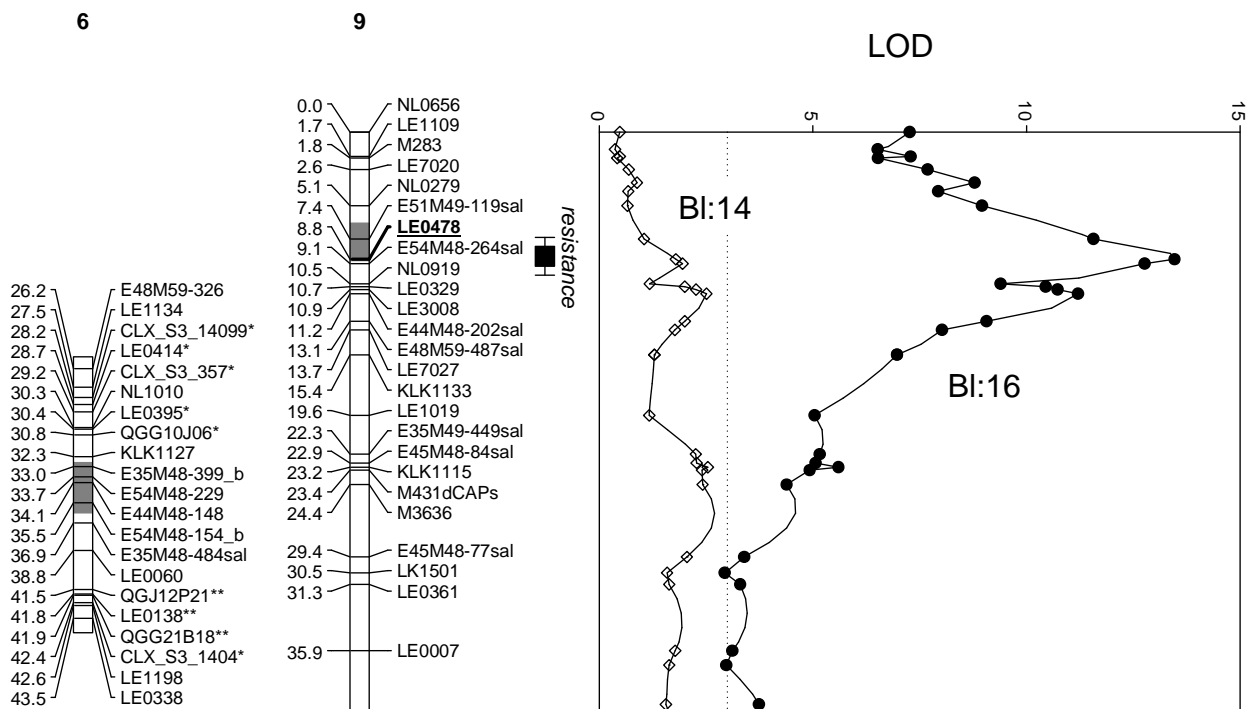


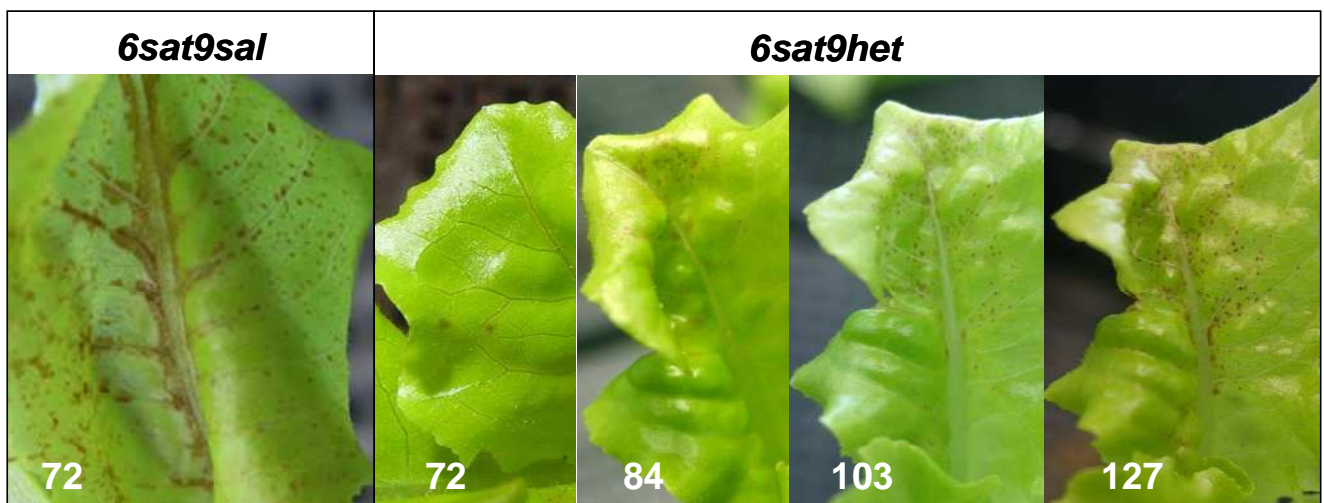
Supplemental Data, Jeuken et al., (2009) Rin4 causes hybrid necrosis and race-specific resistance in an interspecific lettuce hybrid.”



Supplemental Figure 1. Map positions of hybrid necrosis loci.

