
Lobjoits	Yes	2	2	1	2
Iceberg	Yes	-	-	2	2
01-778-1	No	1	10	0	10
Salinas 88	No	0	9	3	9
Glacier	No	0	5	2	5
Imperial	No	1	3	1	3
Grand Rapids	No	0	10	4	10

[a]A sample was declared positive for presence of tombusvirus(es) if its absorbance (405 nm) was higher than the mean absorbance of samples from plants of the same age that were grown in uninfested fields plus three standard deviations

Table 4 Monogenic dominant inheritance of resistance to lettuce dieback in the cultivar 'Salinas'/'Saladin'

	March 2003 (Salinas)[a]			March 2004 (Salinas)[a]		
	H	*D*	*P* (χ^2)[b]	*H*	*D*	*P* (χ^2)
Salinas (S)	82	0		224	0	
Saladin (Sd)	42	0		-	-	
Iceberg (I)	0	104		0	27	
Green Towers (GT)	2	72		4	190	
F_2: S × GT	–	–		251 (*241*)	70 (*80*)	0.19
F_2: GT × S	–	–		249 (*257*)	93 (*86*)	0.35
F_2: S × I	129 (*125*)	38 (*42*)	0.50			
	all *H*	3*H*:1*D*	all *D*	*P* (χ^2)		
$F_{2:5}$ RILs: Sd × I	20 (*22.5*)	4 (*3*)	24 (*22.5*)	0.65		

[a]Genetic populations were evaluated in two experiments. For each, the observed numbers of healthy (*H*) and diseased (*D*) plants are provided

[b]*P* (χ^2) is the probability that the observed data fit the segregation patterns expected for a single dominant resistance gene. Expected data are given in *italics* after observed data. F_2 populations were expected to segregate 3*H*:1D and $F_{2:5}$ RILs were expected to segregate 15H:2segregating (3*H*:1*D*):15*D*

Inheritance and mapping of resistance from 'Salinas'/'Saladin'

In May 2003, 11 'Salinas' × 'Green Towers' and reciprocal F_1 plants remained healthy in an infested field, suggesting dominant inheritance of resistance (data not shown). Despite the moderate escape frequency in that experiment (0.31), the probability that all F_1 plants were susceptible yet escaped infection was extremely low ($P = 7.3 \times 10^{-6}$). Maternal effects were ruled out, because the susceptible 'Green Towers' was the maternal parent for 9/11 healthy F_1 progeny. Reciprocal F_2 populations from this cross and the 'Salinas' × 'Iceberg' F_2 showed segregation patterns that were consistent with monogenic dominant inheritance (Table 4).

To confirm monogenic inheritance and to map the gene conferring resistance in 'Salinas'/'Saladin', resistance was evaluated for 48 $F_{2:5}$ RILs from the cross 'Saladin' × 'Iceberg'. The RILs were planted in a randomized complete block design, with two replicates of 10–30 plants each. Chi-squared tests of independence showed no significant differences in DI between the two replicates. Each RIL was considered resistant (R) if all plants remained healthy (H), susceptible (S) if over 95% of the plants showed symptoms of disease (D), or segregating (seg) otherwise. Within each segregating RIL, 20–50% of the plants showed symptoms. Of the 48 RILs, there were 20R:24S:4seg, which was consistent with the ratios expected for a single resistance gene (15R:15S:2seg) (Table 4). Taken in conjunction with the results observed for the 'Salinas' × 'Iceberg' F_2 and the 'Salinas' × 'Green Towers' reciprocal F_1 and F_2, our results support the conclusion that the resistance in 'Salinas'/'Saladin' is conferred by a dominant allele at a single locus. We have named this locus *Tvr1* for Tombusvirus resistance gene *1*.

A molecular map was generated using DNA from F_2 plants and $F_{2:5}$ RILs as described. To map *Tvr1*, the genotypes of $F_{2:5}$ RILs were determined to be *Tvr1*/*Tvr1*, *Tvr1*/*tvr1*, or *tvr1*/*tvr1* if F_6 progeny were resistant, segregating, or susceptible, respectively. Linkage analysis revealed a single map position for *Tvr1* (Fig. 2). *Tvr1*

Table 5 Monogenic dominant inheritance of resistance to lettuce dieback in PI 491224