Genetic maps with the two interactive loci on chromosome 6 and 9 (C6 and C9) and the LOD profile for race-specific resistance on C9 to *B. lactuca* BI:16 (formerly designated *R39*) are shown. The genetic map and chromosomal designations are based on analysis of a F_2 *L. saligna* x *L. sativa* population (Jeuken et al., 2001). Note that chromosome 6 of this map corresponds to chromosome 8 of the consensus map of *Lactuca* spp. (Truco et al., 2007); chromosome 9 is the same in both maps. The grey bars show the two smallest fine mapped segments that are associated with hybrid necrosis based on F_2 populations and introgression lines from cross 1 and 2. * = Markers that are based on EST sequences with sequence similarity to Resistance Gene Candidates encoding NBS-LRR proteins (McHale et al., 2009). **= Marker that is based on an EST sequence with sequence similarity to MLO (Mildew-resistance LOcus gene in barley). The LOD profiles of race-specific resistance to *B. lactuca* BI:16 (solid bullets) and susceptibility to BI:14 (open diamonds) are shown for C9 with one LOD and two LOD support confidence intervals (approximate multiple QTL mapping, MQM). The highest

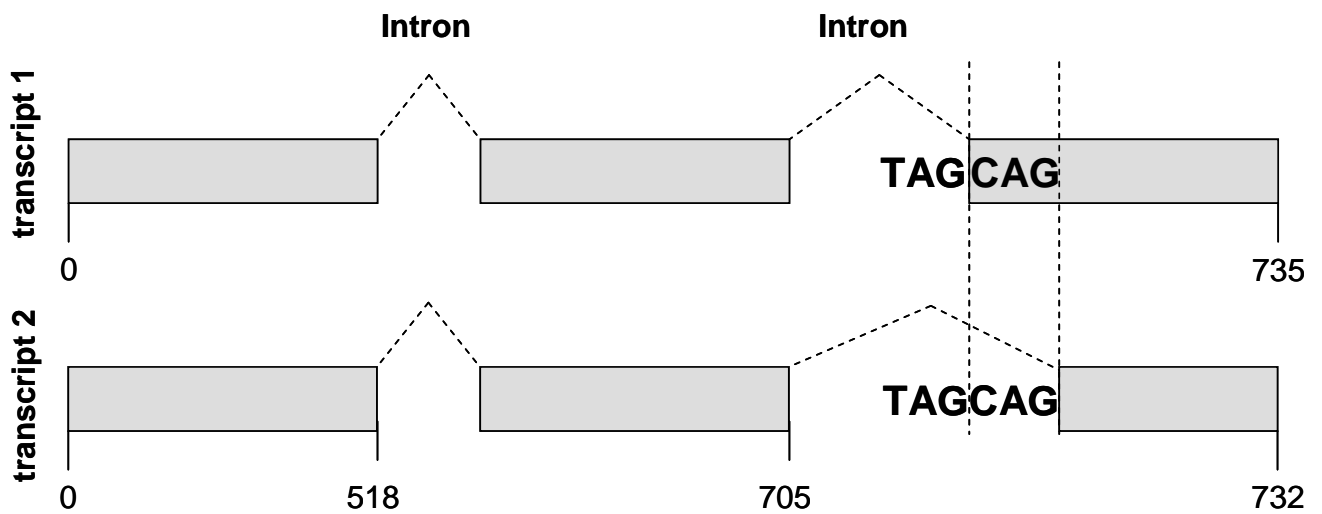
associated marker is underlined and bold. The marker LE0478 that was most tightly associated with the peak on chromosome 9 is underlined and in bold. LE0478, derived from a lettuce unigene with sequence similarity to *Arabidopsis RIN4*, and its derived markers (LE0478INT, LE0478indel not shown) all mapped to the same position.



Supplemental Figure 2. Hybrid necrosis symptoms in youngest leaf after temperature shift. Observations on genotype classes with extreme to severe hybrid necrosis symptoms at lower temperatures are shown: *6sat9sal* and *6sat9het*, in hours after shift from 30° to 15°C. Left: detail of necrotic cells in *6sat9sal* appearing near the major veins. Right: time frame of appearance of necrotic lesions in a *6sat9het* leaf.



Supplemental Figure 3. Hybrid necrosis symptoms in whole plants after temperature shift. Observations on genotype classes with severe to extreme hybrid necrosis symptoms at lower temperatures: *6sat9het* and *6sat9sal*, and genotypes with no hybrid necrosis symptoms: *6sat9sat* *L. sativa* cv. Olof and *6sal9sal*/doubleBIL6.1+9.1, are shown at 5 and 17 days after a shift from 30° to 15°C.



Supplemental Figure 4. Proposed alternative splicing of *Rin4* transcripts.

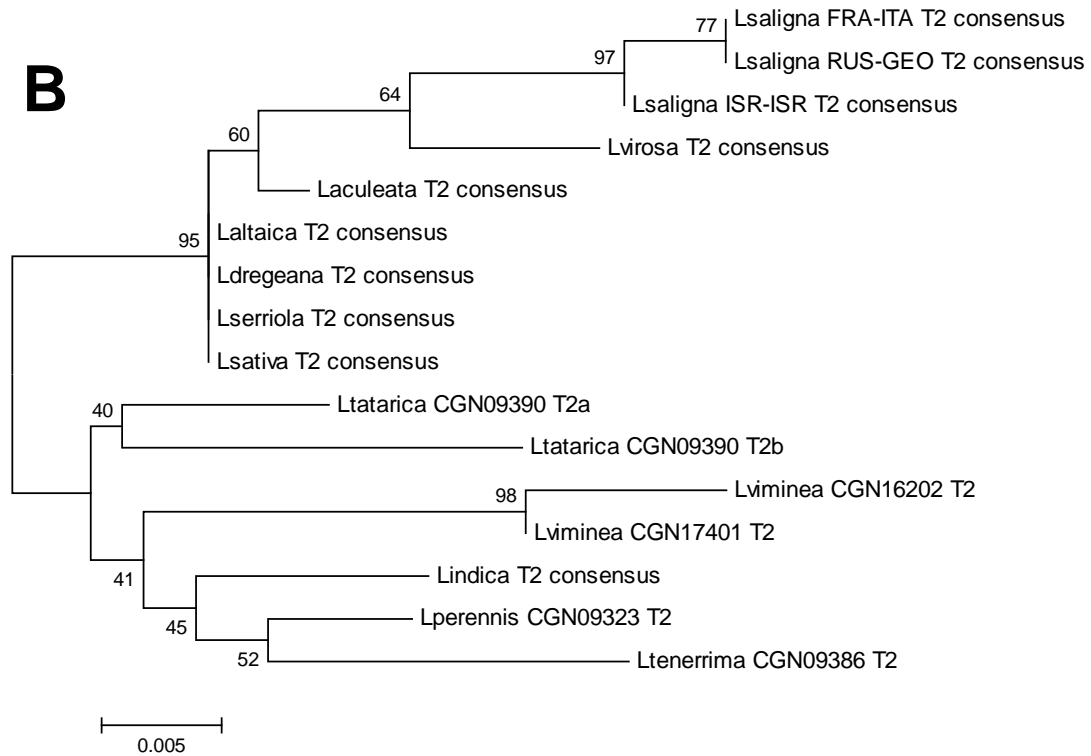
The proposed alternative splicing events that utilize different 3'splice sites within the last intron at a 'TAGCAG' sequence leading to two transcript versions in *L. sativa*, *L. saligna* and *L. virosa* are shown. Transcript1 and transcript2 arose from a splice after the 'TAG' and the 'CAG' motif, respectively. This results in one extra amino acid, namely glutamine at position 237 in transcript1.

A

Lsaligna_FRA-ITA_consensus MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTPRSRPQPEEPVGRRAVRPSRENE 82
Lsaligna_RUS-GEO_consensus MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTPRSRPQPEEPVGRRAVRPSRENE 82
Lsaligna_ISR-ISR_consensus MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTPRSRPQPEEPVGRRAVRPSRENE 82
Lvirosa_consensus MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTPRSRPQPEEPVGRRAVRPSRENE 82
Laculeata_consensus MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTPRSRPQPEEPVGRRAVRPSRENE 82
Laltaica_consensus MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTPRSRPQPEEPVGRRAVRPSRENE 82
Ldregeana_consensus MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTPRSRPQPEEPVGRRAVRPSRENE 82
Lsativa_consensus MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTPRSRPQPEEPVGRRAVRPSRENE 82
Lserriola_consensus MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTPRSRPQPEEPVGRRAVRPSRENE 82
Ltatarica_CGN09390_T2a MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTPRSRPQPEEPVGRRAVRPSRENE 82
Ltatarica_CGN09390_T2b MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTPRSRPQPEEPVGRRAVRPSRENE 82
Lvimineae_CGN16202 MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTAHRSRQPEEPVGRRAVRPSRENE 82
Lvimineae_CGN17401 MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTAHRSRQPEEPVGRRAVRPSRENE 82
Lindica_consensus MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTPRSRPQPEEPVGRRAVRPSRENE 82
Lperennis_CGN09323 MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTPRSRPQPEEPVGRRAVRPSRENE 82
Ltenerima_CGN09386 MAQRPTVPKFGNWESEDNVPYTVYFDKARKKGTGGKMINPNDPQENPEMFADKTPPTPRSRPQPEEPVGRRAVRPSRENE 82

Lsaligna_FRA-ITA_consensus YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--VAAGKGSQSPAYEGKNSYDSSHGTPSR162
Lsaligna_RUS-GEO_consensus YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--VAAGKGSQSPAYEGKNSYDSSHGTPSR162
Lsaligna_ISR-ISR_consensus YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--VAAGKGSQSPAYEGKNSYDSSHGTPSR162
Lvirosa_consensus YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--VTAGKGSQSPAYEGKNSYDSSHGTPSR162
Laculeata_consensus YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--VAAGKGSQSPAYEGKNSYDSSHGTPSR162
Laltaica_consensus YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--VAAGKGSQSPAYEGKNSYDSSHGTPSR162
Ldregeana_consensus YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--VAAGKGSQSPAYEGKNSYDSSHGTPSR162
Lsativa_consensus YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--VAAGKGSQSPAYEGKNSYDSSHGTPSR162
Lserriola_consensus YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--VAAGKGSQSPAYEGKNSYDSSHGTPSR162
Ltatarica_CGN09390_T2a YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--VAAGKGSQSPAYEGKNSYDSSHGTPSR162
Ltatarica_CGN09390_T2b YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--GAAGKGSQSPAYEGKNSYDSSHGTPSR162
Lvimineae_CGN16202 YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--VSAGKGSQSPAYEGKNSYDSSHGTPSR162
Lvimineae_CGN17401 YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--VAAGKGSQSPAYEGKNSYDSSHGTPSR162
Lindica_consensus YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--VAAGKGSQSPAYEGKNSYDSSHGTPSR162
Lperennis_CGN09323 YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAK--AAAGKGSQSPAYEGKNSYDSSHGTPSR162
Ltenerima_CGN09386 YQPPNDIAGRRITSGGSAYQRGGQGTAAAGRPVKISAGSENSFDRSPLHPHYQAKAAAAGKGSQSPAYEGKNSYDSSHGTPSR164

Lsaligna_FRA-ITA_consensus SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMISGSDARPNYNIPRDQKPNKCF CFPSSK 243
Lsaligna_RUS-GEO_consensus SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMISGSDARPNYNIPRDQKPNKCF CFPSSK 243
Lsaligna_ISR-ISR_consensus SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMISGSDARPNYNIPRDQKPNKCF CFPSSK 243
Lvirosa_consensus SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMISGSDARPNYNIPRDQKPNKCF CFPSSK 243
Laculeata_consensus SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMISGSDARPNYNIPRDQKPNKCF CFPSSK 243
Laltaica_consensus SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMISGSDARPNYNIPRDQKPNKCF CFPSSK 243
Ldregeana_consensus SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMISGSDARPNYNIPRDQKPNKCF CFPSSK 243
Lsativa_consensus SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMISGSDARPNYNIPRDQKPNKCF CFPSSK 243
Lserriola_consensus SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMISGSDARPNYNIPRDQKPNKCF CFPSSK 243
Ltatarica_CGN09390_T2a SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMISGSDARPNYNIPRDQKPNKCF CFPSSK 243
Ltatarica_CGN09390_T2b SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMISGSDARPNYNIPRDQKPNKCF CFPSSK 243
Lvimineae_CGN16202 SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMISGSDARPNYNIPRDQKPNKCF CFPSSK 243
Lvimineae_CGN17401 SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMISGSDARPNYNIPRDQKPNKCF CFPSSK 243
Lindica_consensus SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMASGSDARPNYNIPRDQKPNKCF CFPSSK 243
Lperennis_CGN09323 SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMASGSDARPNYNIPRDQKPNKCF CFPSSK 243
Ltenerima_CGN09386 SRMKPARGDDSPDRGAAVPRFGEWDENNPSADNYTHIFNKVREERTGSPMISGSDARPNYNIPRDQKPNKCF CFPSSK 244