Genotype	Experiment 1 [a] (August 2002, Soledad)			Experiment 2 (August 2002, Salinas)[a]			Experiment 3 (March 2003, Salinas)[a]		
	H	*D*	*P* (χ^2)[b]	*H*	*D*	*P* (χ^2)	*H*	*D*	*P* (χ^2)
491224	180	0		329	0		92	0	
Iceberg	9	90		9	151		0	104	
Parris Island Cos (PIC)	2	96		0	133		1	49	
Lolla Rossa							0	87	
Lobjoits							9	39	
Valmaine	29	70							
F_1: Valmaine × 491224							13	0	
F_1: Iceberg × 491224							11	0	
F_2: PIC × 491224	199	96	< 0.01	359	109	0.39			
F_2: Lobjoits × 491224	233	103	0.02						
F_2: Lolla Rossa × 491224							189	79	0.10
							3*H*:1*D*	all *H*	*P* (χ^2)
F_3: PIC × 491224							7	2	0.48
F_3: Lobjoits × 491224							8	4	0.98

[a]Genetic populations were evaluated for resistance in three experiments. For each, the observed numbers of healthy (*H*) and symptomatic (*D*) plants are provided

[b]*P* (χ^2) is the probability that the observed data fit the segregation patterns expected for a single dominant resistance gene. For segregating populations, expected data are given in *italics* after observed data. F_2 populations were expected to segregate 3*H*:1*D*. Each F_3 family represents progeny from a selected healthy F_2 plant, and were therefore expected to fit the ratio 2 segregating (3*H*:1*D*):1 all *H*

Table 6 Relationship between lettuce dieback resistance loci from the cultivar 'Salinas' and PI 491224

Genotype	Observed data [a]		Escape frequency (*f*)		
	H	*D*			
Salinas	224	0	–		
PI 491224	55	0	–		
Iceberg	0	27	0		
Green Towers	4	190	0.02		
Parris Island Cos	4	111	0.04		
			Expected data (two unlinked loci) [b]		Probability of observed data, two unlinked loci
			H	D	
F_2: PI 491224 × Salinas	396	0	374	22	1.11×10^{-10}
F_2: Salinas × PI 491224	409	0	386	23	5.24×10^{-11}
Combined F_2	805	0	758	45	5.83×10^{-21}

[a]Genetic populations were evaluated for resistance in March 2004 in Salinas, Calif. Susceptible control cultivars 'Iceberg', 'Green Towers', and 'Parris Island Cos' were used to estimate *f* or probability of failure of a susceptible plant to develop symptoms

[b]Expected data for F_2 populations were calculated from the frequency of susceptible plants expected for two dominant resistance alleles at unlinked loci (1/16 or 0.0625) multiplied by (1–*f*), where *f* was a conservative estimate of escape frequency (0.1)

was most closely linked (1.2 cM) to the AFLP marker E35M47_260s on one side, and was less than 2 cM from the AFLP markers E40M61_260s and E45M48_76s and the RAPD marker OPA17_1600 on the other side. A total of nine markers were located within the 5 cM flanking *Tvr1* on either side. None of the phenotypic markers segregating in this population, including the downy mildew resistance genes *Dm5/8* and *Dm7* and the gene for seed color, *w*, were located on this linkage group (P. Hand and D. Pink, unpublished results). Using markers in common between this map and other *Lactuca* genetic linkage maps (Jeuken et al. 2001; Kesseli et al. 1994; Michelmore 2004; Waycott et al. 1999), it was possible to identify this linkage group as chromosome 2 of Michelmore (2004). The limited number of common markers prevented accurate estimation of the distance between *Tvr1* and the *Dm1/Dm3* gene cluster that is present on this linkage group.