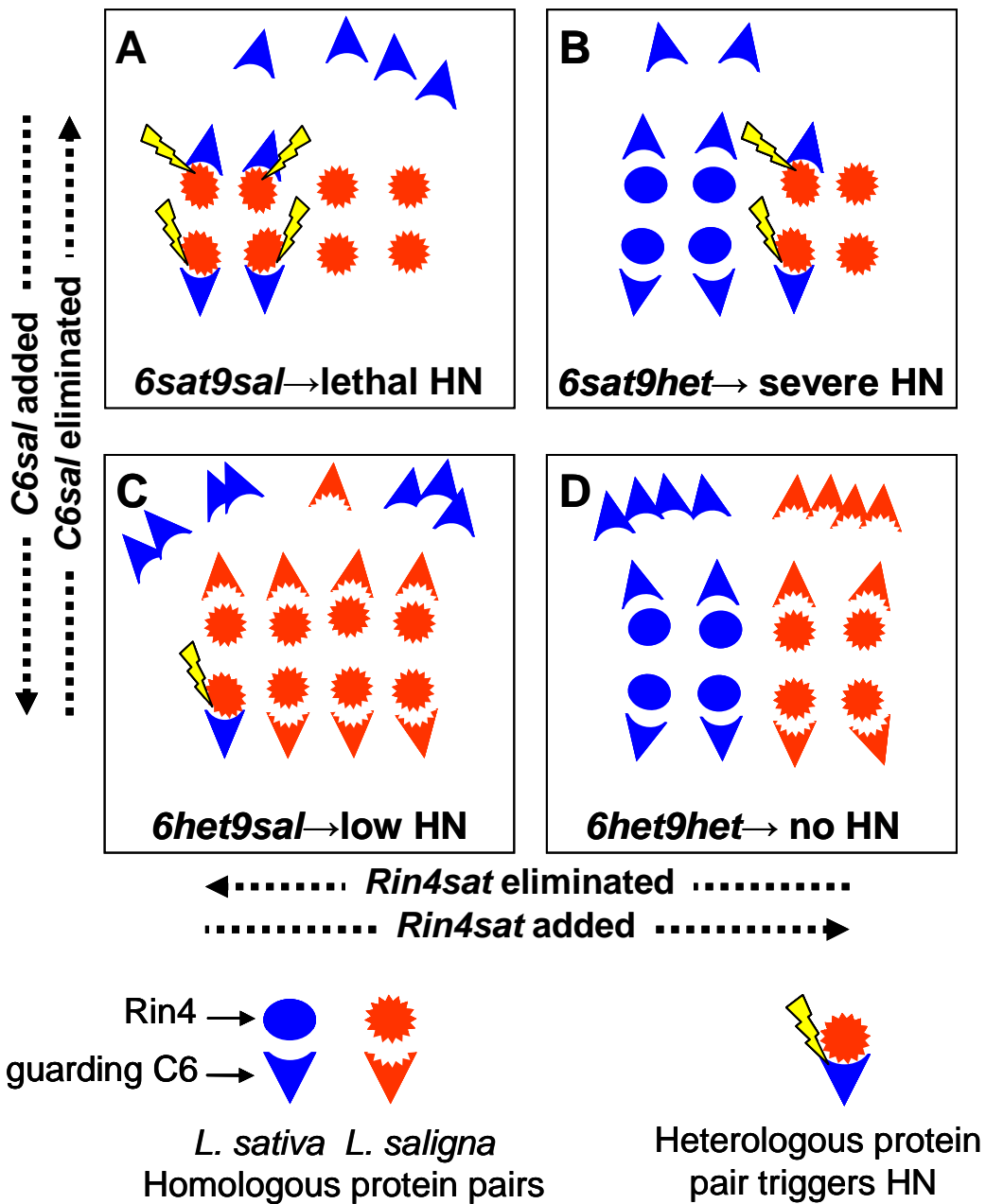


Supplemental Figure 5. Rin4 amino acid haplotypes in *Lactuca* spp.

(A) For cases for which accessions within a species showed identical deduced amino acid sequences, consensus sequences are shown. The eight *L. saligna* accessions were grouped in three consensus sequences. These three haplotype groups are named according to countries of collection: FRA= France, ITA=Italy, RUS=Russia, GEO= Georgia, ISR=Israel. Details of plant material are described in Supplemental Table 6.

Conserved residues that are identical are shaded in black; highly similar residues are shaded in dark grey; quite similar residues are shaded in light grey; residues with low similarity are not shaded. Orange shaded amino acids highlight amino acid differences between *L. saligna* and the other *Lactuca* species. Green shaded amino acids highlight amino acids identical between *L. saligna* and *L. virosa* but different from the other *Lactuca* species. The blue lines represent AvrRpt2 cleavage sites in RIN4 of *A. thaliana* (Kim et al., 2005a). *L. tatarica* expressed two versions of *Rin4T2* transcript, T2a and T2b.

(B) Phylogenetic tree by neighbor-joining.



Supplemental Figure 6. Proposed genetic and molecular model for hybrid necrosis levels in lettuce.

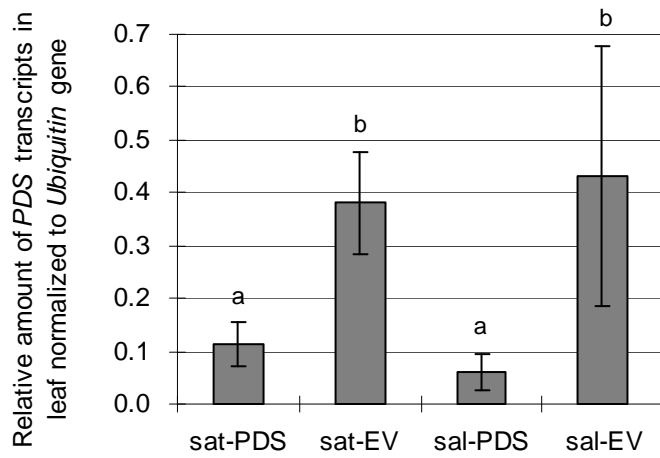
The C6 protein is assumed to be a guard R protein that watches over its target protein Rin4, and when it perceives particular modifications it triggers a hypersensitive response. The C6 protein guards its homologous Rin4 better (100% guarding preference) than its heterologous Rin4 (an arbitrary 50% guarding preference). "HN" = hybrid necrosis.

A. C6sat senses heterologous Rin4sal with a 50% guarding preference and HN is extremely activated (lethal hybrid necrosis). **B.** C6sat proteins prefer to guard their homologous Rin4sat above Rin4sal, less Rin4sal is sensed by C6sat leading to a lower but still severe HN level. In Panel C and D double amounts of C6 proteins were drawn to better illustrate the result of guarding preference differences. **C.** Majority of Rin4sal is guarded by its homologous C6sal proteins. Occasionally, Rin4sal is sensed by C6sat and HN is activated at a low level. **D.** Rin4sat and Rin4sal are both guarded by their homologous C6 proteins; the complex is in balance and does not lead to HN.



Supplemental Figure 7. Silencing of PDS in lettuce by VIGS.

L. sativa cv. Olof (left) and *L. saligna* CGN05271 (right) at 23 days after infiltration are shown. Infection with TRV-*PDS* silences endogenous *PDS* and causes inhibition of carotenoid biosynthesis resulting in the photo-bleaching phenotype.



Supplemental Figure 8. Real-time PCR expression data of *PDS* in lettuce leaves.

mean \pm s.d., sat= *L. sativa* cv. Olof and sal= *L. saligna* CGN05271. The EV addition means plants were infiltrated with empty vector (leaves are green). The PDS addition means that the plants were infiltrated with pTRV2-*PDS* and leaves showing photo bleaching for 75%-100% leaf area were sampled; letters in common, above the error bars, indicate no significant difference between groups ($\alpha=0.05$, Tukey HSD test).

Supplemental Table 1. F₂ segregation ratios and infection severities.

F₂ segregation ratios (panel A), real infection severities to *B. lactucae* Bl:16 per genotype class (panel B) and predicted infection severities to *B. lactucae* Bl:16 per genotype class in case of normal segregation ratios (panel C) are shown. White bold numbers indicate low infection severities to Bl:16. Italic numbers indicate predicted values.

A. Segregation ratios (in %)									
F ₂ cross 1					F ₂ cross 2				
C9 ^a					C9 ^a				
C6 ^a	sat	het	sal	tot	C6 ^a	sat	het	sal	tot
sat	10	11	0	28	sat	9	0	0	9
het	24	22	0	46	het	9	45	4	58
sal	16	9	1	26	sal	4	19	9	32
tot	58	42	1	100 ^b	tot	23	64	13	100 ^c
B. Infection severities ^d									
C9 ^a					C9 ^a				
C6 ^a	sat	het	sal	tot	C6 ^a	sat	het	sal	tot
sat	2.19	0.00		1.37	sat	2.53			2.53
het	1.96	0.53		1.27	het	2.33	0.56	0.00	0.81
sal	1.94	1.50	1.13	1.76	sal	3.33	1.23	1.00	1.41
tot	2.03	0.60	1.13		tot	2.58	0.76	0.71	
C. Predicted infection severity per locus in case of normal segregation ratios (1:2:1 and n=112) ^e									
C9 ^a					C9 ^a				
C6 ^a	sat	het	sal	tot	C6 ^a	sat	het	sal	tot
sat	2.19	0.00	0.00	0.55 ^f	sat	2.19	0.00	0.00	0.55 ^f
het	1.96	0.53	0.00	0.76 ^f	het	1.96	0.53	0.00	0.76 ^f
sal	1.94	1.50	1.13	1.52 ^f	sal	1.94	1.50	1.13	1.52 ^f
tot	2.01	0.64	0.28		tot	2.01	0.64	0.28	

^a Epistatic loci at Chromosome 9 (C9) around 8 cM and Chromosome 6 (C6) around 34 cM. sat=homozygous *L. sativa*, het=heterozygous, sal=homozygous *L. saligna*.

^b 100% equals 113 F₂ plants. Thirteen F₂ plants out of 126 could not be classified as the genotype was unknown for one or both loci.

^c 100% equals 53 F₂ plants. One F₂ plant out of 54 could not be classified as the genotype was unknown for both loci.

^d Average infection severity per genotype class, based on one (cross 2) and two (cross 1) disease tests with *B. lactucae* Bl:16. Scoring classes ranged from 0-4, resistant to susceptible (class 0: no sporulation, class 1: 1-25% of leaf disc area sporulates; class 2: 26-50%; class 3: 50-75% and class 4: 75-100%(Jeuken and Lindhout, 2002).

^e Predicted average infection severities per genotype class per locus. The values of the seven of the nine genotype classes are used from the real data (see above). The

values for genotype 6H9B were predicted to be 0, based on F₂ cross 2. The value of genotype 6sat9sal was predicted to be 0, as 6sat9het and 6sal9het were both 0. A normal segregation ratio of 1:2:1 for both loci and 112 F₂ individuals were used for calculations.

^f In panel C , the predicted infection severities for the genotypes at the C6 locus show a larger difference between sal genotypes and the sat or het genotypes in case of normal segregation ratios than in case of the real segregation ratios in panel B. This implies that a higher LOD value at the C6 locus would possibly have been detected in case of normal segregation.