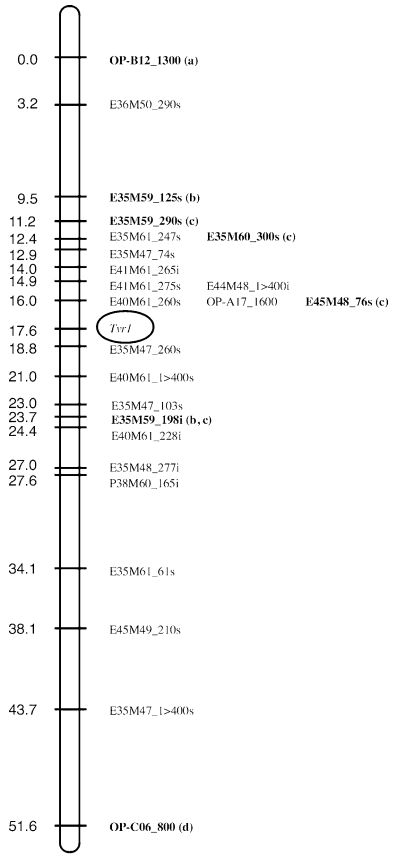

Fig. 2 Genetic map of the region surrounding the tombusvirus resistance locus *Tvr1*. *Tvr1* was mapped to linkage group 2 of an intraspecific map of *Lactuca sativa*, using $F_{2:5}$ recombinant inbred lines of the cross 'Saladin' × 'Iceberg'. Markers in *boldface* are present on other *Lactuca* maps as follows: **a** Waycott et al. 1999, **b** Jeuken et al. 2001, **c** UC Davis Compositae Genome Project map at http://cgpdb.ucdavis.edu/database/genome_viewer/map_data, and **d** Kesseli et al. 1994. *Numerals to the left of the linkage group* show genetic distances in centiMorgans. A single position is given for markers located less than 0.5 cM from one another. Each marker name consists of the primer (random amplified polymorphic DNA markers, starting with "OP-") or primer pair [amplified fragment length polymorphism (AFLP markers)], followed by the size of the amplified fragment in base pairs, and, for AFLP markers, the parent (*i* Iceberg, *s* Saladin) from which the fragment was amplified

Inheritance of resistance from PI 491224

None of the F_1 plants generated by crossing PI 491224 with 'Valmaine' or 'Iceberg' developed symptoms, which established dominant inheritance (Table 5). F_1 progeny from susceptible maternal parents were healthy, ruling out maternal effects. The probability that all F_1 plants were susceptible yet escaped infection was negligible ($P = 5.3\times10^{-15}$), based on the maximum observed frequency of escape (0.19, or 9/48 'Valmaine' plants) in experiment 3. F_2 populations were generated from crosses between PI 491224 and the three susceptible genotypes, 'Parris Island Cos' (PIC), 'Lobjoits', and 'Lolla Rossa'. For F_2 populations grown in Salinas, observed segregation ratios were consistent with monogenic dominant inheritance (Table 5). In Soledad, however, fewer healthy plants were observed than would be expected given a single dominant resistance gene, even for the PIC population, which was grown concurrently in Salinas. To confirm that F_2 scoring was accurate for the healthy plants, F_3 progeny were produced from healthy F_2 plants from the Soledad experiment. The F_3 families were grown and evaluated in Salinas in two replicates of 20–50 plants each. For F_3 families from both of the F_2 populations, the observed data were similar and were consistent with monogenic dominant inheritance, 2seg:1H (Table 5). Within the segregating families, those from the 'Lobjoits' F_2 were consistent with the predicted 3H:1D (570H:201D; $P = 0.5$). Segregating F_3 families from the PIC populations had a slightly higher proportion of diseased individuals than was predicted (444H:213D observed versus 493H:164D predicted). When adjusted to account for the frequency of plant death observed in resistant controls (0.06) however, data were consistent with the predicted 3H:1D (483H:174D; $P = 0.4$). In conjunction with F_1 and F_2 data, these results led us to conclude that dieback resistance of PI 491224 was also conferred by a single dominant allele.

Association between *Tvr1* and the resistance locus from PI 491224

If *Tvr1* and the resistance locus from PI 491224 were distinct and unlinked, approximately 6.25% susceptible offspring would be observed in an F_2 population from the cross 'Salinas' × PI 491224. If the two genes were allelic, all F_2 plants would be resistant. In March 2004, a total of 805 F_2 plants from the two reciprocal F_2 populations were grown in an infested field, and no symptomatic plants were identified (Table 6). The frequency of healthy plants (escapes) was less than 0.05 for all susceptible controls. Using a conservative estimate of 10% escape of infection, the probability of obtaining the observed data, given independently segregating loci, was extremely low ($P = 5.8\times10^{-21}$ overall). Therefore, we concluded that the resistance locus in PI 491224 was either allelic with or linked to *Tvr1*.

Discussion

Susceptibility to lettuce dieback was found to be widespread in the germplasm of cultivated lettuce. Overall, heirloom cultivars and primitive *L. sativa* genotypes were no more likely than modern cultivars to be resistant to dieback. Although all of the *L. saligna* and *L. virosa* tested were resistant, one *L. serriola* accession was susceptible, establishing that susceptibility to LNSV is not unique to *L. sativa*.

Resistance to lettuce dieback was conferred by a dominant allele at a single locus in both 'Salinas', a representative modern iceberg lettuce cultivar (Ryder 1979), and in PI 491224, the source of recently released

resistant romaine germplasm (Grube and Ryder 2003). A single map position was identified for *Tvr1*, the gene from 'Salinas', and this locus appears to be linked to or the same as the gene from PI 491224. To our knowledge, *Tvr1* is the first characterized plant gene to confer resistance to tombusviruses. The absence of effective chemical or cultural control strategies make genetic resistance a prime objective for control of related viruses in tomato, pepper, and other vegetable hosts, in addition to lettuce. A better understanding of the function of *Tvr1* may facilitate the identification of resistance and breeding of resistant varieties in these other crop species.