Supplemental Table 2. Transient expression of *Rin4* alleles and transcripts; macroscopic observations at leaf area at 8 days after infiltration. '+'= necrosis phenotype, '-'= no symptoms

<i>Rin4</i> alleles/transcripts ^a							controls			
A. <i>Rin4</i> alleles and transcripts survey										
Line + genetic code	sat T1	sat T2	sal T1	sal T2 ^b	vir T1	vir T2	H ₂ O	ev ^c	Psoj NIP ^d	
<i>L. sativa</i> cv. Olof	-	-	-	+	-	-	-	-	+	
<i>L. sativa</i> cv. Norden	-	-	-	+	-	-	-	-	+	
<i>L. saligna</i> CGN05271	-	-	-	-	-	-	-	-	+	
<i>L. saligna</i> CGN11341	-	-	-	-	-	-	-	-	+	
BIL6.1+9.1 ^e	6sal9sal	-	-	-	-	-	-	-	+	
BIL6.1 ^f	6sal9sat	-	-	-	-	-	-	-	+	
B. <i>Rin4salT2</i>-associated hybrid necrosis frequency survey in genus <i>Lactuca</i> and other species										
Species + accession nr.					sal T2 ^b			H ₂ O	ev ^c	Psoj NIP ^d
<i>L. sativa</i> cv. Mariska					+			-	-	+
<i>L. sativa</i> cv. Iceberg					+			-	-	+
<i>L. sativa</i> cv. Grand Rapids					+			-	-	+
<i>L. sativa</i> cv. Asparagus					+			-	-	+
<i>L. sativa</i> UcDm10					+			-	-	+
<i>L. sativa</i> cv. Valmaine					+			-	-	+
<i>L. sativa</i> CGN04734					+			-	-	+
<i>L. sativa</i> Ninja					+			-	-	+
<i>L. serriola</i> CGN05939					-			-	-	+
<i>L. serriola</i> PIVT1309					-			-	-	+
<i>L. serriola</i> CGN04796					-			-	-	+
<i>L. serriola</i> CGN05158					-			-	-	+
<i>L. serriola</i> CGN05784					-			-	-	+
<i>L. serriola</i> CGN14295					-			-	-	+
<i>L. altaica</i> CGN04664					-			-	-	+
<i>L. saligna</i> CGN05157					-			-	-	+
<i>L. saligna</i> CGN05314					-			-	-	+
<i>L. saligna</i> CGN05324					-			-	-	+
<i>L. saligna</i> CGN13330					-			-	-	+
<i>L. saligna</i> CGN15726					-			-	-	+
<i>L. saligna</i> CGN16244					-			-	-	+
<i>L. saligna</i> CGN21396					-			-	-	+
<i>L. virosa</i> CGN05869					-			-	-	+
<i>L. virosa</i> CGN05941					-			-	-	+
<i>L. virosa</i> CGN05978					-			-	-	+
<i>L. virosa</i> CGN04683					-			-	-	+
<i>L. virosa</i> CGN13362					-			-	-	+
<i>L. virosa</i> CGN16204					-			-	-	+
<i>L. tenerrima</i> CGN09386					-			-	-	+
<i>L. perennis</i> CGN09323					-			-	-	+
<i>L. viminea</i> CGN16202					-			-	-	+
<i>L. viminea</i> CGN17401					-			-	-	+
<i>L. indica</i> CGN14312					-			-	-	+
<i>L. indica</i> CGN20713					-			-	-	+

<i>Mycelis muralis</i> CGN09327	-	-	-	+
<i>Cichorium intybus</i> L.	-	-	-	+ ^h

^a Alleles: sat=*L. sativa* cv. Olof, sal= *L. saligna* CGN05271, vir=*L. virosa* CGN05978.

T1 and T2 are *Rin4* transcript versions 1 and 2.

^b first symptom visible 6 dpi.

^c empty vector.

^d first symptom visible 4 dpi, caused by *Phytophthora sojae* necrosis-inducing protein.

^e like *L. sativa* cv. Olof but with *L. saligna* introgression at C6 and C9 locus respectively.

^f like *L. sativa* cv. Olof but with *L. saligna* introgression at C6 locus.

^h light necrosis reaction.

Supplemental Table 3. Transient expression of *rin4* mutants.

Six polymorphic residues between *L. saligna* CGN05271 and *L. sativa* cv. Olof at positions 85, 89, 115, 167, 217 and 234, were subject to mutagenesis. A minimum of 5 plants per treatment were used and a minimum of two experiments were performed. Macroscopic observations of necrosis at leaf area were carried out at 8 days after infiltration. '+'= necrosis phenotype, '-'= no symptoms, n.d.= not determined

Mut. residue nr. ^a	<i>Rin4</i> type	Rin4 type name ^b	residue change by site-directed mutagenesis	Infiltration in	
				<i>L. sativa</i> cv. Olof	BIL6.1
	<i>L. saligna</i> allele	Rin4T2sal		+	-
1	mutant	Rin4T2salQ-85	Q→P	+	-
2	mutant	Rin4T2salT-89	T→N	+	-
3	mutant	Rin4T2salQ-115	Q→H	+	-
4	mutant	Rin4T2salT-167	T→P	-	-
5	mutant	Rin4T2salS-217	S→G	-	-
6	mutant	Rin4T2salF-234	F→N	-	-
	<i>L. sativa</i> allele	Rin4T2sat		-	-
4	mutant	Rin4T2satP-167	P→T	-	-
5	mutant	Rin4T2satG-217	G→S	-	-
6	mutant	Rin4T2satN-234	N→F	-	-
4+5	mutant	Rin4T2satP-167 +G-217	P→T, G→S	-	-
4+5+6	mutant	Rin4T2satP-167 +G-218+N-234	P→T, G→S, N→F	-	-
		empty vector		-	-
		PsojNIP ^c		+	+

^a Polymorphic residue number that was changed by mutagenesis.

^b The number is the position of the residue in the protein. See Figure 4.

^c *Phytophthora sojae* necrosis-inducing protein.

Supplemental Table 4. *Lactuca* diversity panel ^a

genus species	CGN acc no, acc name ^b	orig cty ^c	type
<i>Lactuca sativa</i>	CGN05127, Olof ^{de}	Netherlands	butterhead
<i>Lactuca sativa</i>	CGN13390, Norden ^d	Netherlands	butterhead
<i>Lactuca sativa</i>	CGN10966, Mariska ^d	Netherlands	butterhead
<i>Lactuca sativa</i>	CGN04619, Iceberg ^{de}	United States of America	crisp
<i>Lactuca sativa</i>	CGN09352, Grand Rapids ^d	United States of America	crisp
<i>Lactuca sativa</i>	Asparagus, huayesun ^{de}	China	stalk
<i>Lactuca sativa</i>	CGN11339, UCDm10 ^{de}	unknown	latin
<i>Lactuca sativa</i>	CGN05249, Valmaine ^{de}	United States of America	cos
<i>Lactuca sativa</i>	CGN04734 ^{de}	Iran	cutting
<i>Lactuca sativa</i>	CGN04688, Meikoningin ^e	Germany	butterhead
<i>Lactuca sativa</i>	CGN05235, Blonde Lent a Monter ^e	France	cos
<i>Lactuca sativa</i>	CGN20716, Ninja ^d	Netherlands	butterhead
<i>Lactuca serriola</i>	CGN04774 ^e	Egypt	oilseed
<i>Lactuca serriola</i>	CGN05939 ^{de}	Israel	
<i>Lactuca serriola</i>	CGN05099, PIVT1309 ^d	Israel	
<i>Lactuca serriola</i>	CGN04796 ^{de}	Iraq	
<i>Lactuca serriola</i>	CGN05158 ^{de}	Norway	
<i>Lactuca serriola</i>	CGN05784 ^{de}	Slovenia	
<i>Lactuca serriola</i>	CGN14295 ^{de}	Portugal	
<i>Lactuca altaica</i>	CGN15711 ^e	Georgia	
<i>Lactuca altaica</i>	CGN04664 ^{de}	unknown	
<i>Lactuca dregeana</i>	CGN04790 ^e	unknown	
<i>Lactuca dregeana</i>	CGN05805 ^e	unknown	
<i>Lactuca aculeata</i>	CGN09357 ^e	Israel	
<i>Lactuca aculeata</i>	CGN15692 ^e	Turkey	
<i>Lactuca saligna</i>	CGN05271 ^{de}	France	
<i>Lactuca saligna</i>	CGN05157 ^{de}	Italy	
<i>Lactuca saligna</i>	CGN05314 ^{de}	Israel	
<i>Lactuca saligna</i>	CGN05324 ^{de}	Israel	
<i>Lactuca saligna</i>	CGN11341 ^{de}	unknown	
<i>Lactuca saligna</i>	CGN13330 ^d	Turkey	
<i>Lactuca saligna</i>	CGN15726 ^{de}	Russia	
<i>Lactuca saligna</i>	CGN15705 ^e	Georgia	
<i>Lactuca saligna</i>	CGN16244 ^d	Spain	
<i>Lactuca saligna</i>	CGN21396 ^d	Great Britain	
<i>Lactuca saligna</i>	275-5 ^{ef}	France	
<i>Lactuca virosa</i>	CGN05869 ^{de}	Netherlands	
<i>Lactuca virosa</i>	CGN05941 ^{de}	Israel	
<i>Lactuca virosa</i>	CGN05978 ^{de}	Germany	
<i>Lactuca virosa</i>	CGN04683 ^{de}	Netherlands	
<i>Lactuca virosa</i>	CGN13362 ^{de}	France	
<i>Lactuca virosa</i>	CGN16204 ^{de}	Russia	

<i>Lactuca viminea</i>	CGN16202 ^{de}	Armenia
<i>Lactuca viminea</i>	CGN17401 ^{de}	France
<i>Lactuca tatarica</i>	CGN09390 ^e	Russia
<i>Lactuca indica</i>	CGN14312 ^{de}	Indonesia
<i>Lactuca indica</i>	CGN20713 ^{de}	China
<i>Lactuca tenerrima</i>	CGN09386 ^{de}	Spain
<i>Lactuca perennis</i>	CGN09323 ^{de}	Swiss
<i>Mycelis muralis</i>	CGN09327 ^d	Italy
<i>Cichorium intybus</i> L.	wag07 ^{dg}	Netherlands

^a taxonomical relationships and geographical distributions of wild *Lactuca* species are described in Lebeda et al., 2004.

^b accession number in the lettuce collection of the Centre for Genetic Resources, the Netherlands (CGN), <http://www.cgn.wur.nl>, additional specifications available.

^c country where the sample was collected or derived.

^d used in Rin4salT2 transient expression studies.

^e used for *Rin4* cDNA sequencing.

^f provided by A. Beharav from the University of Haifa and collected by Prof. Nevo on the isle of Corsica, France.

^g collected by MJW Jeuken at Wageningen, The Netherlands.

Supplemental Table 5. <i>L. sativa</i>-<i>L. saligna</i> hybrid material		
genotype	accessions	features
<i>L. sativa</i>	cv. Olof cv. Norden	Cultivated lettuce species, host for downy mildew, Olof harbors no known <i>R</i> genes and is susceptible to race Bl:1-Bl:24, Norden harbors <i>Dm3</i> and <i>Dm11</i>
<i>L. saligna</i>	CGN05271 CGN11341	Wild lettuce species, nonhost to downy mildew
<i>L. virosa</i>	CGN05978	Wild lettuce species
preBIL9.1b	Background cv. Olof	Hybrid necrosis phenotype, Heterozygous <i>L. saligna</i> introgression on C9
BIL6.1	Background cv. Olof	Homozygous <i>L. saligna</i> introgression on C6
BIL4.4	Background cv. Olof	Homozygous <i>L. saligna</i> introgression on C4, super susceptible to downy mildew