Although *Tvr1* in crisphead cultivars and the gene from PI 491224 completely prevent development of typical symptoms of lettuce dieback, some asymptomatic plants can support systemic viral infection. In our experiments, however, LNSV was detected in fewer asymptomatic plants compared with symptomatic plants. This suggests that *Tvr1* confers partial resistance rather than tolerance, because tolerant genotypes are those that do not suffer crop losses despite being infected (Agrios 1988). Detection of LNSV in some, but not all, healthy plants could be explained by incomplete penetrance of resistance, higher rates of escape of infection in these genotypes, or viral titers that were near or below the threshold of detection using ELISA. Although the LNSV-L2 antisera reacted with all of the pathogenic TBSV and LNSV isolates that we tested, including L1, L4, BS3, T1 and Cherry (Obermeier et al. 2001), we cannot rule out the existence of tombusviruses that were not detected or were less efficiently detected by the LNSV-L2 antisera. Further research, possibly using more sensitive methods of viral detection such as real-time PCR in conjunction with isolate-specific primers, will be required to elucidate the underlying mechanisms of resistance and to reveal potential isolate by cultivar interactions.

One limitation of field experiments is that the pathogen population is not clearly defined, and the presence of additional pathogens can complicate the interpretation of genetic data. For lettuce dieback, however, the relatively low efficiency of existing greenhouse and growth chamber LNSV inoculation procedures prohibit evaluation of the population sizes required for genetic analysis. Consistent results were obtained using several field sites in which a high incidence of lettuce dieback was observed. Visual assessment of symptoms was more reliable for evaluating dieback infection than ELISA, both because some healthy plants contained LNSV and because the characteristic symptoms of lettuce dieback are easily differentiated from other lettuce diseases.

The genetic base of modern cultivated lettuce germplasm is quite narrow, and modern types of lettuce have experienced extreme population bottlenecks during recent breeding history, particularly following the development of individual "landmark" cultivars (Jagger 1941). The contrast between uniformly resistant crisphead and susceptible romaine groups of cultivars suggests that these gene pools have been reproductively isolated since the fixation of resistance or susceptibility to lettuce dieback. This clearly illustrates that, although vulnerability to future emerging diseases or epidemics may be reduced by broadening the genetic base of modern cultivars, care should be taken in order to avoid reintroduction of susceptibility into resistant gene pools.

A critical question is whether intensive cultivation of resistant germplasm will favor the development of virulent strains of the pathogen that overcome host resistance. Crisphead cultivars have been grown intensively in tombusvirus-infested soils deemed unsuitable for romaine production since the 1980s, without any indication of dieback symptoms. The actual duration and frequency of contact between resistant cultivars and the pathogen are not known, because of the difficulties in accurately determining the prevalence and distribution of the pathogen in soils of the major lettuce growing regions. Several lines of anecdotal evidence suggest that tombusviruses may have caused the epidemic of "brown blight" throughout California and Arizona in the 1920s, and that the gene *Tvr1* was introduced to control this disease in the resistant cultivar 'Imperial' (Jagger 1940; Wisler and Duffus 2000). Because 'Imperial' is in the pedigrees of all modern crisphead germplasm (E. J. Ryder, personal communication), we speculate that *Tvr1* has remained effective despite widespread deployment and hence ,exposure to the pathogen for several decades. If this is the case, understanding the genomic context of *Tvr1* and its relationship with other lettuce gene sequences may reveal clues about what makes a resistance gene durable.

Tvr1 is one of the three virus resistance genes that have been mapped in lettuce (Montesclaros et al. 1997). The other two, *mo* and *Tu*, confer resistance to potyviruses, and neither are linked to *Tvr1* (Kesseli et al. 1994). In addition to *Tvr1*, chromosome 2 also contains the largest cluster of resistance genes identified in lettuce, the *Dm3* cluster (Meyers et al. 1998a). Over 23 genes and pseudogenes with structural similarity to the downy mildew (*Bremia lactucae*) resistance gene *Dm3*, several other functional *Dm* genes, and a gene that confers resistance to the lettuce root aphid (*Ra*) are also found in this region. The orientation of *Tvr1* relative to these genes has not yet been precisely determined, because too few markers in this region are currently present in integrated mapping populations. Despite a demonstrated capacity for rapid evolution (and presumably rapid generation of new specificities) by *Dm3*-related sequences (Meyers et al. 1998b), these genes have generally had short effective life spans, in that virulent isolates of *Bremia* have usually appeared shortly after their release in lettuce cultivars (Crute 1992). In contrast, the Ra gene has been durable in lettuce, and more generally, genes for resistance to virus diseases have in many cases been durable (Ellis et al. 1994; Harrison 2002) Determination of whether *Tvr1* is homologous to *Ra* or any of the *Dm* genes may provide information about the roles of gene structure and plant–pathogen interactions in determining the longevity of resistance genes.

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