Supplemental Table 6. DNA markers at C6 and C9 loci

Marker name ^a	Restrict enzyme	forward primer	reverse primer
C6 locus			
LE1134		AGGATCAAATCCGTGTCAGC	TGTGTTGAAAGAAGCATGGG
CLX_S3_14099		AGAAGCAAGCTCCATGAGGA	TAGTCGGAAAACGCCAGTTC
LE0395	Ddel	GCGTGATGTCGCTTTGTTAAT	ACAGTGAGTGTGTCGCAAGG
LE0414		CGATTGGGAACACATGTCAG	TCCTAAAGAACCACGCAACG
NL1010		CTTCCCAATCTGAAAGCTG	CAAATGCATAAGGGAGCAC
KLK1127		GCAACACCACTTCGGATTCT	CCATGAAAATTGCAAGAAAACA
LE0060	Rsal	GGCACATCTGCAAGAAACAA	ATTGTGCCCAAATCTGAAG
LE0138	Hinfl	GGGCTGTAGCTGGTGTGTTGT	CCAGCTAAAGATCTGCGCTC
CLX_S3_357		GGTGACACCTGATGGGAGTT	CTTTTGAAGCAGACGTGCAG
QGG10J06		GGCAACCAAGAAACAGAACC	TGTGGGAGGGATGTGATGTA
QGJ12P21		TGGGCATCGATAATTCCATT	GTGGCCTTGAGAACCAAAAA
QGG21B18		CCCAAGTCACCTTCCACTGT	CAGTTGTATCCCCTGCCATC
CLX_S3_1404		CCAATGGAAAATTTGGTTGC	CCTCTCAAGTGCAGGTAGCC
LE1198		GAGTGTAGCTGCAGTGGCAA	TCCTGCAGTACCAGCAACAG
LE0338	Alul	GGAAATAAACCATCGAGCCA	TTCAGATTATTCAAATGACGATTTA
C9 locus			
NL0656		GCAATGGAGATGAAAGAGC	TTTTTGGTTTCACTTTCCGG
LE1109		GGACGTTCAAATCCAGCAAT	GCAAATCAGCCGATAAATCC
M283		ATGTGTCTGGGGTGGCTTTA	TTGCCCCATAATCATAAAGAATG
LE7020	Hinfl	CGCTGTCATCGGAGTTGTAA	CCAGTGGAAATTTGGGAAGAA
NL0279		AGCTTGACCAGTTCCACAG	GTCCTTTCTGACTCCTCCC
LE0478 ^b	HinclI	GCATATGGCCCATGAATTCTCGG	GCCCTTTAGAGCTATTCACAACA
LE0478indel		ATAGACCAAATTGCCGTCCA	CCCCTTTCAATTTTGATCGT
LE0478INT		GGTAAAACCGGTGGGAAGAT	TTGGTCACGTGGAATGTTGT
NL0919		CTGAGGGTTGCTCTTTCTG	TGTTCAATTCAAAGTTAACCAC
LE0329	ApoI	GGATATCAACGATCGGAGGA	GTTATCGCTGGCCTCAAGTC
LE3008	MseI	CATGCTGAACTCCACGTAACA	CAAATCCCTGCAGCAATAGA
LE7027	MnII	CTATCGCCGGACTATGGAAA	GCATTAGGACGGATGATGCT
KLK1133		AGGCAGAACACCAACTCCAG	CTCCTTGTGTTGGGAAGA
LE1019		TTTTTCCCGATCTTTGCATC	AGCGAATCTTTGCTTTTTTCG
KLK1115dCAPs	HindIII	GAAGATGGATATTGAAGTTCTGGACAAGCT	ACCCATCACCAAGAATCCA
M431dCAPs	Ddel	GATCGATCGTTCATCGTTCTCTCA	TTGTTGAAACAAGTTCACTATTTGG
M3636		CCAGAGACATTCCACAAGCA	CAACAACACGATAATGGGACA
LK1501	NlaIII	GTTGAACATGCGGTGCTTTG	TAAATCCTCCCATGCAGGTC
LE0361	MnII	GCAGCAATCGATTTTGTATCC	TTGTGCCGATATGTTCACT
LE0007	HinclI	AGGCCGACGTGTTTTATTTG	TCCTTGACTCAGTGCCCTCCT

^a For markers that start their name with M, LE or LK primers were originally designed on EST contigs in the Compositae Genome Project Database (CGPDB). For markers that start with KLK we designed new primers on EST contigs from the Compositae Genome Project Database. Marker names starting with NL are SSR markers.

^b LE0478 is the original primer pair derived from CGPDB; LE0478INT and LE0478indel were developed on additional sequence information of lettuce *Rin4*.

Supplemental Table 7. Primer pairs for <i>Rin4</i> sequencing, cloning and RT-PCR			
	Forward primer	Reverse primer	begin-end bp^a
Sequencing <i>Rin4</i> cDNA and gDNA			
Rin4_UTR	CGGATTTCTTTCAAGCTTCTTC	GCCCTTTAGAGCTATTCACAACA	
Rin4_INT1	CAGACCTCAACCGGAAGAAC	TATGTTTCACCGGACGACCT	183-346
Rin4_INT2	CCATGGGACTCCCTCTAGGT	TGATACTCATTCTCTCCCTGCT	488-229
Rin4_INT3	GGTAAAACCGGTGGGAAGAT	TTGGTCACGTGGAATGTTGT	91-667
Rin4_TOT	ATGGCGCAGCGTCCAAGTGA	TCACTTCGACIATGGGAAG	0-732/735
Rin4_END	GTTTGGTGAGTGGGATGAAAACAA	TCACTTCGACIATGGGAAG	546-732/735
Rin4_STA	ATGGCGCAGCGTCCAAGTGA	GACAGTTCTTCCGGTTGAG	0-208
Agro-assay fragment cloning			
Rin4-topoall	CACCATGGCGCAGCGTCCAAGT	TCACTTCGACIATGGGAAG	
VIGS and RT-PCR			
Is-PDS1 ^b	TACCCGAAGAATGGAAACCA	CAGCTGCAATTTTCATCAGGA	
Is-PDS-RT3 ^c	CCACCCACCATAACATCCATTGAG	TGGCAGAAACATTTCCAAACAGAG	
Is-Ubiquitin ^c	GAAGAAGACCTACACCAAGCCAAAG	ACTCAGCATTAGGGCACTCTTCC	

^a beginning and ending base pair number of PCR product of cDNA sequence *Rin4*.

^b primer pair used for fragment cloning in pTRV2 for VIGS.

^c primer pair used in RT-PCR on VIGS-silenced plants.